

**POLLINATOR MEDIATED REPRODUCTIVE CONSEQUENCES OF ALTERED CO-
FLOWERING UNDER CLIMATE CHANGE DEPEND ON ABIOTIC CONTEXT**

A THESIS SUBMITTED TO THE FACULTY OF THE PROGRAM IN PLANT BIOLOGY
AND CONSERVATION

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IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER
OF SCIENCE IN PLANT BIOLOGY AND CONSERVATION FROM NORTHWESTERN
UNIVERSITY AND THE CHICAGO BOTANIC GARDEN

MAY 24, 2019

Signature Page:

Abstract:

Pollinator mediated reproductive consequences of altered co-flowering under climate change depend on abiotic context

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The abiotic and biotic effects of climate change on plant reproduction are typically studied separately despite their potential to interact. Climate change-induced shifts in phenology provide a framework for studying interactions between abiotic and biotic effects of climate change because phenological shifts can expose organisms to stressful abiotic conditions in addition to altering their biotic interactions. For example, shifts in co-flowering patterns are likely to alter the degree to which plant species interact for pollinators, but how these interactions are affected by changes in abiotic conditions is unclear. Using a short-term field experiment that is based on a long-term flowering phenology data set, I examined how shifts in co-flowering between *Linum lewisii* and *Potentilla pulcherrima* affect the reproductive success of *Linum* in control and water addition scenarios. Long-term shifts in flowering phenology show that *Linum* is co-flowering 25% less than it used to with *Potentilla* as the climate changes. I therefore removed *Potentilla* flowers in the field to alter the degree of co-flowering overlap from 0-100% between the two species; half of these plots were watered to relieve drought stress in a dry year. Finally, I also examined pollen limitation through hand pollination treatments within each plot. I measured pollinator visitation, pollen deposition, and female reproductive success in *Linum*. Pollen limitation was not detected in *Linum*. Pollinator visitation rates and the number of conspecific pollen grains were both unaffected by co-flowering overlap and watering. However, *Linum* plants experiencing less overlap with *Potentilla* had a higher proportion of conspecific pollen on their stigmas in both watered and unwatered plots. Plants experiencing less overlap

also produced a higher proportion of fruits per flower, seeds per ovule, and seeds per plant, but only in the watering treatment; co-flowering did not affect reproductive success in unwatered plants. These results indicate that plants that co-flowered to a lesser extent with *Potentilla* had enhanced reproductive output because they received a higher proportion of *Linum* pollen, but these biotic effects were only detectable when the plants were not water-limited. Thus, if the climate continues to shift towards earlier snowmelt, and severe droughts become more frequent, the positive biotic effects of decreased co-flowering will likely play a lesser role in affecting *Linum* reproduction than drought. Since the abiotic and biotic effects of climate change can interact, studying them in isolation provides an incomplete picture of biological responses to climate change. Only through investigating them in conjunction with one another can we begin to understand and predict how climate change will affect plant reproduction and potentially even population persistence.

Acknowledgements

I would like to extend thanks to many people, across many states and institutions, who very generously contributed to the work presented in this thesis.

Special mention goes to my enthusiastic and dedicated advisor, Dr. Amy Iler. My M.S. at Northwestern University and the Chicago Botanic Garden has been an absolutely wonderful experience. I thank Amy wholeheartedly not only for her tremendous academic support, but also for her faith in me as a student and researcher. Lastly, I thank Amy for giving me the opportunity to do work at the Rocky Mountain Biological Laboratory (RMBL). I am immensely grateful for the chance to experience such a special place. RMBL forever holds a place in my heart.

I am also immensely grateful to my committee members, Dr. Paul CaraDonna and Dr. Krissa Skogen. I thank them for their valuable academic and professional advice. I thank Paul, especially, for being so dedicated to his role as my “secondary advisor.”

Special mention also goes to the members of the Iler-CaraDonna Lab for all of their helpful feedback and moral support. I also thank Dr. Nick Waser and Dr. Mary Price for all of their advice and letting me use their lab space and equipment. Additionally, I am hugely grateful to my REU student, Jessica Robledo, for all the long hours in the field setting up plots and conducting pollinator observations.

I am also very thankful to the funding agencies who made this work possible: The Plant Biology and Conservation Department at Northwestern University and the Chicago Botanic Garden, the Rocky Mountain Biological Laboratory, the American Alpine Club, and Kavu.

Lastly, but definitely not least, thanks go to my mom and dad for all the incredible moral and financial support over the years. I am especially thankful to my mom for counting all of those seeds with me! This work is dedicated to both of you.

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1 **Introduction**

2 Anthropogenic climate change can directly affect plant reproduction through altered
3 temperature and precipitation regimes, and increasing climatic variability at local, regional, and
4 global scales (Inouye 2008; Peñuelas *et al.*, 2004; Scaven and Rafferty 2013). The direct effects
5 of changes in temperature and water availability on plant reproduction are well understood:
6 extreme temperatures and water stress can reduce the capacity of plants to uptake nutrients and
7 directly damage plant tissues, negatively impacting growth and reproduction (Saavedra *et al.*,
8 2003; Caruso *et al.*, 2005; Caruso 2006; Zinn *et al.*, 2010; Scaven and Rafferty 2013). Climate
9 change can also impact plant reproduction indirectly, via changes in biotic interactions with
10 herbivores and pollinators (Singer and Parmesan 2010; Thomson 2010; Liu *et al.*, 2011; Kudo
11 and Ida 2013). The relative importance of the indirect, biotic impacts of climate change
12 compared to the direct, abiotic effects is poorly understood, as they are typically studied
13 separately (Forrest 2015). To better understand how plant species and communities respond to
14 climate change, the abiotic and biotic effects of climate change on plant performance need to be
15 examined in conjunction with one another.

16 Shifts in flowering phenology can be used to examine both the abiotic and biotic effects
17 of climate change because they can expose plants to stressful abiotic conditions and alter biotic
18 interactions (Inouye 2008; Liu *et al.*, 2011; Kudo and Ida 2013). Many species are shifting their
19 flowering phenologies earlier in accordance with the warming climate, however, the direction
20 and magnitude of phenological change is often species-specific because species are differentially
21 sensitive to climate drivers in their local community (Ovaskainen *et al.*, 2013; CaraDonna *et al.*,
22 2014; Theobald *et al.*, 2017; Vilela *et al.*, 2018). Such species-specific shifts in plant phenology
23 can not only directly affect plant fitness through exposing plants to unfavorable abiotic

conditions, but also indirectly by altering the synchrony, or temporal overlap, of plants with antagonists and mutualists, including pollinators, herbivores, and co-flowering plant species (Forrest 2015; Rafferty *et al.*, 2015). In particular, species-specific phenological shifts in response to climate change are altering co-flowering patterns at the community-level (*i.e.*, the temporal synchrony or temporal overlap of co-occurring flowering species) (Forrest *et al.*, 2010; CaraDonna *et al.*, 2014; Theobald *et al.*, 2017). Although climate-driven changes in co-flowering patterns have been widely hypothesized to alter species interactions (Mitchell *et al.*, 2009; Forrest *et al.*, 2010; Theobald *et al.*, 2017), the consequences of these changes are still widely unknown (Miller-Rushing *et al.*, 2010).

The majority of studies examining the biotic effects of climate change on plant reproduction have focused on shifts in synchrony between plants and other organisms, such as between plants and pollinators, as opposed to between different plant species (*e.g.*, co-flowering). In particular, most attention has focused on the potential consequences for plant reproduction of shifts in synchrony between flowering and pollinator activity, where if the phenologies of plants and pollinators are not shifting equally with climate change, “phenological mismatches” might develop (Forrest 2015; Rafferty *et al.*, 2015). Such asynchronies could decrease pollinator visitation to plants, relative to the historic average, and reduce plant reproduction (Forrest 2015; Rafferty *et al.*, 2015). Indeed, field experiments, short-term studies, and modeling approaches find that shifts toward both earlier and later flowering are likely to reduce synchrony between plants and pollinators, reducing pollinator visitation, and decreasing fitness in some plant species (Memmott *et al.*, 2007; Rafferty and Ives 2011; Gezon *et al.*, 2016; Rafferty *et al.*, 2016). Long-term studies have also documented reductions in pollination and seed set in earlier flowering plants, likely because of asynchrony between flowering and

pollinator emergence (Kudo 2004; Thomson 2010; Burkle *et al.*, 2013; Kudo and Ida 2013). Yet, some long-term studies have found no directional change in synchrony between plants and pollinators (Bartomeus *et al.*, 2011; Iler *et al.*, 2013). Therefore, shifts in synchrony between plants and pollinators, and their consequences on reproduction, are not uniform across plant species. However, shifts in plant-pollinator synchrony are not isolated from changes in other species interactions, and therefore, they will likely be mediated by changes in plant-plant interactions, such as co-flowering.

While shifts in co-flowering among plant species can impact pollination and plant reproductive success, little research has been dedicated to the reproductive consequences of climate-driven changes in co-flowering. Shifts in co-flowering can have consequences for plant reproductive success if competitive or facilitative interactions between plant species modify the strength of their interactions with animals. A multitude of studies have shown that changes in co-flowering between plant species can increase or decrease competition for pollinator services by impacting pollinator visits and receipt of compatible pollen (Waser 1978; Johnson *et al.*, 2003; Mitchell *et al.*, 2009). Increases in co-flowering generally tend to increase interspecific competition for pollinator services, resulting in reductions in plant reproductive success via reduced pollinator visitation rates and/or increased receipt of heterospecific pollen (Waser 1978; Brown *et al.*, 2002). Alternatively, increases in co-flowering can create multi-species floral displays that attract more pollinators, resulting in increases fruit and seed set through facilitation (Moeller and Geber 2005; McKinney and Goodell 2011). Despite the wealth of literature documenting the varied impacts of co-flowering on plant fitness, only one study to my knowledge has investigated how *climate-driven* shifts in co-flowering can impact reproductive success. Vilela *et al.*, (2018) found that climate-driven increases in co-flowering between four

Malpighiaceae species over a 10-year period decreased fruit set in one species with a lower rate of autogamy. Thus, there are some data showing that climate-driven shifts in co-flowering between interacting plant species can impact reproductive success by impacting competition for pollinator services, however, we have a poor understanding of how changes in co-flowering may interact with changes in the abiotic environment under climate change to affect plant reproductive success.

To investigate the reproductive consequences of climate-driven shifts in co-flowering and drought stress I conduct a short-term field experiment based on known climate-driven changes in co-flowering from a long-term flowering phenology data set from the Rocky Mountains in Colorado, USA. I examine how altered co-flowering between two subalpine herbs, *Linum lewisii* (*Linaceae*) and *Potentilla pulcherrima* (*Rosaceae*) (hereafter *Linum* and *Potentilla*), affect the pollination and reproductive success of *Linum* in control and water addition scenarios. Long-term shifts in flowering phenology show that *Linum* is co-flowering 25% less than it used to with *Potentilla* as the climate changes (CaraDonna *et al.*, 2014). To experimentally decrease co-flowering between *Linum* and *Potentilla*, I removed *Potentilla* flowers to create different degrees of co-flowering. To investigate potential interactions between co-flowering and climate-induced drought-stress on plant reproduction, I added water to half of the plots during a severe drought. Specifically, I ask how experimental changes in water availability and co-flowering between *Linum* and *Potentilla* affect 1) interactions for pollinator services and 2) fruit and seed set in *Linum*. I expect that plants receiving additional water will produce more fruits and seeds. Additionally, I expect that a reduction in co-flowering will result in an increase in pollinator visitation rates or the proportion of conspecific pollen, a decrease in pollen limitation, and an

increase *Linum* fitness. Lastly, watering and co-flowering may interact, so that co-flowering only affects pollination and plant reproduction in plants receiving additional water.

Methods

Study site

This study took place in the year 2018 in a dry, subalpine meadow encompassing ca 48,000 square meters at the Rocky Mountain Biological Laboratory (RMBL) (38°57' N, 106°59' W, elevation: approx. 2900 m) in Gothic, Colorado, USA. The landscape surrounding the site was a mosaic of conifer-aspen forests, and wet and dry meadows that supports a diverse array of forbs, shrubs and grasses. Forbs and grasses dominated the study site, with *Festuca thurberi*, *Potentilla pulcherrima* and *Linum lewisii* covering the majority of the study plots. Other common forb species included: *Delphinium nuttallianum*, *Erigeron speciosus*, *Veratrum californicum*, *Ipomopsis aggregata*, and *Lupinus argeneus*. The experiment was located within a west facing, dry meadow encompassing ca. 48,000 square meters with soils dominated by fine sand, a neutral pH, and low salinity (Appendix B). The slope and elevation varied across the meadow, with the slope ranging from 1.03 degrees to 14.2 degrees, and the elevation steadily increasing from west to east. The growing season at the site was short, with snowmelt occurring in May and snowfall beginning in early October.

Study Species

I used a 39-year flowering phenology study from the RMBL (Inouye 2008; CaraDonna *et al.*, 2014) to select *Linum lewisii* (Linaceae) and *Potentilla pulcherrima* (Rosaceae) as my study species. *Linum* and *Potentilla* are common, locally abundant species at the RMBL. Across 39 years (1973–2012), *Linum* and *Potentilla* have altered their flowering phenologies as the climate

changes; the first flowering date of *Linum* has shifted earlier, resulting in a longer flowering duration at the population-level, and *Potentilla* has shifted its peak flowering earlier, with no change to flowering duration at the population-level (Figure A1). Consequently, *Linum* is co-flowering 25% less with *Potentilla* (i.e. less co-flowering due to earlier flowering), whereas *Potentilla* is co-flowering 39% more with *Linum* (i.e. more co-flowering due to shift in peak flowering). I also selected this species pair because indirect evidence suggests that these species may compete with one another for pollinators: these two plant species share pollinator species, and early individuals of *Linum* produce more fruits than later individuals, whereas in *Potentilla*, later individuals produce more fruits than early individuals (Iler, *unpublished data*). *Linum* was used as the focal taxon to assess the impact of climate change and co-flowering on plant fitness because *Linum* is an obligate outcrosser and *Potentilla* is capable of selfing. Thus, *Linum* was more likely to be affected by changes in co-flowering than *Potentilla*.

Linum Lewisii is a short-lived, perennial herb that thrives in open areas and well-drained, sandy soils. *Linum* is widely distributed across North America. It extends from Alaska to Louisiana, and establishes in a variety of ecosystems, such as mixed grasslands, sagebrush, piñon-juniper, subalpine-meadows and aspen communities, and in openings in coniferous forests (Ogle *et al.*, 2009, *USDA unpublished data*). *Linum* plants have large taproots from which many new stalks emerge every season (Burkle and Irwin 2009). Each plant produces multiple stalks bearing cymose inflorescences with pale blue flowers. The perfect flowers have five petals, sepals and stamens. Each flower lasts one day; the flower opens in the morning around 09:00 hrs and the petals begin to abscise around 15:00 hrs, depending on the amount of wind or precipitation (Kearns and Inouye 1994). Two ovules are born within each of the five fused carpels, with a total of ten possible seeds. The seeds mature in capsules, which dry and dehisce in

mid-August. *Linum* is self-compatible, however, it is an obligate-outcrossing perennial, where insects are required to transfer self-pollen for seed set (Kearns and Inouye 1994). A variety of bees and flies are known to pollinate *Linum*, including Muscoid, Empidid, Syrphid, Bombyliid, and Nematoceran flies, *Bombus* spp, and small bees, within the Halictidae and Andrenidae families (Kearns and Inouye 1994). Bees are more effective than flies at depositing *Linum* pollen on a per visit basis, however, flies appear to deposit more pollen overall due to their higher visitation rates (Kearns and Inouye 1994).

Potentilla pulcherrima is a self-compatible, long-lived perennial herb that grows in open, well-drained subalpine meadows (Stinson 2004). *Potentilla* is distributed across the intermountain west, and typically establishes in subalpine meadows and open areas of coniferous forests (Stinson 2004). This species tends to dominate or co-dominate in mixed perennial communities; it grows from rhizomes, spreading horizontally and ascending about 20-45cm (Stinson 2004). *Potentilla* is iteroparous, as it produces flowers and fruits every year after the first year of growth, and recruitment usually occurs from locally dispersed seeds (Stinson 2004). It produces many yellow flowers per plant, which have five petals. The flowers are visited by a wide variety of bees and flies (Burkle and Irwin 2010). Although self-compatible, *Potentilla* requires pollinator-assisted outcrossing for maximal seed set (Burkle and Irwin 2010). *Potentilla* can also vegetatively reproduce through producing stalks near the base of the parent plant (Burkle and Irwin 2010).

Experimental Design

Co-flowering treatments. The study was a split-plot, randomized block design (12 blocks total, with 3 co-flowering treatment plots per block, for a total of 36 plots). Each experimental block consisted of three plots that were randomly assigned to a *Potentilla* flower removal

treatment: 100% removal, 50% removal, and a control in which no *Potentilla* flowers were removed. Focal *Linum* plants occurred within a 1×1 m plot that was surrounded by a larger removal area of 4×4 m. The aim of this design was to control for potential edge effects of the flower removal treatment. Experimental plots were established in areas that had at least five *Linum* individuals within the central 1×1 m plot and at least 20 *Potentilla* individuals within the larger 4×4 m area. Each individual *Linum* plant in the 1×1 m plot was assigned a unique identification number and marked with a small, aluminum tag in the ground. Plots were separated by 4–8 m within each block, and blocks were separated by at least 12 m. I removed *Potentilla* flowers from plots by clipping flowers and flower buds with scissors. For the 50% removal treatment, I counted the total number of *Potentilla* flowers and flower buds in each plot and cut half of the flowers and buds evenly throughout the plot. I waited two weeks into the *Linum* flowering season to count and remove *Potentilla* flowers and flower buds in the 50% removal treatment because *Potentilla* flowers later than *Linum*, and there were few *Potentilla* flowers present within the first two weeks of *Linum*'s flowering season. The 100% flower removal treatment was applied before *Potentilla* began to flower to ensure that there was no co-flowering between the two species. In 2018, spring snowmelt (defined as first date on which bare ground was visible) occurred on 24 April in a long-term plot ca. 1 km from the study site, and was the sixth earliest recorded snowmelt date since 1975 (b. barr, *unpublished data*). Therefore, the control treatment (no flower removal) represented an earlier than average year, and the 100% and 50% removal treatments represented a future climate change scenarios with even less co-flowering, respectively.

Watering Addition. Half of the 12 experimental blocks were randomly assigned to a water addition treatment, and half were controls. The watering treatment began two weeks into

Linum flowering during the 8-week flowering season (season length: 18 June – 10 August, first watering date: 2 July). Four liters of water were applied to each 1×1 m plot, 3-4 times per week for a total of four weeks (2 July – 30 July 2018). The water was sourced from Copper Creek adjacent to the study site and applied using plastic watering cans. Water was applied evenly across the 1×1 m plots at a rate to allow the water to be absorbed into the soil (*i.e.*, no water runoff occurred). I watered at the end of the day to minimize evapotranspiration and measured the volumetric water content of the soil in each plot using a FieldScout TDR 100 Meter, (Spectrum Technologies, Aurora, IL, USA) at a probe depth of 7.62 cm. I took a soil moisture measurement before I started watering and then after watering began to ensure I was increasing soil moisture. Spring 2018 was a particularly dry spring at the RMBL because it had the second lowest snowpack ever recorded, and the water content of the snowpack was 39% lower than the long-term average (2018= 42.4cm; 1974-2018 average= 69.4cm; b. barr, *unpublished data*). Thus, the watering treatment alleviated drought conditions relative to the unwatered plots.

Pollen limitation experiment. The 1×1 m plots were split into two pollination treatments: an open-pollinated control and pollen addition. If a flowering individual was located in the pollen addition section of the plot, I hand pollinated its flowers. Specifically, I collected anthers with forceps from *Linum* plants that were located anywhere between 4 and 12 meters from the 4×4 m plot and rubbed the anthers directly onto the stigmas of the flowering individual. Anthers were rubbed against stigmas within two minutes of collection. I recorded and hand pollinated the flowers every day because *Linum* flowers only last for one day (Kearns and Inouye 1994).

Data Collection

Pollinator observations. Pollinator observations occurred on open *Linum* flowers in the 1×1 m plots during the flowering period of *Linum* (25 June – 20 July 2018). Observations were

conducted from 9:00 – 14:00 because the flowers opened mid-morning and the petals fell off around mid-afternoon. On rainy days or days with more than 80% cloud cover, observations did not occur, because pollinators were inactive. For the first two weeks of flowering, each observation period lasted five minutes per plot with one observation period at each plot per day. The observation period was then increased to 10 minutes per plot for the rest of the flowering season because there were few visitors entering the plots during the five-minute observation sessions. Observations were only conducted at plots that had at least one flower present. Before starting an observation, the time, date, cloud cover, wind velocity, air temperature, and the total number of *Linum* flowers within the 1×1 m plot were recorded. Pollinators were then observed on a set number of *Linum* flowers that could be observed for the entire observation session. The number of visits to *Linum* flowers was recorded for each visitor that contacted the reproductive organs of the flower. The visitation rate was calculated as the number of visits per flower per minute. The number of visitors that contacted the reproductive organs of the flower during each observation session was also recorded. Visitors were identified to the family-level when possible based off of the insect collection at the RMBL and by taking pictures for later identification using a Canon EOS Rebel camera. Two people conducted a total of 209 observation sessions, for a total of 1,735 minutes, over 18 days.

Pollen Deposition. A total of 500 stigmas were collected for two weeks during *Linum* peak flowering, and slightly after peak flowering, from plants that did not receive additional pollen. To give pollinators ample time to visit the flowers, the stigmas were collected around 14:00 hrs and after petals had fallen off. The stigmas were removed with forceps, placed in an Eppendorf tube, and stored in a freezer for later analysis. After thawing, the stigmas were stained with fuchsin gel, mounted on slides, and viewed under a compound microscope at 40x

magnification. To distinguish *Linum* pollen from *Potentilla* pollen I measured the size of pollen grains of both species: *Linum* pollen grains ranged from 1.5–2.50 mm and *Potentilla* from 0.5–1.0 mm (Figure A2). I was unable to distinguish *Potentilla* from all other pollen grains on the stigmas, so all non-*Linum* pollen was categorized as “other”. I calculated the proportion of conspecific pollen as the number of *Linum* pollen grains divided by the total number of pollen grains (pollen quality). The total number of *Linum* pollen grains deposited on a stigma was my measure of pollen quantity.

Linum Reproduction. To examine *Linum* reproduction I recorded the number of flowers (reproductive potential) every day from 18 June – 10 August, and the number of stalks (plant size) on each *Linum* plant within the 1×1 m plots. I collected all fruits from experimental *Linum* plants and stored them in paper envelopes from early to mid-August. Lastly, all seeds were removed from fruit capsules by hand in a lab at the Chicago Botanic Garden, and seed viability was determined by appearance, as non-viable seeds were flat, soft, and pale (Figure A3). This visual assessment of seed viability was confirmed via X-Ray (Faxitron X-Ray Corporation MX-20) on a subsample of 30 seeds. I measured female reproductive success per plant as fruits per flower (number of fruits divided by the total number of flowers per plant), the seeds per ovule (number of viable seeds divided by the total ovules), and total seeds per plant (total number of viable seeds).

Data Analysis

Co-flowering Treatments. One third of the *Linum* plants did not flower (34%), and 22% of the plots contained plants that produced little to no flowers per day. Consequently, I removed plots that contained on average less than two flowers per day from the analysis, under the assumption that fewer than two flowers was not enough to detect a response to a change in co-

flowering with *Potentilla*. As a result, I removed eight plots from the analyses and was left with 28 plots across 10 blocks (vs. 36 plots across 12 blocks).

To explore the impact of *Potentilla* flower removal on the amount of co-flowering between *Linum* and *Potentilla* flowers, I first compared the difference in co-flowering between control and 50% removal plots in each block. Co-flowering was calculated based on the abundance of *Linum* and *Potentilla* flowers in each plot during peak flowering. The number of *Potentilla* flowers in each 4×4 m plot was counted once per week during the three weeks of peak flowering and then averaged across the three dates. The number of *Linum* flowers for each 1×1 m plot was averaged across the same three dates. I divided the number of *Potentilla* flowers in each plot by sixteen to calculate the number of flowers per square meter (for direct comparison with *Linum*). Next, I divided the total minimum number of flowers shared between *Linum* and *Potentilla* plants in the plot by the total number of *Linum* flowers in the plot (following CaraDonna *et al.*, 2014). A value of 1 indicates that *Linum* flowering completely overlapped with *Potentilla* flowering at peak flowering. A paired t-test was used to examine the difference in co-flowering overlap between the control and 50% removal plots within each experimental block (there were 16 plots total in the paired t-test after removing the 8 plots that had little to no *Linum* flowers). The 50% removal plots contained on average 29% (95% CI: 28.94–29.5%) less co-flowering compared to control plots ($t = 2.99$, $df = 7$, $p = 0.02$; Figure 1). However, co-flowering among the control and 50% removal plots was highly variable across blocks and was more accurately described as a continuous variable (Figure 1). For example, in four of the 50% removal plots, co-flowering was between 90% and 100%. This result likely occurred because many of these plots contained little to no *Linum* flowers, so that removing 50% of the *Potentilla* flowers had a minor effect on overlap compared to controls. I therefore treated co-flowering

overlap as a continuous variable ranging from 0 to 1 in my analyses, as opposed to a categorical variable.

Water Addition. To investigate the effectiveness of the watering treatment, I compared volumetric soil moisture (% water) between watered and control plots in a linear mixed model (across all 36 plots). The linear mixed model included watering treatment as a categorical predictor, and soil moisture as a continuous response. To account for variation across soil moisture recording dates, I included day of year as a random intercept term. I compared a model including watering treatment to a null model with an intercept only, and I selected the best model based on the lowest AIC score (Table A1).

Pollination & Reproductive Success. I used generalized linear mixed effect models (GLMMs) to analyze the effects of co-flowering on *Linum* pollination (pollen quantity, pollen quality, visitation rate, and number of visitors) and reproductive success (fruits per flower, seeds per ovule and total seeds per plant). A GLM was used for pollinator visitation rate due to model convergence issues (see below). Similarly, all of the analyses except pollinator visitation rate and number of visitors were conducted on plants that did not receive supplemental pollen (pollinator visitation was measured across all open flowers within plots, regardless of whether they received additional pollen). A negative binomial error distribution was used for over-dispersed count data (number of visitors, pollen quantity, and total seeds per plant) and a binomial error distribution for proportion data (visitation rate, pollen quality, fruits per flower and seeds per ovule). I used a chi-square goodness of fit test on the residual deviance to check for over-dispersion. The fixed predictors for all of the analyses differed depending on the response variable, however, every analysis included co-flowering as a fixed, continuous predictor (more detailed descriptions of the fixed predictors are provided below). Plot and block were random intercept terms in all of the

GLMMs unless otherwise stated (plot was not a random effect for visitation rate or number of visitors because these were already plot-level responses). Treating block as a random effect allowed me to account for variation across the meadow, and treating plot as a random effect allowed me to use data at the individual plant-level in my analyses. For each response variable, I performed model selection based on AIC values, to determine the model of best fit from a set of candidate models. The null model was an intercept-only model for each response variable, in addition to any random effects. The best model for each response variable was selected based on the lowest AIC value. To accept a more complex model over the null, ΔAIC had to be ≥ 2 . Models other than the null that were within 2 AIC units from one another are discussed in the

For the number of visitors and visitation rate, co-flowering and the mean number of *Linum* flowers per plot per observation day were continuous predictors. I included floral abundance to account for potential responses of pollinator behavior to flower density in both of these models. Watering treatment was analyzed separately because watering commenced two weeks after pollinator observations started. Watering was not included as a fixed predictor in the GLMMs described here because watering had no effect on pollinator visitation (see Appendix C for details). Day of year was included as a random effect to account for variation across observation dates; however, models for visitation rate failed to converge. To achieve convergence, I averaged visitation rates across observation dates at the plot-level and made block a fixed predictor in all candidate models rather than a random effect (the number of *Linum* flowers, a continuous predictor, was also averaged across all dates for each plot). There were four candidate models: the full model included an interaction between co-flowering and mean number of flowers per plot with block as an additive term. In addition to the null model, all possible combinations of predictors that also include the co-flowering treatment composed the

remaining models because understanding the effects of co-flowering was the focus of the study (see Table A2 for full set of candidate models).

To assess the quality and quantity of pollen deposited by pollinators, the co-flowering and watering treatments were fixed predictors. I added an observation-level random effect to account for over-dispersion in pollen quality (Harrison 2014; Harrison 2015), and I consequently removed plot as a random effect because the observation-level random effect accounts for pseudoreplication within each plot. Thus, there were four candidate models each for pollen quantity and quality, where the full model included an interaction between the co-flowering treatment and watering treatments. Similar to above, I then included all possible combinations of predictors that also include the co-flowering treatment, in addition to a null model (Table A2).

For the reproductive analyses, the fixed effects of the GLMMs included co-flowering as a continuous predictor, watering treatment as a categorical predictor, and plant size (number of stalks) as a continuous predictor, because larger plants were expected to have higher reproductive output (Elzinga *et al.*, 2007). For fruits per flower, an observation-level random effect was added to account for over-dispersion, and as for pollen quality, plot was removed as a random effect. For each reproductive response, my candidate set included eleven different models: the full model included a three-way interaction among all three fixed effects (co-flowering, watering, and plant size). As above, I then included all possible combinations of predictors that also include the co-flowering treatment and a null model (see Table A3 for list of candidate models).

Pollen Limitation. To examine whether reproduction in *Linum* was pollen limited, I calculated the average magnitude of pollen limitation at the plot-level. Specifically, I determined the difference in effect size between control plants and plants supplemented with pollen for mean

fruits per flower, mean seeds per ovule, and mean seeds per plant. I ran linear models where the co-flowering treatment was a continuous predictor and watering treatment and block were categorical predictors, and the magnitude of pollen limitation (*i.e.*, the difference in effect size) was the response variable. Block was included as a fixed effect in all models because the models did not converge when it was added as a random effect. I then compared four models that contained co-flowering, where the full model included an interaction between water and co-flowering and the addition of block, plus the null model (Table A4).

Results

Across the study period, 313 of the 467 *Linum* plants produced a total of 9,339 flowers, with an average of 30 flowers produced per flowering plant over the 8-week flowering season. *Linum* plants grew an average of 13.7 stalks per plant, which were an average of 33.1 cm tall ($n = 467$ plants). *Linum* plants produced a total of 4,200 fruits and 9,720 seeds, with an average of 19.3 fruits and 50.4 seeds per plant. A total of 209 pollinator observation sessions were conducted over 18 days, for a total of 1,735 minutes. The watering treatment increased soil moisture by 3.04% (9.48 ± 0.96 , $t=9.87$; Figure 2; Table A1). The pollen limitation experiment showed that *Linum* was not pollen limited (Figure 3; Table 1; Table A4).

Pollination

Visitation to *Linum* was primarily by bees (37% of visits, mostly Halictidae and Apidae) and flies (36% of the visits, Muscidae, Syrphidae, and Bombyliidae), and to a lesser extent by Lepidoptera (10%), Coleoptera (11%), wasps (3%), and unknown visitors (3%). Neither watering nor co-flowering affected visitation rate or the number of visitors (Figure 4). The

number of visitors depended on conspecific floral abundance (Table A2), with the mean number of visitors increasing as the number of flowers per plot increased (Table 2).

Watering did not affect pollen quantity or quality, but co-flowering affected pollen quality (Figure 5). The best model for pollen quality contained co-flowering, whereas the best model for pollen quantity was the null (Table A2). As co-flowering increased, the proportion of conspecific pollen deposition (*i.e.* pollen quality) decreased (Table 2; Figure 5). For pollen quality, a model containing the addition of water was within 2 AIC units of the best model ($\Delta\text{AIC} = 1.9$); however, the additive effect was weak (-0.16 ± 0.41 , $z=-0.4$, $p=0.69$), showing that the model with solely the co-flowering treatment best described the data.

Reproductive Success

Co-flowering affected fruits per flower and its effect depended on both plant size and watering (Table 3). There were two competing models within 2 AIC units of the best model: one contained only the interaction between co-flowering and watering ($\Delta\text{AIC} 1.4$) and the other only between co-flowering and size ($\Delta\text{AIC} 1.7$; Table A3). These results suggest that both interactions are important, so I present the model with both interactions here (Table 3). In *Linum* plants that were not watered, fruits per flower increased as co-flowering increased in small plants, whereas in large plants, fruits per flower decreased as co-flowering increased (Figure 6). In watered *Linum* plants, fruits per flower decreased as co-flowering increased, and large plants had more fruits per flower than small plants (Figure 6).

The effect of co-flowering on seeds per ovule depended on watering (Table 3). There were two competing models within 2 AIC units of the best model: one included additive effects of co-flowering and water ($\Delta\text{AIC} 0.7$), and the other included an interaction between co-flowering and watering plus an additive effect of plant size ($\Delta\text{AIC} 1.7$). These results suggest

that the interaction between co-flowering and watering may be weak (additive effect of watering: 0.77 ± 0.29 , $z=2.7$, $p=0.008$), and that size had a weak effect on seeds per ovule after accounting for the interaction between co-flowering and watering (-0.004 ± 0.008 , $z= -0.478$, $p=0.63$). Therefore, I present the best model containing only the interaction between co-flowering and water here. In *Linum* plants that were watered, the seeds per ovule decreased as co-flowering increased, consistent with fruits per flower; in unwatered plants, there was no effect of co-flowering on seeds per ovule (Figure 6).

The effect of co-flowering on total seeds per plant depended on both plant size and watering (Table 3). No other models were within 2 AIC units (Table A3). The GLMM predicted that the total number of seeds per plant decreased with increasing co-flowering, but only in individuals that received extra water (Figure 6). The model also predicted that plants with more stalks produced more seeds, as expected (Figure 6).

Discussion

My results suggest that climate-driven shifts in co-flowering overlap between plant species can impact plant reproduction, yet this effect is strongly mediated by abiotic factors. Specifically, a reduction in co-flowering between *Linum* and *Potentilla* ultimately increases reproductive output in *Linum*, but only with sufficient water availability. These results indicate that climate change may have positive effects on the pollination and reproduction of some plant species via reductions in temporal synchrony of co-flowering species; however, these positive biotic effects will likely depend on abiotic conditions. Furthermore, these results contribute to our limited knowledge of the reproductive consequences of climate change-induced shifts in synchrony in plant-plant interactions (Faust and Iler, *in prep*; Vilela *et al.*, 2018). The majority of

studies examining the reproductive impacts of climate-driven shifts in synchrony have focused on interactions between plants and other organisms, rather than interactions among different plant species (Faust and Iler, *in prep*; Vilela *et al.*, 2018). This gap in the literature likely exists because demonstrating changes in temporal synchrony between species, and associated reproductive consequences, often requires long-term observational studies, of which there are few (Miller-Rushing *et al.*, 2010). I address this knowledge gap by using a field experiment informed by a 39-year flowering phenology dataset.

Although the direct abiotic effects of climate change are relatively well studied, little effort has been made to compare and integrate their importance to that of the biotic impacts of shifts in synchrony between interacting species (Ockendon *et al.* 2014; Forrest 2015; but see Gezon *et al.*, 2016, Gallagher and Campbell 2017). Here I find that climate-driven alterations in co-flowering overlap may not correspond to changes in plant reproduction when abiotic factors limit reproduction, such as when drought or spring frosts occur. Climate-induced drought and spring frosts can directly impact plant reproduction through reducing floral abundance, plant size (drought) and seed production (drought: Peñuelas *et al.*, 2004; Miller-Rushing and Inouye 2009; Liancourt *et al.*, 2014; Gallagher and Campbell 2017; frosts: Inouye 2008; Boggs and Inouye 2012; CaraDonna and Bain 2016; Iler *et al.*, 2019). Both drought and spring frosts are projected to increase at my study site because earlier snowmelt and less snowfall are predicted in the southwestern United States under future climate change (Inouye 2008; Pederson *et al.*, 2011; b. barr, *unpublished data*). Specifically, for subalpine communities, earlier snowmelt is projected to increase the length of the dry period occurring before the onset of mid-summer monsoons, and consequently, impact soil moisture for months (Pederson *et al.*, 2011; Blankinship *et al.*, 2014; Sloat *et al.*, 2015). Thus, if the climate continues to shift towards earlier snowmelt, and severe

droughts become more frequent, the positive biotic effects of decreased co-flowering will likely play a lesser role in affecting *Linum* reproduction than drought. These results pertain to the meadow that I studied but are likely to extend to other *Linum* individuals in subalpine communities within the Rockies, given the consistent predictions for climate change in these areas.

The year of this study, 2018, had the second lowest snowpack recorded from 1974 to 2018, leading to a very dry spring, with the total water input from spring snowmelt in 2018 being much lower than average (b. barr, *unpublished data*). The drought severely reduced *Linum* growth and reproduction (Appendix B), despite *Linum* being classified as a drought tolerant species (Reeves 2006, USDA *unpublished data*; Ogle *et al.* 2009, USDA *unpublished data*). Over one third of the *Linum* plants did not flower, and the average number of flowers per plant was about 1/3 of *Linum* flower production recorded from other studies at this site in previous years (Kearns and Inouye, 1994; Burkle and Irwin, 2009). Therefore, water likely limited reproduction in unwatered plants, decreasing their ability to take advantage of pollen loads with a high proportion of conspecific pollen under less co-flowering overlap. Conversely, watered plants which experienced less co-flowering overlap were able to utilize pollen loads with a high proportion of conspecific pollen because they received sufficient water to mature more fruits and seeds during a drought period.

The increase in the proportion of conspecific pollen on *Linum* stigmas as co-flowering declined was likely due to changes in competition for shared pollinator services between *Linum* and *Potentilla*. My field observations indicate that *Linum* and *Potentilla* shared pollinators, and during flower observations, I often saw visitors move back and forth between *Linum* and *Potentilla* flowers. Pollinator sharing among co-flowering plant species can affect pollinator

services such as pollinator visits and compatible pollen receipt (Waser 1978; Johnson *et al.*, 2003). The outcome of pollinator sharing on these pollinator services can be positive or negative, depending on the type of interaction present (*e.g.* competition, facilitation). Here, decreases in pollinator sharing had positive effects on pollinator services on *Linum* by increasing the proportion of conspecific pollen. However, in cases of facilitation, a decrease in pollinator sharing could decrease pollinator services. Therefore, the fitness outcomes of shifts in synchrony between co-flowering species will depend on whether the plant species compete for pollination or facilitate pollination (Waser 1978; Brown *et al.*, 2002; Johnson *et al.*, 2003; McKinney and Goodell 2011).

High percentages of heterospecific pollen deposition are likely the main cause of reduced reproduction in *Linum* plants experiencing more overlap with *Potentilla* because increases in heterospecific pollen deposition have been shown to negatively impact plant reproduction (Waser 1978; Mitchell *et al.*, 2009). Female plant fitness can be negatively impacted by heterospecific pollen deposition through a variety of mechanisms including: stigma clogging and closure (Waser and Fugate 1986; Galen and Gregory 1989), pollen allelopathy (Murphy and Aarssen 1995), style clogging (Arceo-Gómez and Ashman 2011), and ovule usurpation (Harder and Cruzan 1993). This study does not elucidate the mechanism behind a reduction in *Linum* fitness from heterospecific pollen deposition, as this was not the focus of the study. Yet, it demonstrates that naturally deposited heterospecific pollen can have a negative impact on plant reproduction, which is important because very few field studies have examined the reproductive consequences of naturally deposited heterospecific pollen (but see: Briggs *et al.*, 2015; Thomson *et al.*, 2018). The vast majority of what we understand about the mechanisms and impacts of

heterospecific pollen comes from greenhouse and potted plant studies that use hand pollination, which often do not reflect the reality of natural communities (Briggs *et al.*, 2015).

The highly intermixed pattern in which *Linum* and *Potentilla* grew is likely a critical factor explaining why increased co-flowering lead to a lower proportion of conspecific pollen but no change in pollen quantity, visitors, or visit rate. The direction and magnitude of effects of pollinator sharing on plant reproduction depend to some extent on the spatial distribution of plant species because interactions among plant species for pollinator services vary with the abundance, density, and distance of interacting plant species (Ghazoul, 2006; Caruso *et al.*, 2002; Seifan *et al.*, 2014; Jakobsson *et al.*, 2015). If the species are highly intermixed, flower constancy- the tendency of a pollinator to only visit one species- can decrease, resulting in increases in heterospecific pollen deposition (Waser 1986; Thomson, 1982). Thus, species living in dense, intermingled patches might receive the same amount of pollinator visits, but higher heterospecific pollen deposition, as I find here. Furthermore, my results are consistent with findings from a recent review, which found that the spatial arrangement of interacting native and alien plants species was an important factor affecting native plant reproductive output, with intensive intermingling of species tending to reduce conspecific pollen deposition and reproductive output, but not visitation rates (Charlebois and Sargent, 2017).

This study occurred over one-year and is based on changes in co-flowering from a long-term dataset, as opposed to a long-term experimental study on the consequences of changes in co-flowering. Therefore, pollinators in this study responded to local-scale changes in co-flowering over a short time period as opposed to directional changes through time and over a much broader spatial scale. Yet, the spatial scale of the study was sufficient to detect a change in heterospecific pollen transfer and *Linum* reproduction. Furthermore, I expect decreased co-

flowering through time to lead to higher proportions of conspecific pollen on *Linum* even at broader spatial scales, as these two species grow in highly intermingled patches throughout the area. An additional caveat is that I did not change synchrony per se by driving *Linum* to flower earlier (*e.g.*, Rafferty and Ives 2011, 2012), but rather removed flowers to simulate a future climate change scenario in which *Linum* is co-flowering less with *Potentilla* because of reduced temporal synchrony.

Understanding the consequences of shifts in co-flowering among interacting species is important for advancing our knowledge of how plant communities will respond to the warming climate, because changes in synchrony among other interaction types (*i.e.*, plant-pollinator, plant-herbivore) have been implicated in population declines, and even a few cases of species extinctions (Burkle *et al.* 2013; Cahill *et al.* 2013). The extent to which changes in reproduction will affect plant population growth rates and extinction probabilities will depend on how sensitive population growth rate is to perturbations in reproduction vs. other important vital rates like vegetative growth and survival (Iler *et al.*, 2019). This study suggests that climate-driven decreases in co-flowering between interacting plant species can increase plant female reproductive fitness through decreasing competition for pollinator services. More generally, the magnitude and direction of the reproductive impacts of climate-driven changes in co-flowering will depend on whether interactions for pollinator services between co-flowering plant species are competitive or facilitative. Lastly, this study demonstrates that direct climate-driven abiotic stressors may supersede indirect biotic effects if abiotic stressors reduce plant reproduction and growth. To gain a comprehensive understanding of the relative importance of both biotic and abiotic effects of climate change on plant reproduction, future studies should also examine the two types of effects in conjunction with one another (Forrest 2015). Only by considering both the

527 abiotic and biotic effects of climate change can we begin to understand the overall consequences
528 of climate change on plant reproduction, and make informed predictions about how climate-
529 induced shifts in co-flowering will affect plant reproduction and potentially even population
530 persistence and community structure.

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Figures

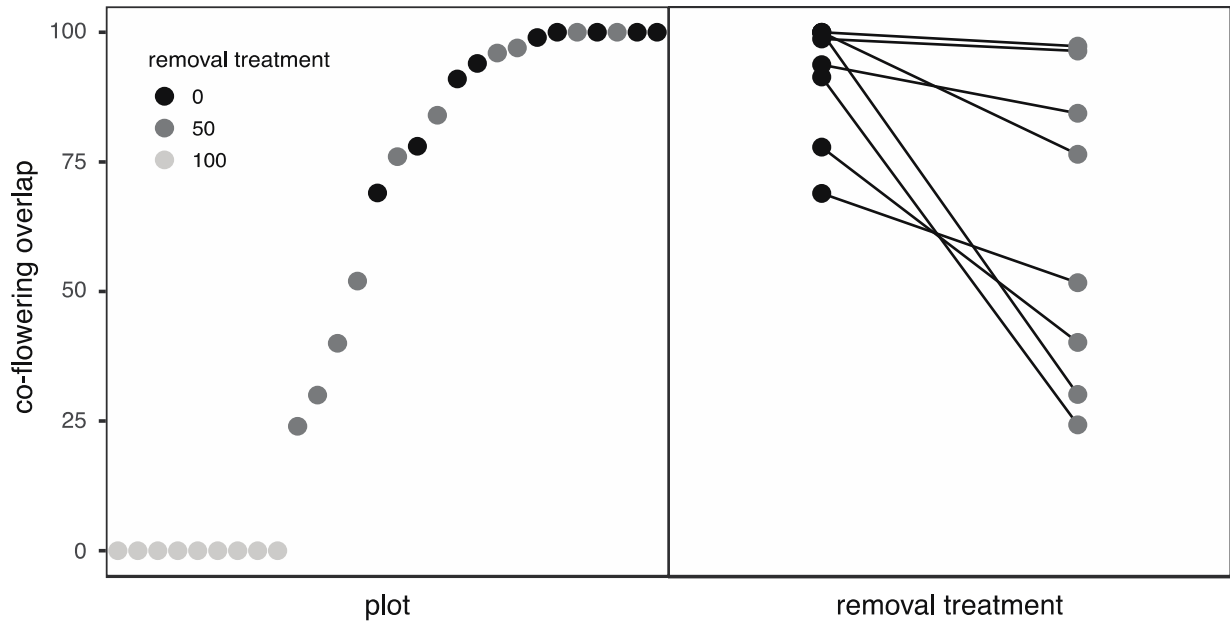


Fig. 1. Co-flowering treatments. (Right) The mean percentage of co-flowering overlap between *Linum* and *Potentilla* flowers in the control and 50% removal treatment plots (the 100% *Potentilla* removal is always zero overlap). Each dot is a study plot (8 blocks). Four blocks with treatment plots that contained no *Linum* flowers were excluded. (Left) The percentage of co-flowering overlap between *Linum* and *Potentilla* flowers shown for each plot in our analysis (28 plots; 8 plots were removed with little to no *Linum* flowers).

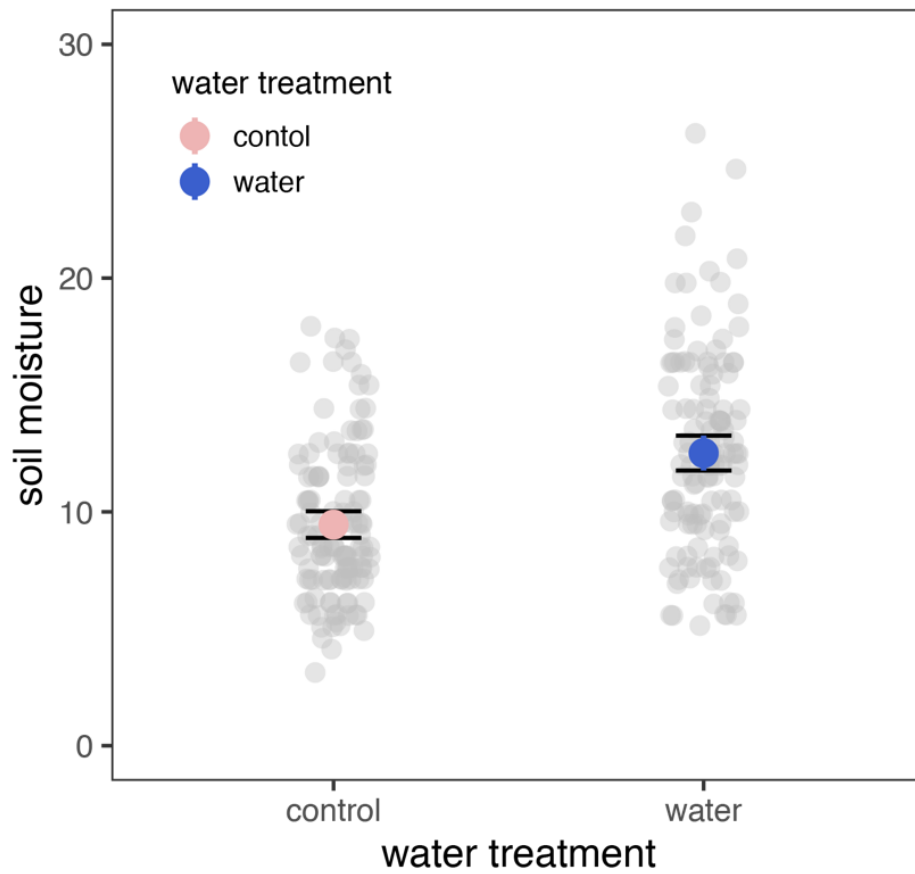


Fig. 2. Mean soil moisture in a water addition and control treatment across 36 plots, with 95% confidence intervals. Gray dots represent all soil moisture measurements taken across multiple weeks and are jittered across the x-axis for visualization (n=252 moisture readings across 36 plots; control: 9.46%, 95% CI: -7.6% to +11.3%; water: 12.5%, 95% CI: -10.6% to +14.4%).

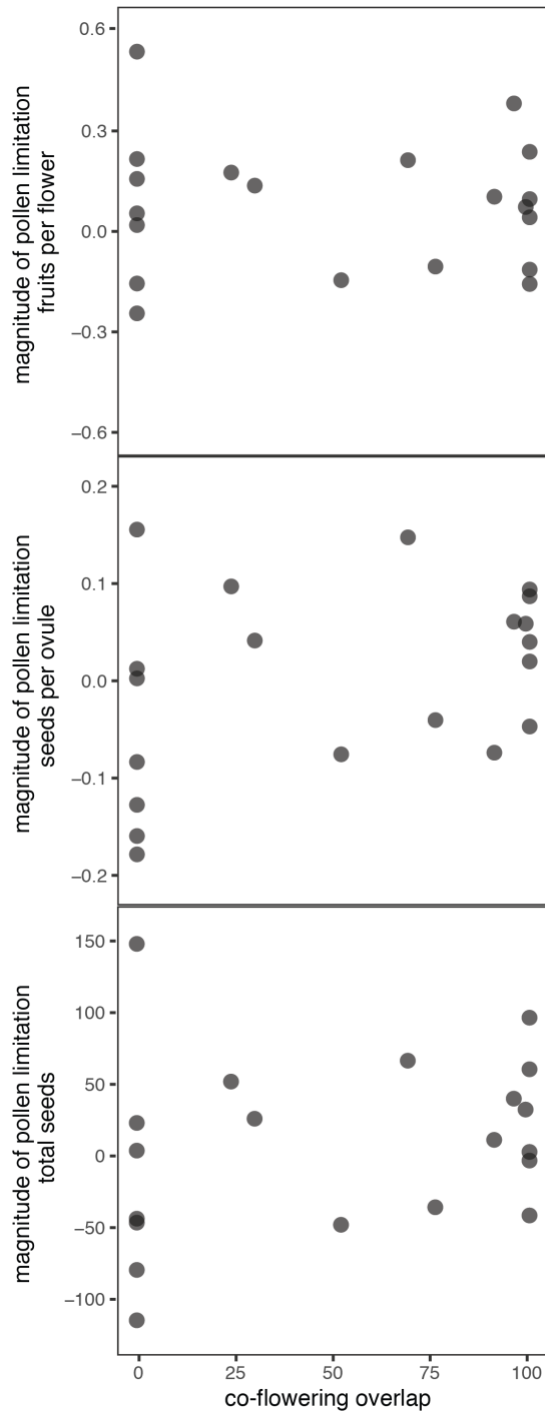


Fig. 3. Pollen limitation of *Linum* reproduction in response to the percentage of co-flowering overlap. Each dot represents a plot-level mean of the magnitude of pollen limitation, for each response variable (n= 20; 20 plots over 7 blocks). The magnitude of pollen limitation was calculated as the difference in reproductive success (top: fruits per flower, middle: seeds per ovule, and bottom: total seeds per plant) between the control plants and plants supplemented with pollen.

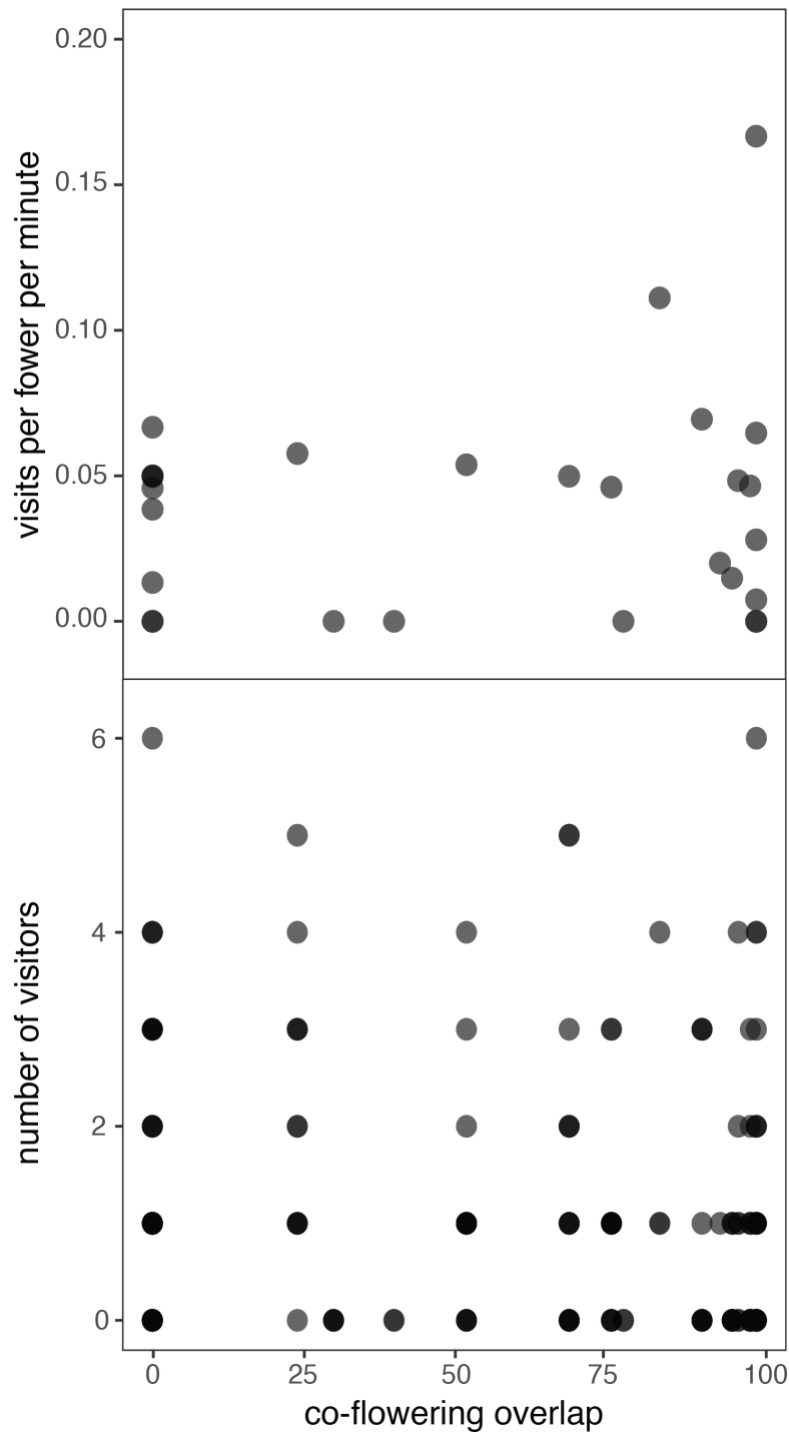


Fig. 4. Pollinator visitation to *Linum* in response to the percentage of co-flowering overlap. (Top) Mean pollinator visitation rate per plot, under co-flowering treatments ranging from 0-100% (n= 28, block=10, plot=28). (Bottom) Mean number of visitors per observation period, under co-flowering treatments ranging from 0-100% overlap, with 95% confidence intervals (n= 182, 28 plots over 10 blocks).

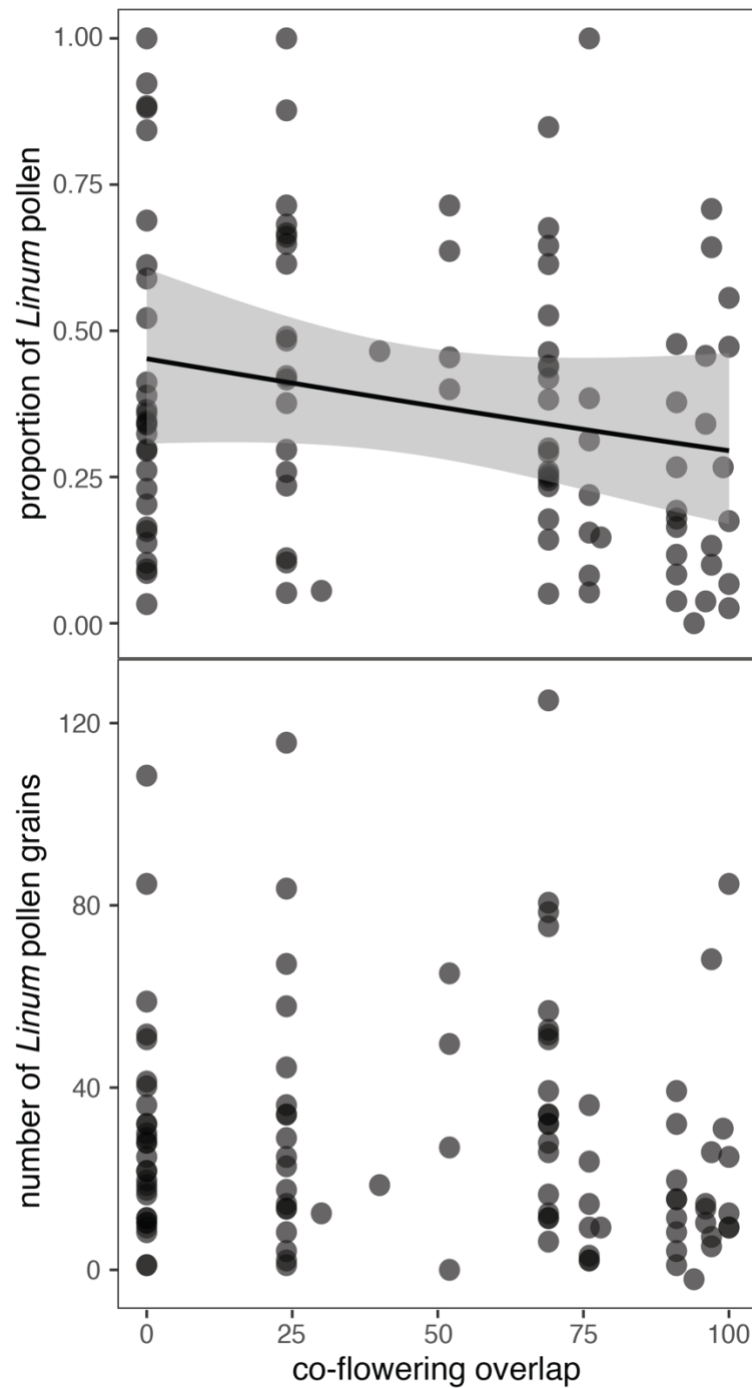


Fig. 5. Pollen deposition on *Linum* stigmas in response to the percentage of co-flowering overlap. (Top) Predicted mean conspecific pollen deposition under co-flowering treatments ranging from 0-100% overlap, with 95% confidence intervals (n=105, 19 plots over 9 blocks). (Bottom) Predicted mean number of *Linum* pollen grains per individual, under co-flowering treatments ranging from 0-100% overlap, with 95% confidence intervals (n=105, 19 plots over 9 blocks).

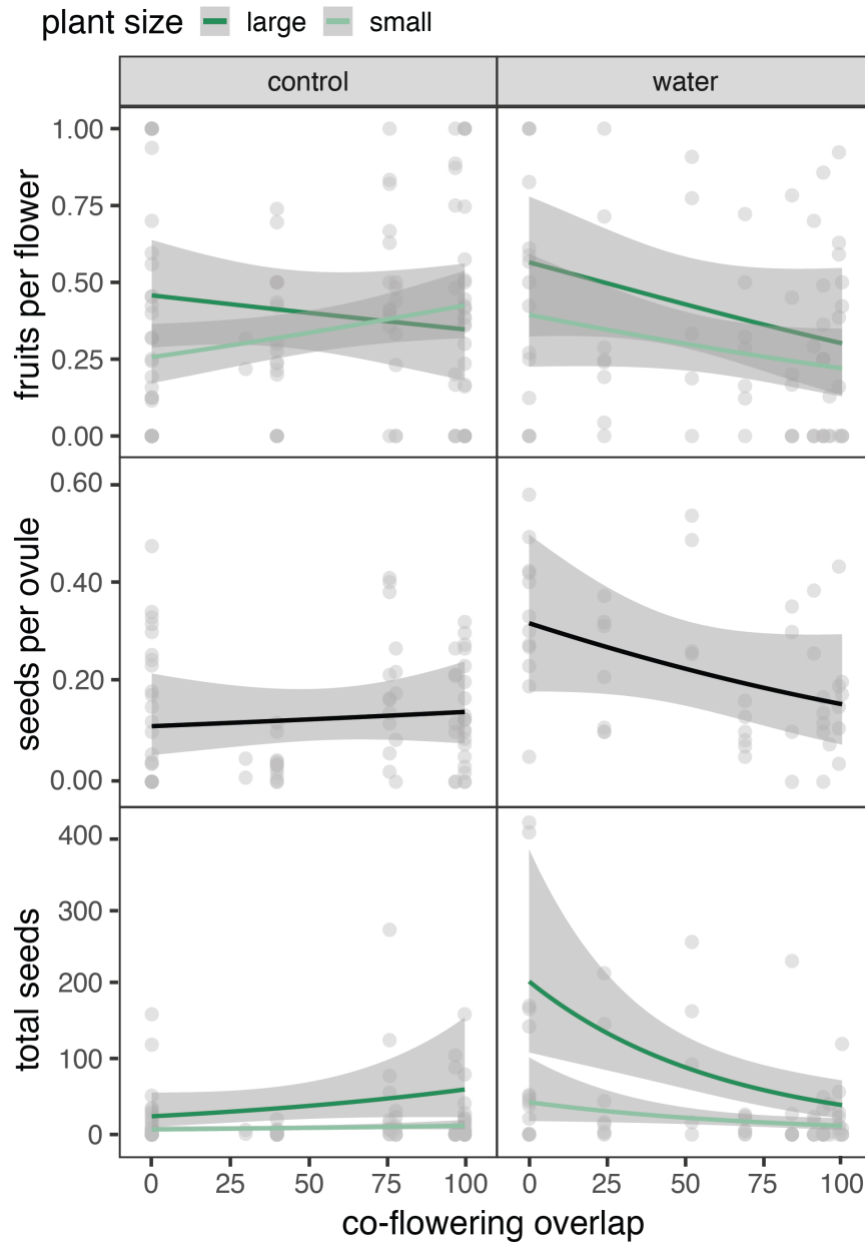


Fig. 6. Female reproductive success in *Linum* in response to the percentage of co-flowering overlap in watered and unwatered control plots. Plant size is indicated by different colored lines when it was included in the best model. “Large” indicates a plant with 20 or more stalks, and “small” indicates a plant with less than 20 stalks. (Top) Mean fruits per flower for each individual plant under co-flowering treatments ranging from 0-100% overlap, and two watering treatments versus the number of stalks per plant, with 95% confidence intervals (n=191, 28 plots over 10 blocks). (Middle) Mean seeds per ovule for each individual plant under co-flowering treatments ranging from 0-100% overlap, with 95% confidence intervals (n= 128, 28 plots over 10 blocks). (Bottom) Total number of seeds for each individual plant under co-flowering treatments ranging from 0-100% overlap, and two watering treatments, versus the number of stalks per plant, with 95% confidence intervals (n=191, 28 plots over 10 blocks).

Tables

Table 1. Pollen limitation results. The magnitude of pollen limitation is the difference in effect size between the control plants and plants supplemented with pollen.

Response	Predictor	Coefficients	T-value	P-value
Magnitude of pollen limitation: Fruits per flower	Intercept	0.08 ± 0.04	1.74	0.10
Magnitude of pollen limitation: Seeds per ovule	Intercept	0.002 ± 0.02	0.07	0.94
Magnitude of pollen limitation: Total seeds per plant	Intercept	7.49 ± 13.9	0.54	0.60

Table 2. Results from models of best fit for pollination and pollen deposition. Pollen quantity is the number of conspecific pollen grains on *Linum* stigmas (mean per plant), and pollen quality is the proportion of conspecific pollen grains on stigmas (mean per plant). I used Johnson's (2014) extension of Nakagawa and Schielzeth's (2013) methodology to calculate an R-squared value for pollen quality. Only the marginal R-squared value (fixed effects only) is reported here because the model included an observation level random effect, which has been shown to inflate conditional R-squared (fixed and random effects) values (Harrison 2014; Harrison 2015).

Response	Predictor	Coefficients	Z- value	P-value	R ²
Visitation rate	Intercept	-3.15 ± 0.45	-7.08	<0.001	NA
Number of visitors	Intercept	-0.21 ± 0.21	-0.96	0.336	NA
	# Flowers	0.02 ± 0.01	2.92	0.003	
Pollen quantity	Intercept	3.33 ± 0.12	28.39	<0.001	NA
Pollen quality	Intercept	-0.18 ± 0.28	-0.65	0.517	0.04
	Co-flowering	-1.22 ± 0.44	-2.78	0.005	

Table 3. Reproductive Success results. Fruits per flower is the proportion of fruit produced per flower (mean per plant), seeds per ovule is the proportion of seeds produced per ovule (mean per plant), and total seeds per plant is number of seeds produced per plant. I used Johnson's (2014) extension of Nakagawa and Schielzeth's (2013) methodology to calculate R-squared values for reproductive response variables. Only the marginal R-squared values (m, fixed effects only) are reported for fruits per flower and seeds per ovule because these models included an observation level random effect, which has been shown to inflate conditional (c) R-squared (fixed and random effects) values (Harrison 2014; Harrison 2015).

Response	Predictor	Coefficients	Z-value	P-value	R ²
Fruits per flower	Intercept	-1.67 ± 0.48	-3.49	<0.001	m: 0.04
	Co-flowering	1.70 ± 0.68	2.50	0.013	
	Stalks	0.05 ± 0.02	2.05	0.041	
	Water	0.84 ± 0.52	1.60	0.11	
	Co-flowering * stalks	-0.08 ± 0.03	-2.29	0.022	
	Co-flowering * water	-1.76 ± 0.75	-2.33	0.02	
Seeds per ovule	Intercept	-2.25 ± 0.28	-7.96	<0.001	m: 0.04
	Co-flowering	0.23 ± 0.39	0.58	0.56	
	Water	1.34 ± 0.43	3.12	0.002	
	Co-flowering * water	-1.04 ± 0.62	-1.69	0.09	
Total seeds per plant	Intercept	1.50 ± 0.390	3.80	<0.001	m: 0.30 c: 0.33
	Stalks	0.06 ± 0.02	3.37	<0.001	
	Co-flowering	0.40 ± 0.45	0.89	0.37	
	Water	1.91 ± 0.63	3.05	0.002	
	Co-flowering * Water	-1.89 ± 0.86	-2.19	0.03	

Appendix A.

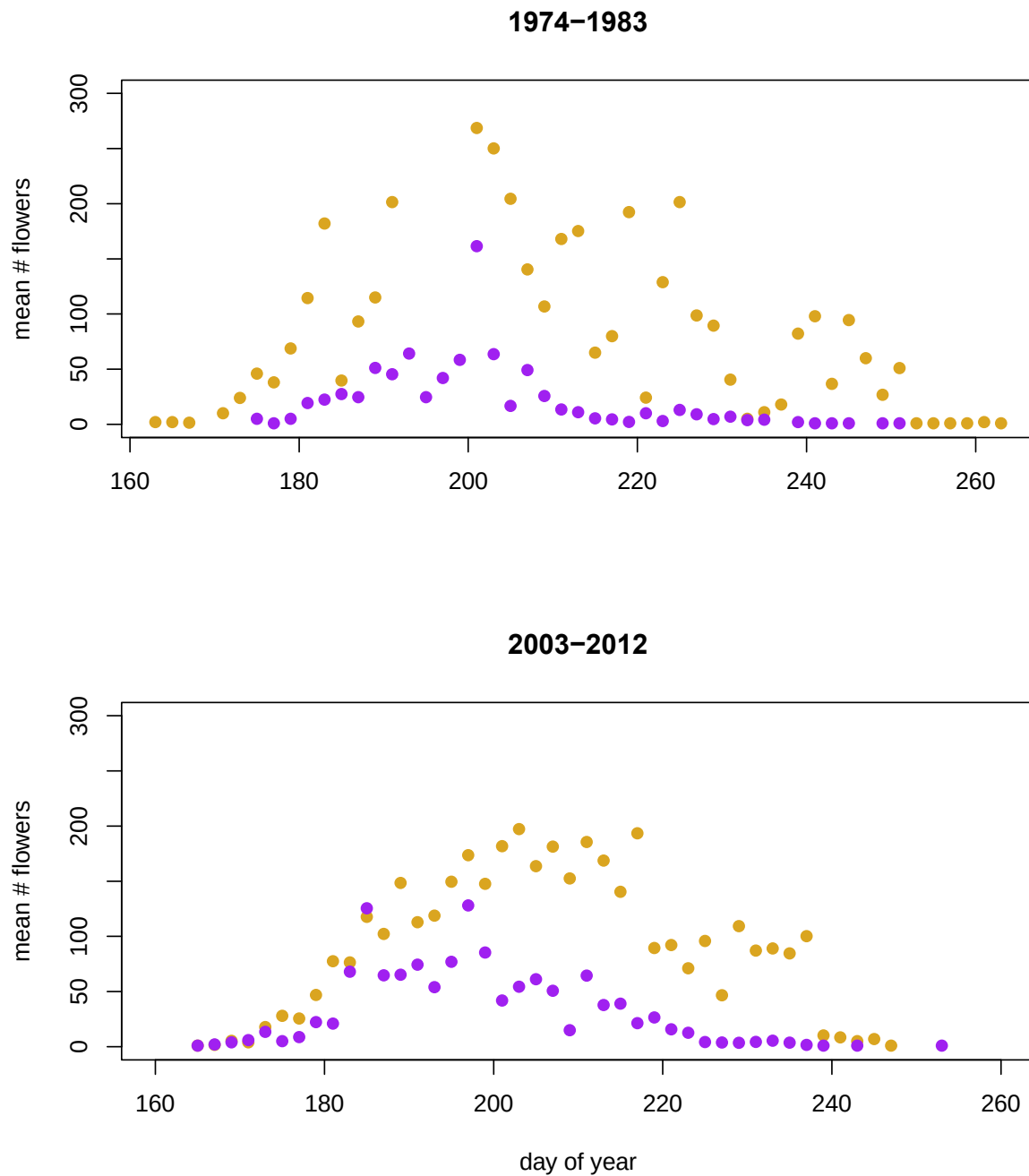


Fig. A1. Co-flowering overlap for *Linum* (purple) and *Potentilla* (yellow) during the flowering season in two time periods (1974-1983 and 2003-2012) at RMBL. First flowering in *Linum* has shifted earlier, leading to a longer duration of flowering at the population level. Peak flowering in *Potentilla* has shifted earlier.

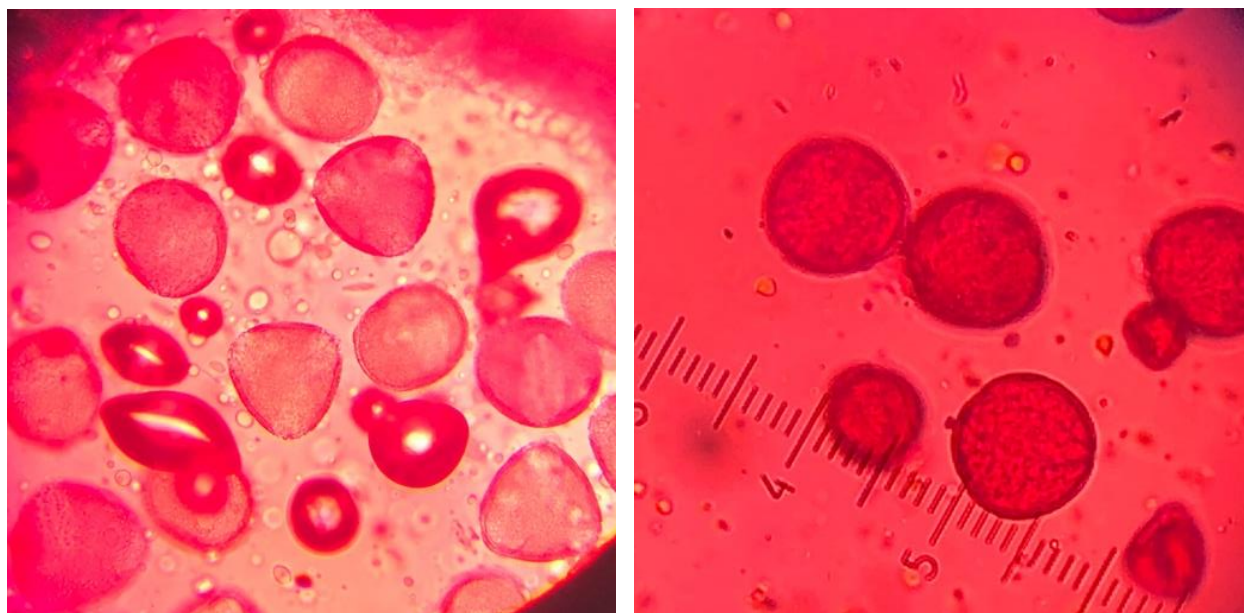


Fig. A2. (Left) *Linum* pollen grains under 40x magnification. (Right) *Potentilla* pollen grains under 40x magnification.



Fig. A3. Viable *Linum* seeds indicated by dark color and hard seed coat.

Table A1. Set of candidate models for a LMM examining the impact of water on soil moisture in each plot. The null model included an intercept as the only fixed effect.

Response	Model Statement	AIC	dAIC
Soil moisture	Water	1301.2	0.0
	Null	1356.2	55.0

Table A2. Candidate set of models for GLMMs & GLMs examining *Linum* pollination responses.

Response	Model Statement	AIC	dAIC
Pollinator visitation rate	Null	15.5	0.0
	Co-flowering + block	34.1	18.6
	Co-flowering + flowers + block	35.9	20.4
	Co-flowering * flowers + block	37.4	21.9
Total pollinator visits	Co-flowering + flowers	525.7	0.0
	Co-flowering * flowers	527.6	1.9
	Null	429.5	3.8
	Co-flowering	531.4	5.7
Pollen quality	Co-flowering	896.3	0.0
	Co-flowering + water	897.3	1.8
	Null	899.6	3.3
	Co-flowering * water	899.7	3.4
Pollen quantity	Null	924.4	0.0
	Co-flowering	924.4	0.0
	Co-flowering + water	927.8	3.4
	Co-flowering * water	929.2	4.8

Table A3. Sets of candidate models for GLMMs examining *Linum* reproductive success.

Response	Model Statement	AIC	dAIC
Fruits per flower	Co-flowering * stalks + co-flowering * water	984.4	0.0
	Co-flowering * water	985.8	1.4
	Co-flowering * stalks	986.1	1.7
	Co-flowering * stalks * water	987.0	2.6
	Null	987.4	3.0
	Co-flowering + stalks + co-flowering * water	987.7	3.3
	Co-flowering * stalks + co-flowering + water	987.9	3.5
	Co-flowering	989.1	4.7
	Co-flowering + stalks	990.9	6.5
	Co-flowering + water	991.1	6.7
	Co-flowering + stalks + water	992.8	8.4
Seeds per ovule	Co-flowering * water	944.3	0
	Co-flowering + water	945.0	0.7
	Co-flowering + stalks + co-flowering * water	946.1	1.8
	Null	946.8	2.5
	Co-flowering + stalks + water	946.8	2.5
	Co-flowering * stalks + co-flowering * water	948.0	3.7
	Co-flowering	948.5	4.2
	Co-flowering * stalks + co-flowering + water	948.8	4.5
	Co-flowering + stalks	950.3	6.0
	Co-flowering * stalks * water	951.7	7.4
	Co-flowering * stalks	952.3	8.0
Total seeds per plant	Co-flowering + stalks + co-flowering * water	1274.7	0.0
	Co-flowering * stalks + co-flowering * water	1276.7	2.0
	Co-flowering + stalks + water	1277.5	2.8
	Co-flowering * stalks + treatment + water	1279.3	4.6
	Co-flowering + stalks	1279.7	5.0
	Co-flowering * stalks * water	1280.5	5.8
	Co-flowering * stalks	1281.3	6.6
	Co-flowering * water	1284.2	9.5
	Co-flowering + water	1290.2	15.5
	Null	1292.0	17.3
	Co-flowering	1293.5	18.8

Table A4. Candidate set of models for the magnitude of pollen limitation for fruits per flower, seeds per ovule, and total seeds per plant.

Response	Model Statement	AIC	dAIC
Magnitude of pollen limitation: fruits per flower	Null	-5.6	0.0
	Water * co-flowering + block	0.7	6.3
	Co-flowering + water + block	1.5	7.1
	Co-flowering + block	1.5	7.1
Magnitude of pollen limitation: seeds per ovule	Null	-33.6	0.0
	Water + co-flowering + block	-30.0	3.6
	Co-flowering + block	-30.0	3.6
	Water * co-flowering + block	-28.9	4.8
Magnitude of pollen limitation: total seeds per plant	Null	225	0.0
	Co-flowering + water + block	230.5	5.5
	Co-flowering + block	230.5	5.5
	Co-flowering * water + block	232.4	7.4

Appendix B. Characterization of soils and variation in flowering across the study plots

Soils

We examined the soil pH, salinity and type to determine the soil characteristics of the study site. We sampled approximately 1 pint of soil from four random points spaced at least 50cm from one another in each plot. Soil measurements were taken at a depth of 15cm and mixed together into one sample per plot (Perkins *et al.*, 2013). To determine the soil type of our samples we used the hydrometer method (Bouyoucos 1962). Specifically, we mixed 50g of our soil sample with 100mL of Calgon and rinsed it with deionized water, and then placed the mixture into a 1 L cylinder. Next, we plunged the mixture until a uniform suspension was obtained, and recorded the mixture's hydrometer value and temperature. After 1 hour and 40 minutes we recorded the mixture's hydrometer value and temperature again. From these values we calculated the percentage of sand, silt and clay within our soil samples, thereby obtaining both soil texture and type. To determine soil pH and salinity we mixed 5g of soil sample with 50mL of distilled water, and recorded pH and salinity values using a Vantakool pH meter and a YL - TDS2 portable digital total dissolved solids (TDS) tester pen for water quality.

All of the soil samples had a soil type and texture of fine sand. The average pH across all soil samples was 6.6, with the highest reading at 7.0 and lowest at 6.2. These results show that the soil in the study site had a natural pH, and that the pH did not vary greatly across the plots. The average soil salinity was 148.2 ppm with the highest salinity measurement at 225 ppm and the lowest at 129 ppm, indicating very small amount of total dissolved solids in the soil and very low soil salinity across the plots.

Linum flower production

Linum flower production appeared to vary across the study site and was lower compared to previous studies at the RMBL. One third of the plants within the study plots did not flower (34%), and the flowering plants in this study produced an average of 30 flowers per plant. In contrast, Kearns and Inouye (2004) found that *Linum* plants grown in a laboratory setting produced about 88 flowers per plant. Similarly, Burkle and Irwin (2009) found that 72 *Linum* plants growing in a dry, subalpine meadow under a control treatment produced a little more than 100 flowers per plant. To better understand the variation in *Linum* flower production across the site, and determine why *Linum* plants produced drastically fewer flowers than plants in past studies, I conducted a survey of all the flower buds on each individual plant in my study.

I surveyed flower buds for evidence of drought and herbivory, and recorded the percentage of buds on each plant experiencing, drought, herbivory, and drought plus herbivory. I recorded a bud as “experiencing drought” if it was shriveled, had a white-yellow tint, and if it felt crusty (Figure B1). I recorded a bud as “experiencing herbivory” if an herbivore was directly feeding on the bud, if there were herbivore feeding marks, or if there was insect webbing surrounding the bud (Figure B1). Both the percentage of buds experiencing drought and the percentage of buds experiencing herbivory had scales of damage ranging from 0 (no drought/no herbivory) to 100 percent (no healthy buds). The “drought plus herbivory” category indicated buds that were experiencing both drought and herbivory. The percentage of damage within the drought plus herbivory category was added to both the drought and herbivory totals, so that the percentages could sum to above 100 percent for an individual plant. The survey took place just prior to peak flowering and lasted for two days. In addition to the survey, we examined soil moisture (methods described in main text) in each plot to determine if differences in soil

moisture had an impact on flower production. We performed one round of measurements on 3 July prior to applying the watering treatment.

We expected soil moisture to explain variation in flower production across the meadow, so we first examined whether soil moisture varied significantly across experimental blocks using a linear model. Block was the predictor variable (factor, 12 levels), and soil moisture was the response variable. Next, we examined whether soil moisture had an effect on flower production using generalized linear mixed models (GLMM) with a negative binomial error structure, where soil moisture was a continuous predictor and the total number of flowers per plant was the response. In addition, the GLMM included plot and block as random effects. The candidate set of models included a soil moisture treatment model and a null model (intercept only). In both analyses, we compared the AIC values between the two models to select the best model.

We then examined the effects of drought stress and herbivory on flower production, using results from the floral survey. We used GLMMs with a negative binomial error structure to determine whether drought, herbivory, and plant size (number of stalks per plant) had an effect on flower production. The GLMM included percent buds experiencing drought, percent buds experiencing herbivory, and plant size as continuous predictors, and total flowers per plant as the response variable. Also, block and plot were included as random effects. We had a set of thirteen candidate models that included: a full model with an interaction between all three predictors, and a null model, which was an intercept-only model. We compared the AIC values to select the best model, and presented models within 2 AIC units in the results.

The best model for the impact of block on soil moisture was the null model, indicating that soil moisture did not differ across blocks (Table B1; Table B2). The best model for the effect

of soil moisture on the total number of flowers per plant was the null model as well (Table B1; Table B2).

The best model for the impact of drought, herbivory, and plant size on total flowers included an interaction between stalks and herbivory and the addition of drought (Table B3; Table B4). Two models were within 2 AIC units of our best model, one model containing an interaction between stalks and drought and an additive effect of herbivory, and one model containing an additive effect of all three predictors. The interaction between drought and stalks was weak (0.0003 ± 0.0004 , $z=0.8$, $p=0.43$), so we only present the model containing drought as an additive effect. Plants with a higher percentage of herbivory and drought produced fewer flowers. Larger plants had more flowers and a higher percentage of their flower buds damaged by herbivory compared to smaller plants (interaction between size and herbivory).

My floral survey results show that drought stress and floral herbivory had a negative impact on *Linum* flowering. The spring of 2018 was extremely dry and received less precipitation from snowmelt than the long-term average (2018= 42.4 cm; 1974-2018= 69.4 cm, b. barr, *unpublished data*). Past studies at the RMBL have recorded higher numbers of *Linum* flowers per plant in springs that received more precipitation from snowmelt compared to 2018 (1988= 54.4cm; 2004= 62.4cm, b.barr, *unpublished data*) (Kearns and Inouye 2004; Bukle and Irwin 2009). Thus, the low amount of precipitation from spring snowmelt is likely the main driver of increased drought stress present in *Linum*, and as indicated by my floral survey results, a key factor in decreased *Linum* flower production in 2018. However, our soil moisture analysis does not support this conclusion. *Linum* flower production likely did not vary with soil moisture because this analysis was only based on one round of soil moisture measurements taken at the end of spring. Therefore, we may not have captured the soil moisture conditions and effects on

flower production that occurred across the season. Thus, the floral survey results are likely a more reliable indication of the impact of drought on *Linum* flower production. In conclusion, it was likely a combination of drought stress and herbivory that lead to low *Linum* flower production in 2018.



Fig. B1. (Top-Left) A *Linum* plant exhibiting drought stressed flower buds. (Top-Right) A *Linum* plant exhibiting drought stressed flowers and buds. (Bottom-Left) A species of *Lepidoptera* feeding on *Linum* flower buds. (Bottom-Right) *Linum* flower buds experiencing both drought and herbivory.

Table B1. Results from models of best fit for soil moisture and flower production.

Response	Predictor	Coefficients	T-value	P-value
Soil moisture	Intercept	7.05 ± 0.37	19.2	<0.001
Total flowers	Intercept	2.12 ± 0.40	5.26	<0.001

Table B2. Candidate set of models for linear model examining soil moisture across blocks, and a GLMM examining the effect of soil moisture on total flowers per plant.

Response	Model Statement	AIC	dAIC
Soil moisture	Null	162	0
	Block	166.7	4.7
Total flowers	Null	2627	0
	Soil moisture	2628	1

Table B3. Results from models of best fit for drought and herbivory.

Response	Predictor	Coefficients	Z-value	P-value
Total Flowers	Intercept	3.14 ± 0.44	7.08	<0.001
	Stalks	0.06 ± 0.01	4.34	<0.001
	Drought	-0.03 ± 0.004	-6.25	<0.001
	Herbivory	-0.01 ± 0.005	-2.80	0.005
	Herbivory * drought	0.001 ± 0.0003	1.70	0.09

Table B4. Candidate set of models for GLMM examining the effect of drought, herbivory and plant size on total flowers per plant.

Response	Model Statement	AIC	dAIC
Total flowers	Stalks + drought + stalks * herbivory	2515.65	0
	Stalks + drought + herbivory	2516.048	0.4
	Stalks * drought + stalks + herbivory	2517.491	1.8
	Stalks * herbivory + stalks * drought	2517.636	2.0
	Stalks + drought	2519.143	3.5
	Stalks * drought * herbivory	2519.606	4.0
	Stalks * drought	2520.66	5.0
	Stalks + herbivory	2553.047	37.4
	Stalks * herbivory	2553.952	38.3
	Stalks	2578.676	63.0
	Drought	2583.66	68.0
	Herbivory	2612.53	96.9
	Null	2626.951	111.3

Appendix C. Analysis of the effects of watering on pollinator visitation rate (per flower per minute) and the number of visitors to *Linum* (per observation period).

Pollinator observations began before I applied the watering treatment. Therefore, I conducted an independent analysis examining the effect of the watering treatment on the number of visitors and visitation rate (as opposed to including watering as a predictor in models that also contained co-flowering overlap as a predictor). To assess whether watering affected visitation rate and the number of visitors, I only included visit data from dates after the watering treatment was applied. I used a generalized linear model (GLM) with a binomial error distribution for visitation rate, and a generalized linear mixed effects model (GLMM) with a zero-inflated negative binomial error distribution for visitors. Both analyses included watering treatment as a fixed predictor, and plot, block, and day of year as random intercepts. Day of year was included as a random effect to account for variation across observation dates; however, models for visitation rate failed to converge so I averaged visitation rates across observation dates at the plot-level. Additionally, block was switched to a categorical predictor (rather than a random effect) for mean pollinator visitation rate, because the models did not converge with it as a random effect. As a result, for pollinator visitation rate, there were two candidate models: a treatment model with the addition of block as a fixed effect and an intercept only model (*i.e.*, no treatment). For the number of visitors there were also two candidate models: a treatment model and an intercept only model (Table C1). The null model was the best model for both visitation rate and visitors (Table C1; Table C2). Therefore, when I examined effects of co-flowering on pollinator visitation and the number of visitors, I did not include watering treatment as a fixed predictor, allowing me to use my full dataset on pollinator visitation.

Table C1. Set of candidate models for a GLM and GLMM examining the impact of water on pollinator visitation rate (GLM binomial), and the impact of water on pollinator visitors (GLMM zero inflated binomial).

Response	Model Statement	AIC	dAIC
Pollinator visitation rate	Null	9.6	0.0
	Water + block	25.7	16.1
Pollinator visitors	Null	377.5	0.0
	Water	381.2	3.7

Table C2. Effects of watering treatment on pollinator visitation rate (per flower per minute) and the number of visitors to *Linum* (per observation period).

Response	Predictor	Coefficients	Z-value	P-value
Pollinator visitation rate	Intercept	-3.12 ± 0.53	-5.93	<0.001
Number of visitors	Intercept	-2.50 ± 2.14	-1.17	0.244