Ecological interactions in a changing world

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Abstract

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21st-century ecology is necessarily a science of change. Understanding how and why communities and interacting species respond to transformations in global climate and human land use can help improve our ability to forecast and manage shifts in ecological interactions. In this dissertation, I present work that asks these kinds of questions as they relate to plants, insects, and their relationships. In chapter one I use a Bayesian hierarchical modeling approach to examine how fruiting and seed dispersal phenology is influenced by climate across the subalpine wildflower community on Mt. Rainier. I find that the kinds of changes we expect to occur in snowmelt timing, temperature, and soil moisture are likely to shorten the time it takes for plants to transition from flowering to seed dispersal. This work highlights the importance of increased attention on post-flowering phases of plant reproductive phenology, and suggests that the future may shift relationships between plants and their animal associates, including frugivores and invertebrate seed predators. In chapter two I ask how the interaction between an ecologically important species (subalpine lupine, *Lupinus latifolius* var. *subalpinus*) and its primary insect herbivore (the Cascade grasshopper, *Prumnacris rainierensis*) changes with elevation, and use a combination of observations, experiments, and trait measurements to tease apart the drivers of these patterns. The results of this chapter show that grasshopper herbivory has meaningful impacts on lupines, and that the strength of this relationship increases with elevation due to a complex suite of interactions between abiotic factors and plant and insect traits that influence levels of consumption. These findings point to the difficulty of predicting how species interactions will change in systems where warming is not the sole variable of interest and where climatic history shapes populations' responses to environmental change. Finally, in chapter three I take a meta-analytic approach to test the hypothesis that ecological restoration is an effective means of invertebrate conservation, using a dataset comprising 59 studies of restoration impacts on invertebrate biodiversity with a wide geographic and taxonomic scope. On average, I find that the restoration of native vegetation is associated with significant increases in species richness, abundance, and diversity. However, high-among study heterogeneity suggests an urgent need for more research on neglected taxa, habitats, and geographic regions, more careful study design and reporting, and a move toward sharing raw data from restorations assessments. Overall, this work makes novel contributions to our knowledge about biotic responses to climate change from the perspective of plants and insects, and underscores the fundamental complexity of ecological dynamics.

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DEDICATION

To Ross, who did it all first.

Chapter 1. Early Snowmelt and Warmer, Drier Summers Shrink Flowering Transition Times in Subalpine Wildflowers

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ABSTRACT

Plant reproductive phenology is shifting rapidly with global climate change. Many studies focus on flowering responses to climate, but few investigate how postflowering processes, such as how quickly plants develop from flowering to seed dispersal, respond to environmental factors. We examined the climatic drivers of postflowering phenology in 28 species of western North American subalpine meadow plants over large spatial and temporal climate gradients. We took a Bayesian hierarchical approach to address whether and how climate influences the time it takes for wildflower populations to transition from flower to seed. Our previous work on the same species demonstrated that the initiation of flowering depends on snowmelt timing, with warmer temperatures and soil moisture also playing a role. Here, we found that for the majority of the flowering community, the same climate drivers also affected the time it takes to move from flowering to seed dispersal. Climate-sensitive species shortened flower–seed transitions when snow melted earlier, temperatures were warmer, and/or soil dried down more quickly—conditions we expect with higher frequency under climate change. Our work underscores the fact that predicting the impact of climate change on plant reproductive phenology demands empirical data on phases beyond flowering. Additionally, it suggests that some species face a future in which multiple environmental factors will push them towards more rapid transitions from

flowering to postflowering phases, with potential effects on plants themselves and the many animal associates that rely on them, including frugivores and seed predators.

1.1 INTRODUCTION

Because the outcomes of an organism's interactions with its environment during key life events are to a large extent dictated by when they occur, phenology powerfully affects individual fitness, population dynamics, and species distributions. For instance, plants may face reduced reproductive success unless they synchronize flowering and seed set with the activity of pollinators and seed dispersers (Kudo and Ida 2013, González-Varo et al. 2019), while avoiding fruit or seed predators and exposure to droughts or frosts (Boulay et al. 2007, Pardee et al. 2019). Because the developmental processes of plants are driven by seasonal changes in climate, especially air and soil temperatures (Badeck et al. 2004), they are particularly sensitive to global warming (Parmesan 2006). Considerable research has looked at climatic effects on leaf phenology (Zohner et al. 2016, Xie et al. 2018), and flowering (Miller-Rushing and Inouye 2009, Richardson et al. 2017). However, as noted in Wolkovich and Ettinger (2014), we lack phenological research on stages of plant reproduction beyond flowering. This bias impoverishes our understanding of how postflowering phenology responds to environmental factors, and how such responses may affect relationships with interactors other than pollinators, such as frugivores, seed predators, and dispersers.

Studies on flowering phenology alone could be adequate for understanding climatic influences on reproductive phenology as a whole if postflowering events are solely constrained by endogenous factors like plant physiology or genetics, such that the timing of seed dispersal is well predicted by the timing of flowering. For example, an arboretum-based study of reproductive phenology in North American trees found the strongest predictor of flowering and fruiting events was interphase duration (i.e., the time between budburst and flowering, or flowering and fruiting), suggesting that minimum fruit-development times may dictate the overall shape of reproductive phenology (Ettinger et al. 2018). Evidence for endogenous controls has also been found by Marco and Páez 2002, and Semenchuk et al. 2016. However, phenologically plastic responses to climate do appear to shape the duration of fruit and seed development in some systems (Stinson 2004, Hülber et al. 2010, Li et al. 2016). For example, alpine forbs and graminoids experimentally transplanted to warmer or cooler locations adjust the timing of fruiting relative to flowering so that dates of fruit set remain relatively stable (Jiang et al. 2016). Similarly, a temperate mistletoe shortens the duration of fruit maturation over the course of the growing season (Aizen 2003). In both cases, these strategies may favor successful animal seed dispersal (cf. flowering phenology being influenced by pollinator-mediated selection; e.g., Sandring and Ågren 2009, Chen et al. 2017).

Although plasticity in flowering has been observed globally (Levin 2009, Iler et al. 2017), it is not clear if postflowering plasticity is similarly widespread, and/or if in some species postflowering phenology could be sensitive to climate in ways that exacerbate or mitigate the impacts of shifts in flowering. To address these unknowns, we investigated climatic influences on postflowering reproductive phenology in high mountain meadows in western Washington (Mt. Rainier National Park, Washington). Climate-induced phenological shifts in these plants could have profound ecological impacts because they host a unique wealth of biodiversity (Körner 2000). Such shifts are likely, as growing seasons in high mountain meadows are strongly constrained by snow cover, and snowmelt timing is a key driver of reproductive phenology (Inouye 2008, Caradonna et al. 2014, Theobald et al. 2017). In addition, these communities are being invaded by lower-elevation forest species moving up (Rochefort and Peterson 1996, Zald et al. 2012), but have minimal opportunity to shift their own distributions because their survival and growth is limited by the lack of climatically suitable habitat (Dirnböck et al. 2011) and by soil quality (Ford and HilleRisLambers 2020). Therefore, how these plant communities respond to changes in climate is important for them and for animal associates. Between 2010 and 2015, we collected an observational data set of reproductive phenology in 48 perennial wildflower species in this system and found flowering responds strongly to climate; all species flowered earlier with earlier snowmelt and warmer temperatures, but differing sensitivities led to the reassembly of coflowering communities (Theobald et al. 2017). We now use a 28-species subset of the same data to ask: Does climate influence the time between peak flowering and peak seed dispersal (henceforth "flower–seed transitions")? If so, how do climatic effects differ across species?

1.2 MATERIALS AND METHODS

1.2.1 *Study system and sampling design*

Sampling took place in Mt. Rainier National Park, on the southern slope of Mt. Tahoma (a.k.a. Mt. Rainier, 46.8529° N, 121.7604° W). This volcano is the highest peak in western Washington State, United States (4,392.5 m), and it has a temperate maritime climate. Summers are warm and dry and winters are cool and wet, with most precipitation falling as snow between October and May (Rochefort and Peterson 1996). Old-growth coniferous forests blanket the mountain's lower reaches (530–1,450 m), and its highest elevations (>1,900 m) are partly covered by permanent snow, bare rock, and ice, and partly by sparsely distributed alpine vegetation. Our work focuses on the midelevation (subalpine) zone, which ranges from 1,490 to 1,900 m and hosts lush, herbaceous perennial wildflower meadows.

We collected phenological data over six growing seasons (2010–2015) at five sites roughly evenly spaced along an elevational transect, spanning the distribution range of subalpine meadow habitat on the south side of the park. Snowmelt timing is influenced by both elevation and local topography; for example, ridgetops become snow-free earlier than depressions in the landscape (Ford et al. 2013). Thus, rather than locating plots randomly within sites, at each elevation we looked for a natural gradient at each site and established five parallel subtransects along it. In general, each subtransect comprised three 1-m2 plots: one located on a hilltop, one on a slope, and one in a depression. Because of differences in landscape and species distributions, plot number per elevation varied from 12 to 6. We monitored a total of 73 plots.

1.2.2 *Phenology data collection*

We recorded the reproductive phenology of all wildflower species in our study plots, visiting plots weekly beginning at snow disappearance and ending when either seed dispersal had occurred for every species, or at the first return of snowfall, whichever came sooner. At each visit, we recorded the species-specific presence/absence of budding, flowering, fruit-producing, or seed-releasing plants within each plot, regardless of abundance. For example, if 10 avalanche lilies (*Erythronium montanum*) were flowering and three releasing seed, we recorded 0, 1, 0, 1 for that species in that plot on that day of the year (DOY). Only open blooms (neither buds nor senescing flowers) with pollen clearly available to pollinators were recorded as flowering, and seed dispersal was only present if seeds were clearly vagile—for example, cracked-open pods containing dry seeds/fluffy seed heads with some seeds already detached. For this analysis, we extracted presence/absence observations for flowering and seed dispersal from the original data set, excluding species appearing in fewer than four plot–year combinations or whose seed-dispersal phase was observed in fewer than 30% of all plot–year combinations. Twenty-eight species had sufficient data (Table S1.1.) After cleaning and holding back 10% of data for model testing, the training data set included 45,430 presence–absence values each for a single year-plot-species-DOY-phenophase combination.

1.2.3 *Microclimate data collection*

We monitored snow disappearance date (SDD), growing degree days (GDD), and soil moisture (SoilMoist). We did not consider photoperiod because day length is less influential than snow cover at high elevations, where it does not reliably indicate seasonality (Hülber et al. 2010, Lewandowska-Sabat et al. 2017). Moreover, in 90% of the plot–year combinations in our data set, day lengths at SDD surpassed the 15-h threshold below which most photoperiod-sensitive alpine species react (Keller and Körner 2003).

Near-surface soil temperatures in systems with deep snow cover are stable at 0°C when insulated by snow, and show diurnal oscillations when snow melts (Lundquist and Lott 2008). To determine SDD, we therefore followed an approach first validated using fiberoptic sensor technology (Tyler et al. 2008) and modified to use inexpensive temperature sensors (Lundquist and Lott 2008). We buried either an iButton (Maxim Integrated, San Jose, California, USA) or HOBO Pendant (Onset Computer Corporation, Bourne, Massachusetts, USA) 1–4 cm below the soil at each plot and recorded temperature every 4 h. We assumed any calendar day with a maximum temperature of <2°C and range of <1°C indicated snow cover. Some (9.5%) sensors failed over the study period. However, plot-to-plot differences in snowmelt dates were consistent across years, so we estimated missing values with a linear interpolation model based on a more extensive sensor network throughout the park (>125 sensors per year, Kroiss et al. 2015).

We recorded air temperature to estimate GDD. At each elevation we hung two sensors in trees near the meadow plots, mounted under white plastic funnels and recording temperature every 2 h. This approach gives similar readings to traditional weather station measurements of air temperature (Lundquist and Lott 2008). We calculated early- and late-season GDD at each plot based on when snow melted at that location, defining GDDearly as the daily temperature sum above 0°C during the first 25 d post-SDD, and GDDlate as a parallel metric for days 25–50 post-SDD. The use of a rolling window after snowmelt for calculating degree days rather than fixed calendar period/s is common in studies of high-altitude meadows (e.g., Sedlacek et al. 2015, Semenchuk et al. 2016), because air temperature can only exert an influence on herbaceous plant reproduction after the insulation of snow has melted (Ward et al. 2018). This does mean that SDD and GDD are related, because early snowmelt is associated with cooler early-season temperatures and late snowmelt with cooler late-season temperatures (Supplementary materials: Fig. S1.1). However, collinearity between these and other explanatory variables was well below the threshold of $r < |0.7|$ commonly used to avoid distortion of model estimations and predictions (Dormann et al. 2013). We chose a 50-d window because only 6.5% of the observations in the final data set captured reproductive activity of any kind occurring more than 50 d after snowmelt, and divided it into two spans because species that emerge early vs. late in the season may have different responses to warming (e.g., Sherry et al. 2007).

Finally, we took weekly point measurements of volumetric soil moisture at each plot using an EC-5 or GS-3 Sensor paired with a ProCheck reader (Decagon Devices, Pullman, Washington, USA). We converted raw measurements into an estimate of the number of days between snowmelt and soil drying down to ≤5% volumetric water content (SoilMoist). We use this as a proxy for the length of the growing season during which plants are free from drought stress, because this is an ecosystem in which summer precipitation is low and much of the ecologically available moisture is derived from snowmelt. More details on soil moisture measurements and conversion can be found in Theobald et al. (2017).

Our microclimate measurements captured a high degree of spatial and temporal variation. SDD ranged from late April to early September, a 130-d period; GDDearly ranged from 114 to 393 (median: 313); and GDDlate from 86 to 403 (median: 330). SoilMoist ranged from 151 d after snowmelt with ≥5% moisture for one plot in 2011, the year with the greatest snowpack, to just 22 d for one plot in 2015. We note that climatic conditions at our sites in 2015, an exceptionally warm and dry year, were similar to those projected for the region in 2080, and those in 2010–2014 were generally similar to current typical conditions (Mauger et al. 2015, Theobald et al. 2017). We therefore use site conditions in 2015 as a "climate change analogue" to help us predict the potential scale of the effects we found (Fig. 1.2).

1.2.4 *Statistical analysis*

Our objective was to quantify the relationships between climate and flower–seed transitions, which we defined as the number of days between a species' maximum probability of flowering and its maximum probability of releasing seed. We make two important notes: First, flower–seed transitions are only one possible way to characterize species' postflowering phenology. We selected this metric because the peaks of phenological phases (in comparison to first, last, or duration) are more robust to variation in plant abundances between plots and reduce the impact of outlier individuals. Second, because we made observations at the plot level, flower–seed transitions do not necessarily reflect the time it takes for individual flowers to move from pollination through seed dispersal. Climate-driven changes in the length of flower–seed transitions detected using this data set may result from changes in developmental rates of individual flowers, or population-level changes in the abundance or synchrony of reproductive stalks. However, this would be a limitation of any phenological measure derived from plot-level data. Although not perfect, we believe flower–seed transitions are the best simple, straightforward proxy for the overall time a species spends on reproduction. We address limitations of interpretation based on this metric in the discussion.

We adapted the hierarchical Bayesian model originally developed in Theobald et al. (2017) to characterize flowering phenology. As previously, we implemented a Bayesian approach to pool information across species, plots, and years, allowing for estimates of climate sensitivity for groups with relatively fewer observations and reducing the impact of outliers. Additionally, it allowed us to fit complex nonlinear relationships with nonnormal distributions, while taking into account uncertainty in our phenological observations.

Our hierarchical model had three levels. First, we modeled the probability of observing either flowering or seed dispersal for a given species, at a given plot, in a given year, on a given day in the growing season. In Eqs. 1a and 1b, we treated the presence–absence observations in our data set (*Y*) as Bernoulli-distributed random variables drawn from the expected probabilities of observing either flowering (*f*) or seed dispersal (*s*) on DOY *j* of year *k* in plot *l* for species *m*:

$$\text{Equation 1a: } Y_{fjklm} \sim \text{Bernoulli}(\alpha_{fjklm})$$

$$\text{Equation 1b: } Y_{sjklm} \sim \text{Bernoulli}(\alpha_{sjklm})$$

Second, we used logit-quadratic functions to describe the underlying shape of flowering and seed-dispersal phenology and ensure the probability of observing each phase fell between 0 and 1. Given the seasonal climate and natural history of this community, we assumed the probability of flowering and seed dispersal for each species to be a unimodal function of time over the growing season, with parameters describing the duration, peak, and height of each curve. We show this and other model components in Fig. S1.2:

$$\text{Equation 2a: } \text{logit}\,(\alpha_{fjklm}) = \text{duration}_{fklm} \times (\text{DOY} - \text{peak}_{fklm})^2 + \text{height}_{fklm}$$

$$\text{Equation 2b: } \text{logit}\,(\alpha_{sjklm}) = \text{duration}_{sklm} \times (\text{DOY} - \text{peak}_{sklm})^2 + \text{height}_{sklm}$$

Third, we modeled climatic effects on the peak of the flowering phase and on flower–seed transitions. Our focus was on the latter, but we fit climatic effects on flowering because we know from our original model that they are important determinants of flowering phenology (Theobald et al. 2017), and it allowed us to compare the relative sizes of climatic effects on flowering vs. postflowering phenology. We did not model relationships between climate and the height and duration of the flowering and seed-dispersal curves, a departure from our earlier work, because we were primarily interested in the effects of climate on the distance between curve peaks.

In Eq. 3a we modeled peak flowering for each species in a given plot and year (peak_{fklm}) as a function of SDD, GDDearly, GDDlate, and SoilMoist, with species-specific coefficients reflecting climate sensitivities ($\beta 1_m$–$\beta 4_m$). We included a random intercept α comprising normally distributed and zero-centered year, plot, and species effects:

Equation 3a: $$\text{peak}_{fklm} = \alpha + \beta 1_m * \text{SDD} + \beta 2_m * \text{GDDearly}_{kl} + \beta 3_m * \text{GDDlate}_{kl} + \beta 4_m * \text{SoilMoist}_{lk}$$

Equation 3b shows our key unknowns of interest: the species-specific climate sensitivities of flower–seed transitions (f2s_{klm}); that is, regression coefficients $\beta 5_m$– $\beta 8_m$. In Eq. 3c, we defined peak seed dispersal (peak_{sklm}) as an additive combination of the timing of peak flower (peak_{fklm}) plus this flower–seed transition (f2s_{klm}).

We fit and updated all models in JAGS v4.3.0 (Plummer 2017) with the *runjags* package (Denwood 2016). As with our previous models, we used diffuse normal priors (mean = 0, variance = 100) for parameter means and uniform priors (min = 1×10^{-6}, max = 20) for variances. We ran three parallel Markov chain Monte Carlo (MCMC) chains of 200,000 iterations each after a burn-in time of 30,000 iterations, each chain being initialized with different starting values for all key parameters. Post-burn-in chains were thinned by keeping every 10th value. To assess model convergence, we used a combination of visual inspection of chain history plots for key parameters and two summary statistics for all parameters: effective sample size and the multivariate Gelman–Rubin potential scale reduction factor (Brooks and Gelman 1998). We assessed model fit by holding back 10% of the original data set and testing the fitted model's ability to predict held-back data points correctly. We followed the recommendation of Saito and Rehmsmeier (2015) for unbalanced data sets in which absences far outnumber presences, and used the AUC of the precision-recall curve as a diagnostic (PR-AUC = 0.66, Supplementary Materials: Fig. S3). By all metrics, our model converged well and possesses good predictive power.

We used general linear models to test whether either a species' flower–seed transition time or the sensitivity of its flower–seed transition to climate variables could be predicted by the timing of its flowering, by flowering sensitivity to the same variables. We also used linear models to test for significant relationships among the species-specific climate sensitivities ($\beta 5_m$– $\beta 8_m$) themselves. We ran all analyses in R version 3.6.0 (R Core Team 2019), and the data set is publicly available (see *Data Availability*).

1.3 RESULTS

We asked whether climate influences postflowering phenology (in the form of flower–seed transition times), and if so, how these effects differ across species. Climate variables affected postflowering phenology for 93% ($n = 26$) of our focal wildflower species (Fig. 1.2, Table S1.2), meaning that for these species, at least one of the coefficients $\beta 5m$– $\beta 8m$ representing climate sensitivities was credibly different from 0. There were clear generalities in the directionality of these sensitivities. Earlier snowmelt, the climate variable with the largest magnitudes of effects on postflowering phenology, was uniformly associated with reductions in flower–seed transition times for all of the 17 species (61%) in our data set that were credibly affected by SDD (Fig. 1.1a, mean 0.17 d shorter per day of advanced snowmelt, across-species SD: 0.09). Note that Fig. 1.1 shows changes in flower–seed transitions per unit increase in each climate variable, so positive values for SDD indicate that later snowmelt lengthens transitions, and vice versa.

Warmer air temperatures (larger GDD sums) were uniformly associated with reductions in flower–seed transition times for the 25 (89%) of focal species credibly affected by temperature changes in either the early (Fig. 1.1b and c, mean 0.08 d shorter per unit increase in GDDlate, across-species SD: 0.02) or late season (mean 0.05 d shorter per unit increase in GDDlate, across-species SD: 0.01). And, fewer moist days were uniformly associated with reductions in flower–seed transition times for the nine species (32%) that were credibly affected by SoilMoist (Fig. 1.11d, mean 0.06 d shorter per day of advanced drought conditions, across-species SD: 0.04). Each climate variable drove postflowering phenology for multiple species but varied in how broadly it affected the community as a whole (Fig. 1, Table S1.2). Only two species (7%), *Kalmia microphylla* and *Micranthes tolmei*, were not credibly affected by any climate variables; but these were two of the rarest species in the data set, with tight spatial distributions experiencing relatively little environmental variation. Species sensitivities to SDD were slightly correlated with sensitivities to SoilMoist and GDDearly (Fig. S1.4).

To translate our model-estimated climate sensitivities to biological impacts, we scaled them by the approximate size of the changes we expect at our field sites under climate change (Fig. 1.2). To do so, we subtracted average values of each climate variable in typical years (2011–2014) from average values in 2015, our “climate change analogue” year. As species distributions vary across elevation and topography (and thus in the climate they are exposed to), we calculated species-specific estimates using only sites where that species was found. Multiplying these estimates of future conditions by each species' climate sensitivities and summing them gave us a prediction of the total days of change in flower–seed transition times that might result under climate change. Most species had shorter transition times under these conditions (Fig. 1.2).

Because SDD had larger effects on postflowering phenology than other climate factors, we conducted additional analyses of the relationships between a species' exposure to snow and its flower–seed transitions (Fig. 1.3). Species spent less time transitioning from flower to seed if their date of peak flower was later (i.e., closer to the return of snow, $P = 0.04$, $R^2 = 0.13$), if they grew in plots with shorter average snow-free windows (Fig. 3b $P = 0.01$, $R^2 = 0.19$), and especially if both were true ($P = 0.01$ for the interaction, R^2 for the complete model = 0.57). Finally, species were more sensitive to SDD if there was a shorter "buffer" period between their date of peak seed dispersal and the return of snow (Fig. 1.3c $P = 0.3$, $R^2 = 0.15$).

There were no significant correlations between the climate sensitivities of flowering and postflowering phenology (Fig. S1.5). Both flowering and postflowering were more strongly affected by snowmelt timing than by temperature or soil moisture, but the sizes of these impacts differed. Overall, flowering was more sensitive to climate than flower–seed transitions. But for at least some species, model-estimated flower–seed transitions were more sensitive to GDDlate than flowering (Fig. S1.5c), and for the majority of species, SoilMoist had larger effects on flower–seed transitions than on flowering (Fig. S1.5d).

1.4 DISCUSSION

1.4.1 *Climatic effects on postflowering phenology*

Climate, particularly the timing of snow disappearance and temperature during the first 25 d after snowmelt, influences flower–seed transition times for the vast majority of our subalpine meadow wildflower community. Earlier snowmelt, greater warmth, and a reduced period of soil moisture availability (all conditions expected with climate change) were each associated with shortened flower–seed transitions. This implies that in this high-elevation wildflower community, most species do not follow an internally constrained timeline that begins at flowering and continues at the same pace regardless of external conditions. Instead, postflowering phenology is plastic—although we find it is less sensitive to climate than flowering, consistent with the findings of Hülber et al. 2010 (Fig. S1.5a-c). This plasticity may be due in part to the relatively large magnitude of year-to-year variability in climate in high mountain ecosystems, particularly in terms of the timing and duration of the snow-free growing season. In regions with less interannual variation—for example, in a tropical dry forest with high seasonal variation in climate but relatively predictable start and end dates for the rainy season (Marco and Páez 2002)—phenology may be more strongly controlled by endogenous traits than by the environment.

The effects of the different climatic variables were remarkably consistent across the 28 species we examined. When either GDDearly or GDD late was higher, for example, flower–seed transitions in all temperature-sensitive species shortened (Fig. 1.1b, c). If this in fact reflects faster fruit/seed maturation, it is an intuitive result. Temperature controls both the rate of plant hormonal and metabolic processes and overall photosynthetic output (Sage and Kubien 2007), so if a minimum number of GDDs is needed for fruit and seed development, in warmer years the threshold will be met more rapidly. This has been observed in both agricultural and natural settings; a review of climate effects on crop maturation found higher temperatures hastened fruit ripening (Moretti et al. 2010) and *Campanula americana* transplanted to warmer sites completed seed maturation about 20 d faster (Haggerty and Galloway 2011). Rarely, the opposite pattern is seen; four out of six alpine species lengthened fruiting in response to warming in the Qilian Mountains of China, but this is a system where heavy snow is rare and summers are cool, so plants experiencing a lifting of temperature constraints can increase their overall allocation to reproduction without risking the seed-dispersal stage being cut off by the return of snow (Li et al. 2016). We believe this is not the case for our study system, as highlighted below.

Similar to temperature, species sensitive to soil moisture all responded by shifting postflowering phenology in the same direction: drier conditions were associated with shorter postflowering transitions (Fig. 1.1d). This may be because plants experiencing signals of drought complete this process before water availability is reduced (Aronson et al. 1992). In very dry 2015, some species, notably *Lupinus latifolius* var. *subalpinus* (subalpine lupine) also aborted many fruits (E. J. Theobald, *personal observations*); so water stress may trigger a shift of resources away from developing new fruits and towards maturing existing ones. This could be adaptive in environments where summer precipitation is low, and the amount of moisture held in the soil decreases steadily—and in some years rapidly—over the growing season.

For species sensitive to snowmelt timing, this climatic variation had the largest relative effects on flower–seed transitions (Fig. 1.1a), with earlier snowmelt uniformly leading to shorter transitions even after accounting for postsnowmelt temperatures. This suggests species may not be taking advantage of a longer growing season even though lengthening fruit- and seed-development times might have fitness benefits (Moles and Westoby 2006), and longer flowering

(Theobald et al. 2017, Pardee et al. 2019) and fruiting phases (Li et al. 2016) have been observed in response to earlier snowmelt and increases in growing season length. We believe this is because the primary resource constraint on growth and reproduction in this system is snow-free time, not heat or water availability. Later phenological phases are especially constrained vs. earlier ones because the onset of winter snow is abrupt and can have catastrophic consequences on seed maturation. The phenological responses of our focal plants are likely to have been shaped by their history in an environment where the growing season is profoundly limited by long periods of snow cover (up to 281 d in some plots and years). We show evidence for this kind of environmental filtering in Fig. 1.3, which suggests that the average snow-free window a species experiences constrains the length of time it devotes to postflowering phases (Fig. 1.3b), and the higher its risk of confronting the return of snow before it completes seed dispersal, the more sensitive it is to the timing of snowmelt (Fig. 1.3c). In other words, with growing seasons as brief as they are in these high-elevation meadows, perennial plants are under pressure to reach the end of reproduction as quickly as possible after snow melts. This would give them a higher chance of releasing propagules before snow returns, and remaining photosynthetically active time could be used to add to stored energy for the following year.

We emphasize again that our use of plot-level presence–absence data means what we are calling flower–seed transitions are not equivalent to individual development rates, though likely influenced by them. Climate may also have direct or interactive effects on floral abundance, the probability of flowers producing fruit or seeds, or phenological synchrony between individuals, all of which could affect our estimates. Finally, without data on individual seed set we can only speculate about fitness implications (Inouye et al. 2019 highlight the general failure to measure individual variation in phenological distributions and reaction norms). That said, we believe this metric captures the time spent on the major phases of reproduction at a species level and is a good proxy for the timing and duration of fruit and seed availability to animal associates.

1.4.2 *Ecological implications*

As the climate warms, we expect shorter flower–seed transitions, driven by three out of the four environmental variables tested in our study: earlier snowmelt times, warmer late-season temperatures, and lower soil moisture availability (Mauger et al. 2015). Perhaps counterintuitively, if snow melts earlier, we expect to see cooler temperatures immediately after snow disappearance, at least in the shorter term. These cooler early-season temperatures would drive longer flower–seed transitions. However, the effect of early-season temperatures is unlikely to offset the larger effects of other climate variables, especially earlier snowmelt (see Fig. 1.1a vs. b). Shorter flower–seed transitions could have implications for individual species if they reflect faster rates of development. Fruit- and seed-development time is sometimes associated with final seed size (Moles and Westoby 2006), with intraspecific variation in seed size potentially affecting plant fitness via effects on germination rates (Veloso et al. 2017), seedling survival, and final plant size (Simons and Johnston 2000). However, reaching peak seed dispersal sooner rather than later could have the benefit of protecting propagules from late-season freezes and for many of our study species, ensuring effective dispersal (Supplementary Materials: Table S1.1) before wet, snowy conditions return.

Shorter flower–seed transitions among this wildflower community could exacerbate phenological mismatches with animal associates by pushing the peak of fruit and seed availability earlier in the season. For example, though the majority of our study species produce dry fruits that are spread by wind or ballistic dispersal, two of the more abundant and ecologically important—the huckleberries *Vaccinium deliciosum* and *Vaccinium* *membranaceum*, found in 58% and 35% of plots respectively—produce sweet, fleshy fruit (Supplementary Materials: Table S1.1) that are important sources of nutrition for many mammals, particularly American black bears (Welch et al. 1997). These animals are also important seed-dispersal mutualists for *Vaccinium* sp. (Willson 1993), making the potential for phenological mismatches problematic for all species. The most widespread wildflower in our study—the legume *L. latifolius* var. *subalpinus*, present in over 70% of plots—has seeds that are predated on, rather than dispersed, by mammals such as yellow-bellied marmots; reproductive females and juveniles are especially reliant on food that is available late in the season, like lupines, to attain hibernation mass (Armitage 2003). In addition, *L. latifolius* is an important host for a multitude of insect seed parasites. These insects, which include muscoid flies (Kaye 1999), sawflies, and leaf beetles (M. L. Sethi, *personal observations*), lay their eggs inside seed pods while they are still attached to the plant, and larvae feed on the ovules as they develop. Shortened or mismatched windows of fruit/seed availability could have negative impacts on insect associates, but be beneficial for plants—in some years, up to 40% of *L. latifolius* seed pods show signs of invertebrate predispersal seed predation (M. L. Sethi, *personal observations*).

1.4.3 *Conclusions*

Our results support prior work (Stinson 2004, Jiang et al. 2016, Li et al. 2016) showing climate impacts on postflowering phenology independent of the responses of flowering phenology to the same variables. We therefore caution against generalizing about patterns of climatic control on reproductive phenology without empirical data on the entirety of the process. It is also clearly difficult to generalize across systems, especially with different constraints on reproductive timing (e.g., snow cover, temperature, animal dispersal). However, climate variables may have consistent cross-species effects within a given system. In the case of this subalpine wildflower community, the earlier snowmelt, warmer temperatures, and reduced soil moisture availability expected with climate change are all likely to compress postflowering phenology in time, with potential implications for plant fitness and species interactions. We demonstrate a methodology for disentangling the unique effects of climate on consecutive reproductive phases (but see Clark et al. 2014 for a considerably more complex treatment) and call for increased efforts to capture the responses of all reproductive phases to the environment, in multiple habitats that face different climatic constraints. In particular, to interpret the impacts on postflowering phenological shifts on development rates and reproductive fitness adequately, studies should include individual-level observations and quantitative data on fruit and seed set.

1.5 ACKNOWLEDGEMENTS

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1.6 DATA AVAILABILITY

Data used in this study are freely available on Zenodo:

http://doi.org.offcampus.lib.washington.edu/10.5281/zenodo.1001772

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1.8 FIGURES

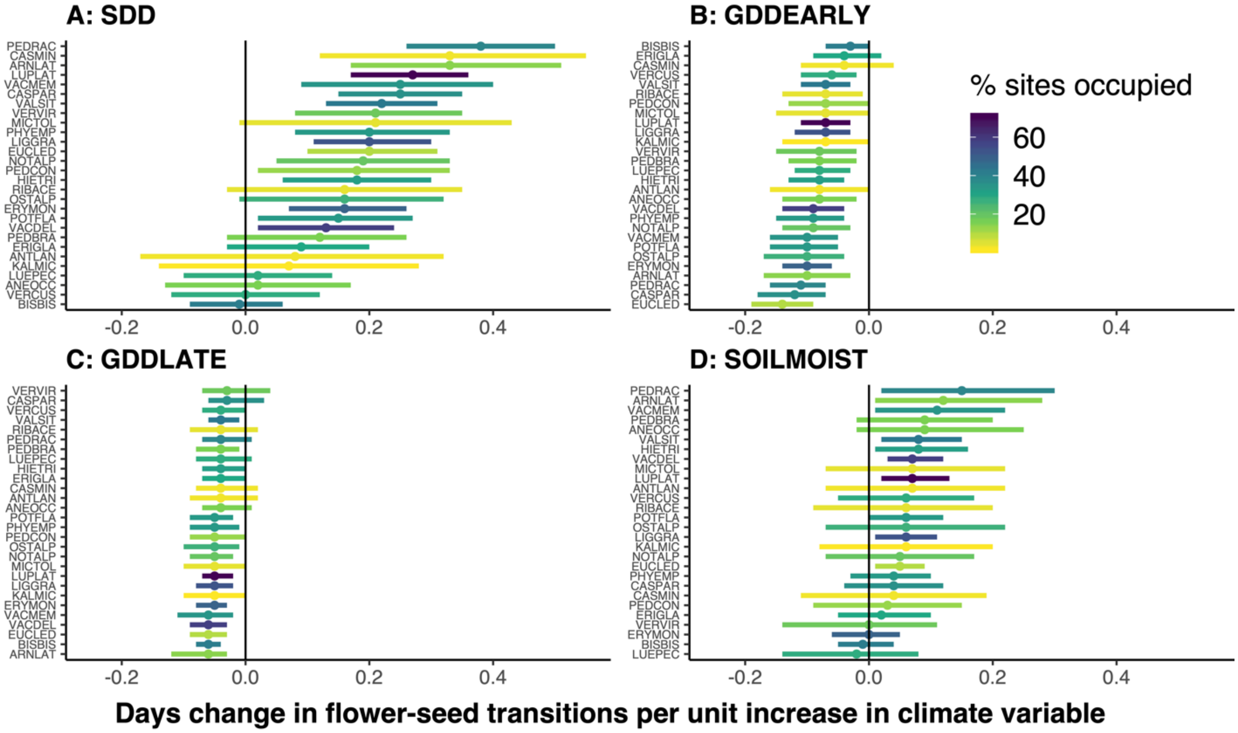

Figure 1.1 (A–D): Model-estimated species-specific sensitivities to climate (regression coefficients $\beta 5m$– $\beta 8m$) scaled to show days of change in flower–seed transitions per 1 SD increase in the climate variable. Note that positive values for snow disappearance date (SDD) indicate that later snowmelt lengthens transitions, and vice versa. Because climate variables were centered and scaled before model fitting, these scaled coefficients are interpretable as relative effect sizes. Points mark medians. Colors reflect proportional abundance (as the scale darkens, species occupy an increasing percentage of study sites). Lines mark Bayesian 95% credible intervals (CI).

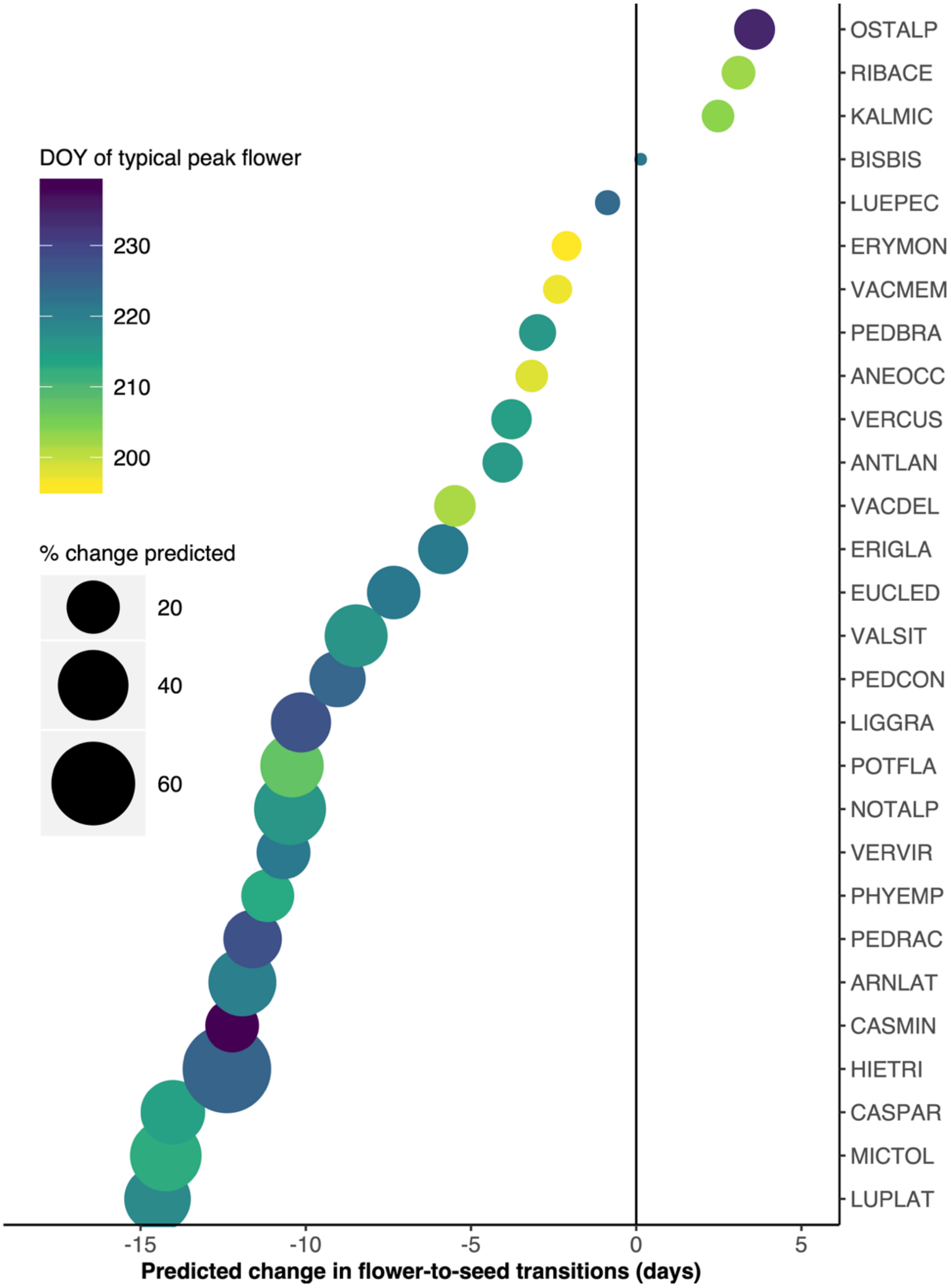

Figure 1.2: Change in flower–seed transition times under hypothetical climate change scenarios. For each species we calculated the average difference in each climate variable between typical years (2011–2014) and 2015, our "climate change analogue" year, at only the sites where that species is found. We multiplied each species' climate sensitivities by these differences and summed them to predict the total days of change in flower–seed transitions under this climate change scenario. Point sizes reflect the proportional size of changes; that is, a species that typically transitions in 14 d and is predicted to shorten by 5 d has a larger point than a species that typically transitions in 40 d and is predicted to shorten by 5 d. Colors reflect seasonality (darker colors indicate later day of the year [DOY] of typical peak flowering).

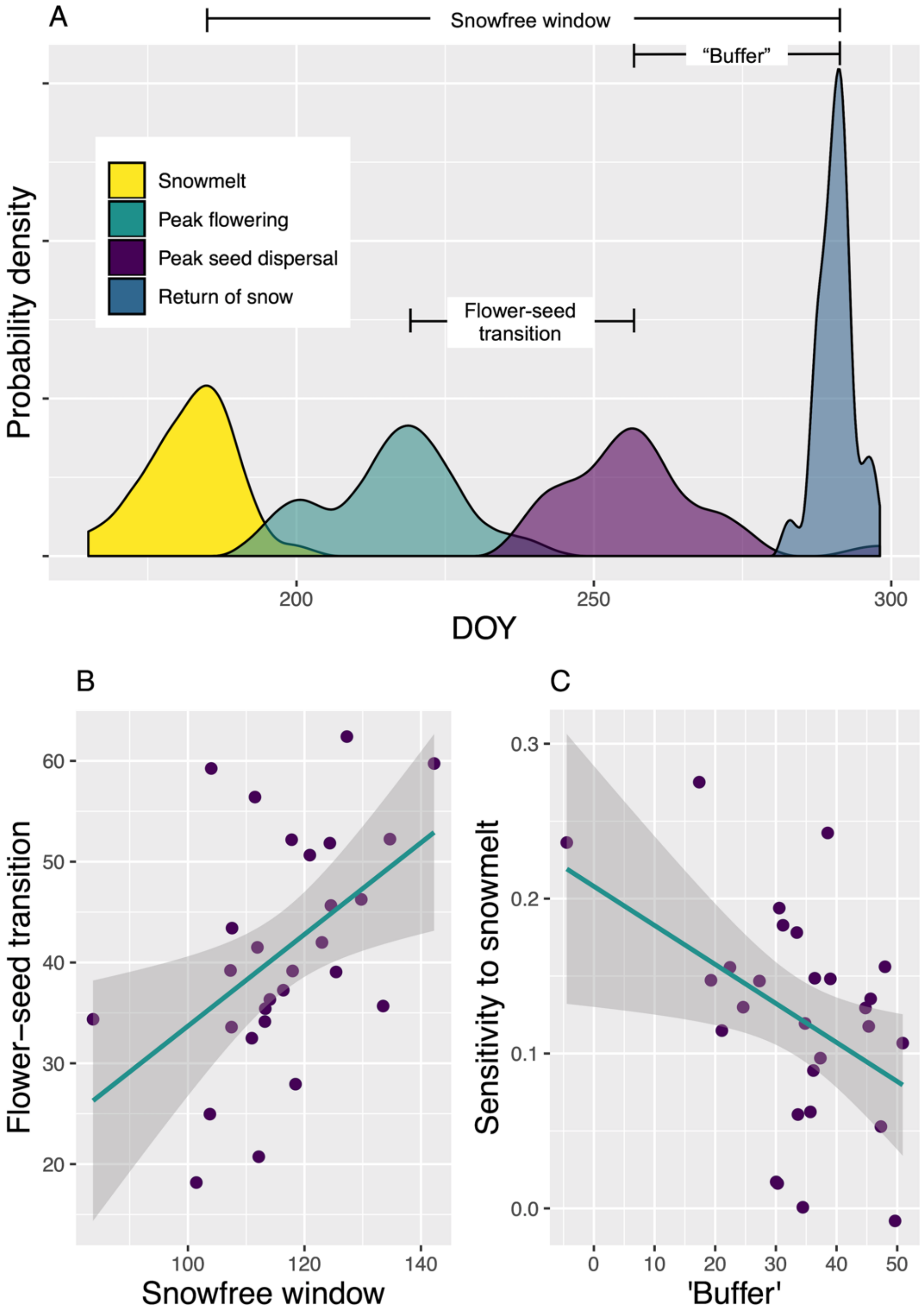

Figure 1.3: (A) Density plots of the mean days of the year (DOYs) of snowmelt, peak flowering, peak seed dispersal, and return of snow each species experiences. (B) The relationship between the mean snow-free window a species experiences and the mean length of its flower–seed transition. (C) The relationship between the sensitivity of flower–seed transition times to snowmelt timing and the typical days of "buffer" time a species has between peak seed dispersal and the return of snow.

1.9 SUPPLEMENTARY MATERIALS

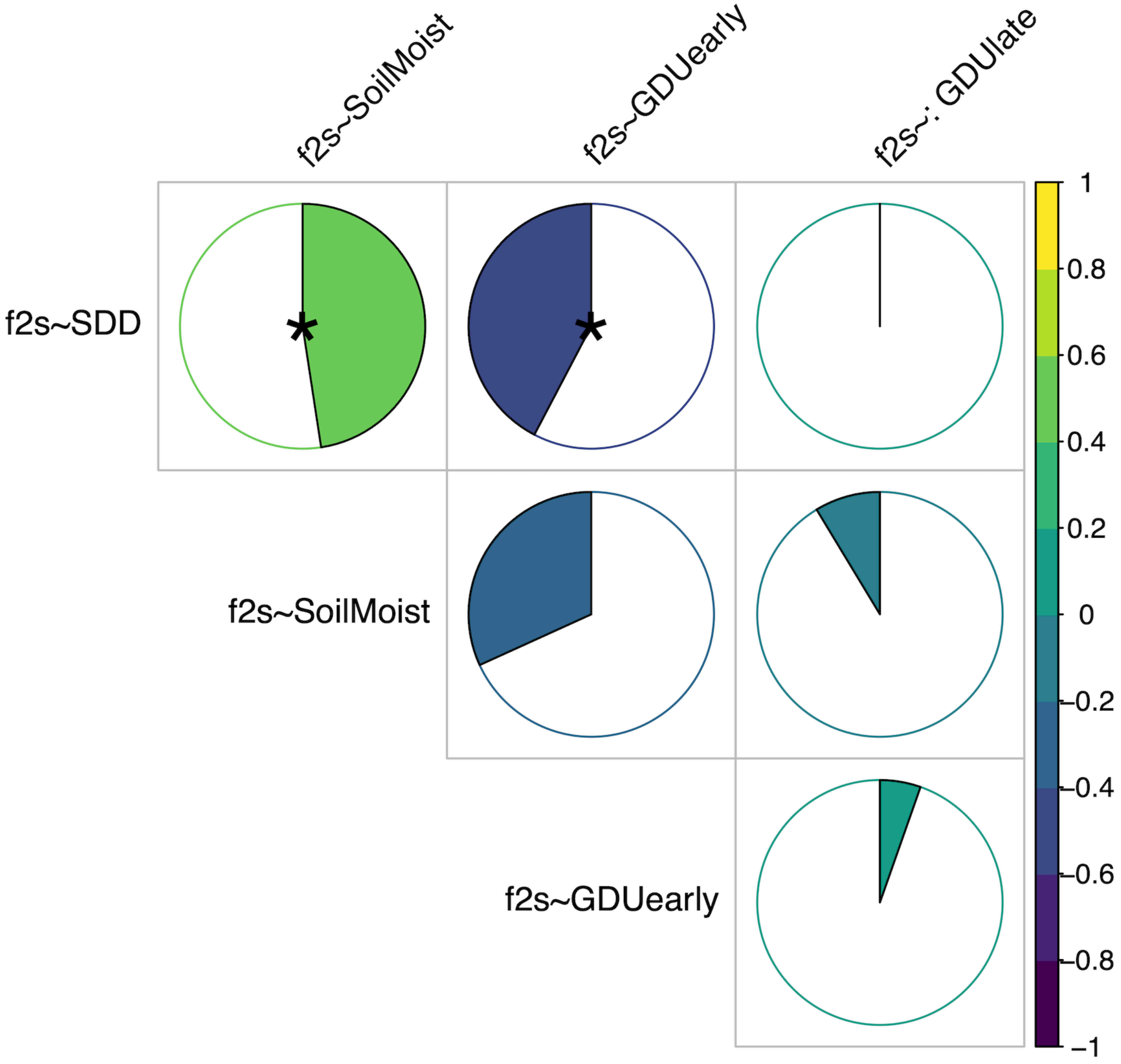

Figure S1.1: Correlations between explanatory parameters. Significant correlations are marked with *; pie slices correspond to the absolute values of the correlation coefficients and the color scale highlights the directions of the relationships. The largest correlations were between SDD and GDUearly (0.54, i.e. if snow melted later, the first half of the growing season tended to be slightly warmer) and SDD and GDUlate (-0.49, i.e. if snow melted later, the second half of the growing season tended to be slightly cooler).

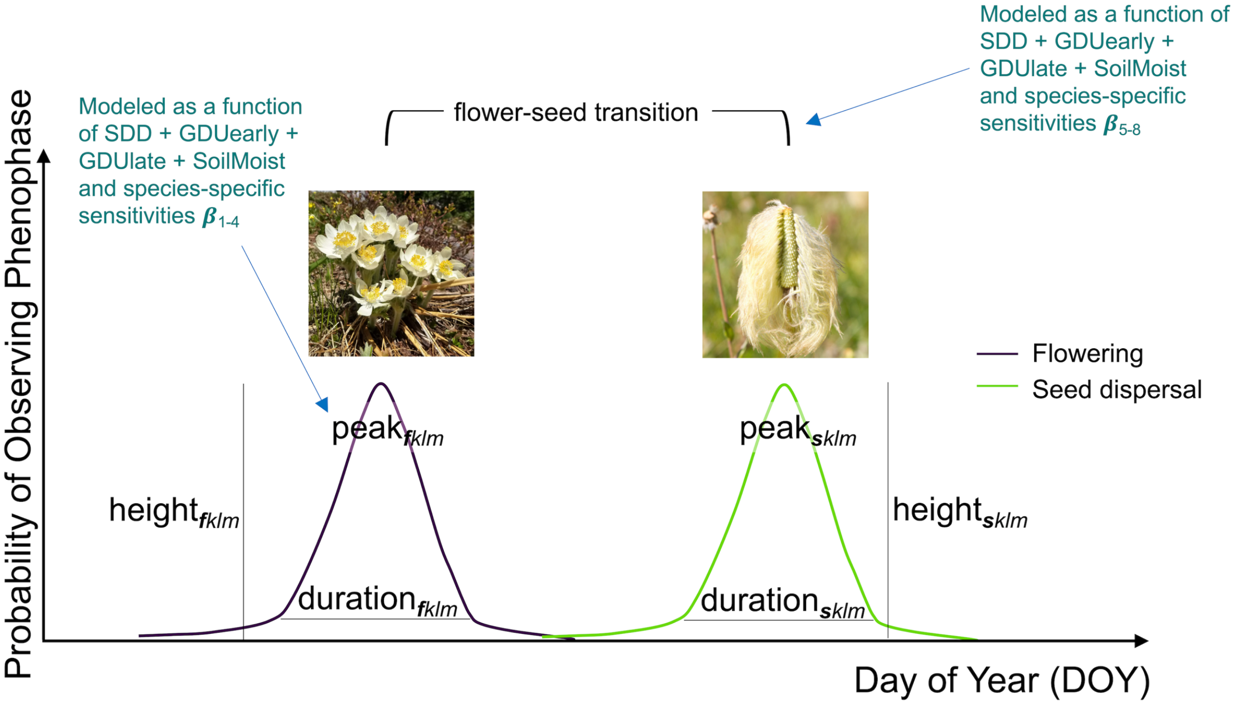

Figure S1.2: Schematic showing how we fit unimodal curves, defined by the parameters *peak*, *height*, and *duration*, to flowering (*f*) and seed dispersal (*s*) observations for each plot-year-species (*klm*) combination. *f2s* = Days between peak flowering and peak seed dispersal; *SDD* = Date of snow disappearance; *GDDearly* = Cumulative temperature sum above 0°C in days 0-25 after SDD; *GDDlate* = Cumulative temperature sum above 0°C in days 25-50 after SDD; *SoilMoist* = Days between SDD and soil drying down to ≤5% water by volume. Inset photos show flowering and seed dispersal phases of *Anemone occidentalis.*

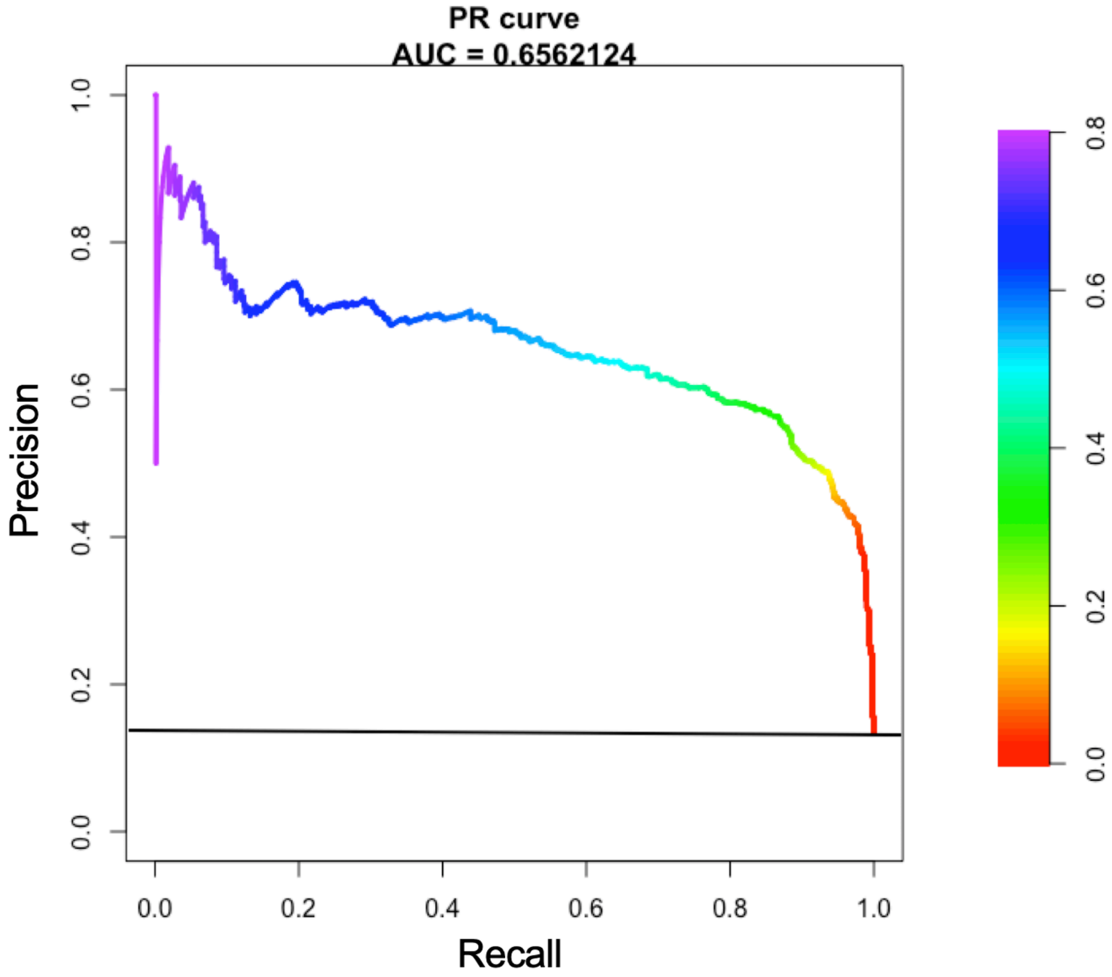

Figure S1.3: Precision-Recall Curve showing the performance of our model on the 10% of data that were held back from model fitting. The black line falls at 0.13 on the y axis and represents the baseline performance we would expect from a random classifier tested on our unbalanced dataset, which has a presence:absence ratio of nearly 7:1 (Saito and Rehmsmeier, 2015).

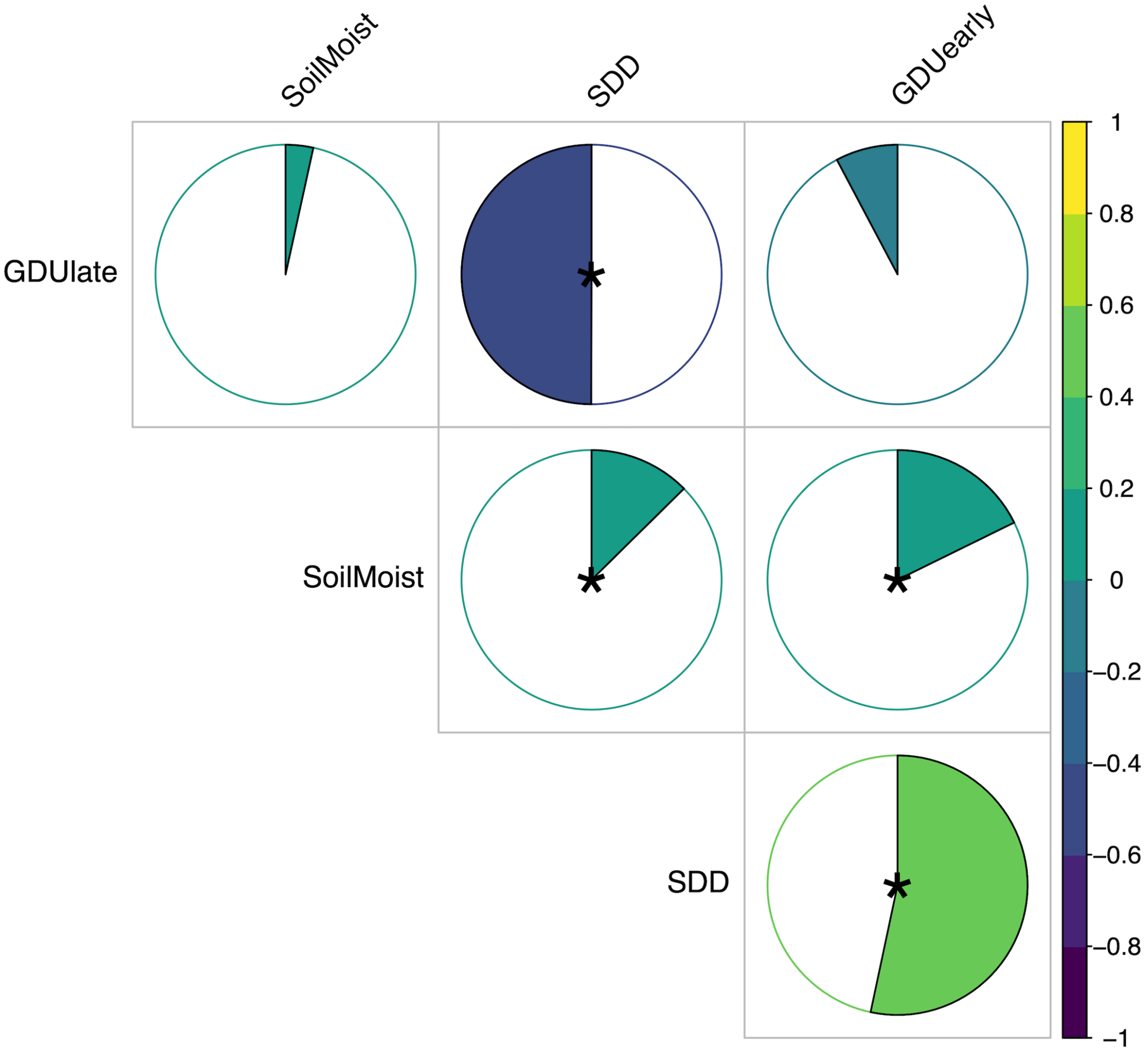

Figure S1.4: Correlations between species-specific climate sensitivities. Significant correlations are marked with *; pie slices correspond to the absolute values of the correlation coefficients. Two correlations were significant; species whose flower-seed transition times had more positive relationships with SDD also had slightly more positive relationships with SoilMoist (corr=0.48) and slightly more negative relationships with GDUearly (corr=-0.42).

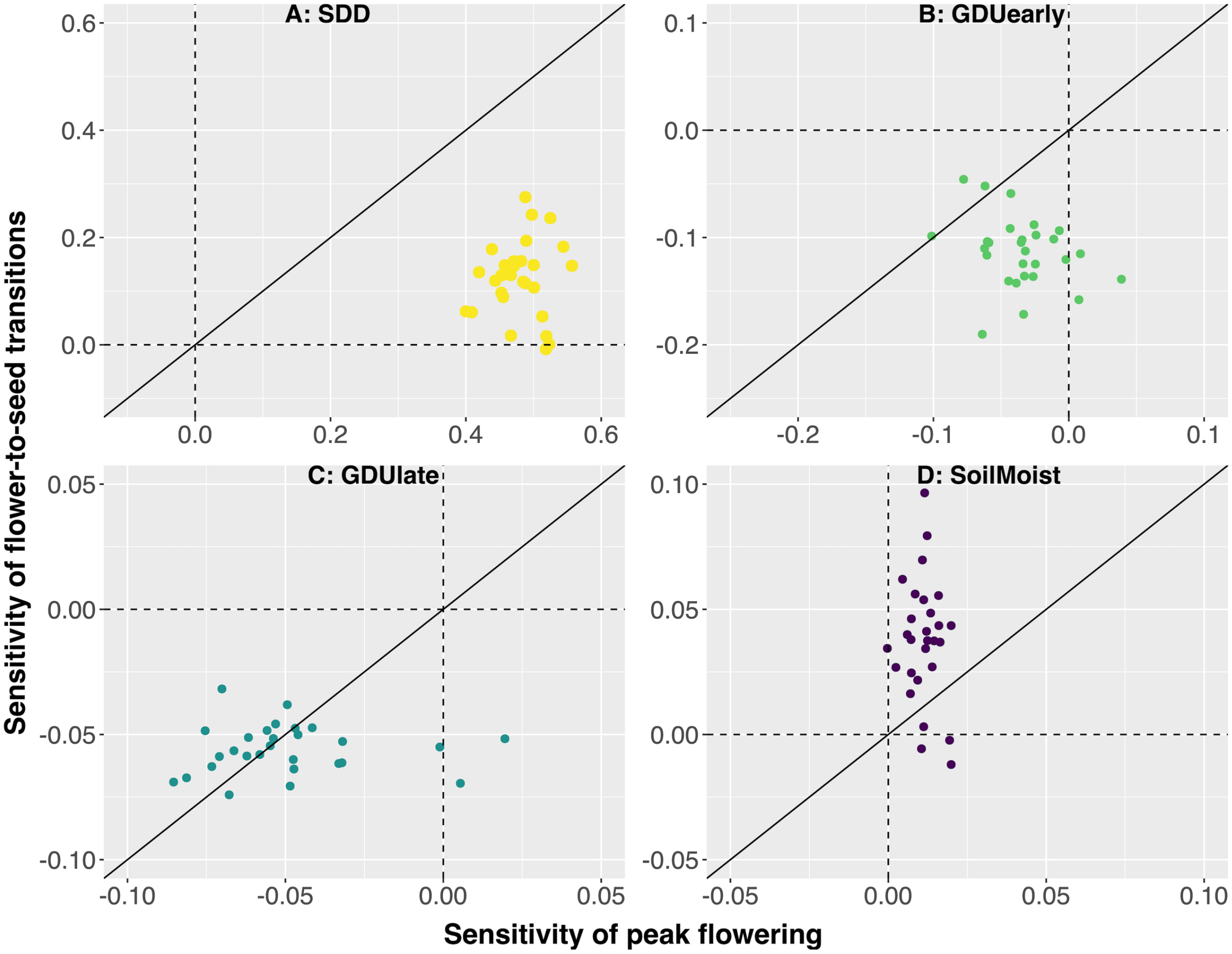

Figure S1.5: Median estimated climate sensitivities of post-flowering vs. flowering phenology. Dashed lines at 0 help highlight positive and negative sensitivity values and the 1:1 line is solid. (all relationships ns). To account for uncertainty in the estimations of these sensitivities we also calculated the regression relationships for each posterior sample of the climate sensitivities and found the proportion of samples with significant relationships; for all sensitivities, this was below 5%.

Table S1.1: Species included in the final analysis, along with traits of interest. Elev and % cover refer to the average elevation and average percent cover of the species at study sites where it was present (we measured cover at each 1 m2 site by laying a 1 m2 framed gridded quadrat over it comprising 10 cm x 10 cm squares, and counting the number of squares within which each species was found).

Species	**Code**	**Form**	**Fruit**	**Dispersal**	**Elev (m)**	**% sites occupied**	**% cover**
Anemone occidentalis	ANEOCC	Herb	Dry	Wind	1817	15.3	3.7
Antennaria lanata	ANTLAN	Herb	Dry	Wind	1791	2.8	1
Arnica latifolia	ARNLAT	Herb	Dry	Wind	1667	13.9	9.5
Bistorta bistortoides	BISBIS	Herb	Dry	Wind	1671	41.7	24.3
Castilleja miniata	CASMIN	Herb	Dry	Ballistic/Wind	1624	2.8	1
Castilleja parviflora	CASPAR	Herb	Dry	Ballistic/Wind	1795	36.1	4
Erigeron glacialis	ERIGLA	Herb	Dry	Wind	1697	29.2	4
Erythronium montanum	ERYMON	Herb	Dry	Ballistic	1609	48.6	2.8
Eucephalus ledophyllus	EUCLED	Herb	Dry	Wind	1567	9.7	19.6
Hieracium triste	HIETRI	Herb	Dry	Wind	1782	31.9	1.5
Kalmia microphylla	KALMIC	Woody	Dry	Wind	1490	1.4	16
Ligusticum grayi	LIGGRA	Herb	Dry	Wind	1660	52.8	7.9
Luetkea pectinata	LUEPEC	Woody	Dry	Wind	1754	27.8	13.2
Lupinus latifolius var. subalpinus	LUPLAT	Herb	Dry	Ballistic	1687	70.8	11.6
Micranthes tolmei	MICTOL	Herb	Dry	Wind	1853	4.2	1
Nothocalais alpestris	NOTALP	Herb	Dry	Wind	1788	19.4	3.7
Oreostemma alpigenum	OSTALP	Herb	Dry	Wind	1679	26.4	2.7
Pedicularis bracteosa	PEDBRA	Herb	Dry	Ballistic/Wind	1691	15.3	6.5
Pedicularis contorta	PEDCON	Herb	Dry	Ballistic/Wind	1800	12.5	23.3
Pedicularis racemosa	PEDRAC	Herb	Dry	Ballistic/Wind	1658	38.9	5
Phyllodoce empetriformis	PHYEMP	Woody	Dry	Ballistic/Wind	1727	33.3	12.3
Potentilla flabellifolia	POTFLA	Herb	Dry	Wind	1656	33.3	1
Ribes acerifolium	RIBACE	Woody	Fleshy	Animal	1670	4.2	6
Vaccinium deliciosum	VACDEL	Woody	Fleshy	Animal	1653	58.3	39.1
Vaccinium membranaceum	VACMEM	Woody	Fleshy	Animal	1576	34.7	48.6
Valeriana sitchensis	VALSIT	Herb	Dry	Wind	1626	43.1	18
Veratrum viride	VERVIR	Herb	Dry	Wind	1637	18.1	6.7
Veronica cusickii	VERCUS	Herb	Dry	Wind	1836	27.8	7.8

Table S1.2: Median values and 95% credible intervals for the species-specific climate sensitivities $\beta5_m$—$\beta8_m$, reported as days of change in flower-to-seed transition times per unit increase in the climate factor i.e. relative effect sizes). The scaling coefficients are as follows: SDD: mean 181.15, SD, 28.05; GDDearly: mean 301.10, SD 53.67; GDDlate: mean 324.00, SD 47.08; SoilMoist: mean 55.93, SD 25.34; DOY mean 215.73, SD 38.75.

Species	**β1:SDD (95% CI)**	**β2:GDDearly (95% CI)**	**β3:GDDlate (95% CI)**	**β3:SoilMoist (95% CI)**
Anemone occidentalis	0.02 (-0.13,0.17)	-0.08 (-0.14,-0.02)	-0.04 (-0.07,0.01)	0.09 (-0.02,0.25)
Antennaria lanata	0.08 (-0.17,0.32)	-0.08 (-0.16,0)	-0.04 (-0.09,0.02)	0.07 (-0.07,0.22)
Arnica latifolia	0.33 (0.17,0.51)	-0.1 (-0.17,-0.03)	-0.06 (-0.12,-0.03)	0.12 (0.01,0.28)
Bistorta bistortoides	-0.01 (-0.09,0.06)	-0.03 (-0.07,0)	-0.06 (-0.08,-0.04)	-0.01 (-0.05,0.04)
Castilleja miniata	0.33 (0.12,0.55)	-0.04 (-0.11,0.04)	-0.04 (-0.08,0.02)	0.04 (-0.11,0.19)
Castilleja parviflora	0.25 (0.15,0.35)	-0.12 (-0.18,-0.07)	-0.03 (-0.06,0.03)	0.04 (-0.04,0.12)
Erigeron glacialis	0.09 (-0.03,0.2)	-0.04 (-0.09,0.02)	-0.04 (-0.07,0)	0.02 (-0.05,0.1)
Erythronium montanum	0.16 (0.07,0.26)	-0.1 (-0.14,-0.06)	-0.05 (-0.08,-0.03)	0 (-0.06,0.05)
Eucephalus alpigenus	0.2 (0.1,0.31)	-0.14 (-0.19,-0.09)	-0.06 (-0.09,-0.03)	0.05 (0.01,0.09)
Hieracium triste	0.18 (0.06,0.3)	-0.08 (-0.13,-0.04)	-0.04 (-0.07,0)	0.08 (0.01,0.16)
Kalmia microphylla	0.07 (-0.14,0.28)	-0.07 (-0.14,0)	-0.05 (-0.1,0)	0.06 (-0.08,0.2)
Ligusticum grayi	0.2 (0.11,0.3)	-0.07 (-0.12,-0.03)	-0.05 (-0.08,-0.02)	0.06 (0.01,0.11)
Luetkea pectinata	0.02 (-0.1,0.14)	-0.08 (-0.12,-0.03)	-0.04 (-0.08,0.01)	-0.02 (-0.14,0.08)
Lupinus latifolius var. subalpinus	0.27 (0.17,0.36)	-0.07 (-0.11,-0.03)	-0.05 (-0.07,-0.02)	0.07 (0.02,0.13)
Micranthes tolmei	0.21 (-0.01,0.43)	-0.07 (-0.15,0)	-0.05 (-0.1,0)	0.07 (-0.07,0.22)
Nothocalais alpestris	0.19 (0.05,0.33)	-0.09 (-0.14,-0.03)	-0.05 (-0.09,-0.02)	0.05 (-0.07,0.17)
Oreostemma alpigenum	0.16 (-0.01,0.32)	-0.1 (-0.17,-0.04)	-0.05 (-0.1,-0.01)	0.06 (-0.07,0.22)
Pedicularis bracteosa	0.12 (-0.03,0.26)	-0.08 (-0.13,-0.02)	-0.04 (-0.08,-0.01)	0.09 (-0.02,0.2)
Pedicularis contorta	0.18 (0.02,0.33)	-0.07 (-0.13,0)	-0.05 (-0.09,0)	0.03 (-0.09,0.15)
Pedicularis racemosa	0.38 (0.26,0.5)	-0.11 (-0.16,-0.07)	-0.04 (-0.07,0.01)	0.15 (0.02,0.3)
Phyllodoce empetriformis	0.2 (0.08,0.33)	-0.09 (-0.15,-0.04)	-0.05 (-0.09,-0.01)	0.04 (-0.03,0.1)
Potentilla flabellifolia	0.15 (0.02,0.27)	-0.1 (-0.16,-0.05)	-0.05 (-0.09,-0.02)	0.06 (0,0.12)
Ribes acerifolium	0.16 (-0.03,0.35)	-0.07 (-0.14,-0.01)	-0.04 (-0.09,0.02)	0.06 (-0.09,0.2)
Vaccinium deliciosum	0.13 (0.02,0.24)	-0.09 (-0.14,-0.04)	-0.06 (-0.09,-0.03)	0.07 (0.03,0.12)
Vaccinium membranaceum	0.25 (0.09,0.4)	-0.1 (-0.16,-0.05)	-0.06 (-0.11,-0.02)	0.11 (0.01,0.22)
Valeriana sitchensis	0.22 (0.13,0.31)	-0.07 (-0.11,-0.03)	-0.04 (-0.06,-0.01)	0.08 (0.02,0.15)
Veratrum viride	0.21 (0.08,0.35)	-0.08 (-0.15,-0.02)	-0.03 (-0.07,0.04)	0 (-0.14,0.11)
Veronica cusickii	0 (-0.12,0.12)	-0.06 (-0.11,-0.02)	-0.04 (-0.07,0)	0.06 (-0.05,0.17)

1.9.1 *References for supplementary material*

Saito, T., & M. Rehmsmeier. 2015. The Precision-Recall Plot Is More Informative than the ROC Plot When Evaluating Binary Classifiers on Imbalanced Datasets. *PLOS ONE*, 10(3), e0118432.

Chapter 2. When ‘Higher’ Means ‘Hungrier’: Climate and Population Trait Differences Drive Increased Insect Herbivory with Elevation in a Perennial Subalpine Wildflower

ABSTRACT

Because short growing seasons severely constrain plant growth and biomass accumulation in high elevation habitats, herbivory can have profound impacts on both individual fitness and community dynamics in these settings. Climate change is expected to increase the activity of insect herbivores as their metabolic rates rise. However, montane communities may not respond in the same ways as agricultural or lowland ecosystems in which temperature is the dominant driver of herbivory. From 2016-2018 we conducted field observations of grasshopper herbivory on subalpine lupines in Mt. Rainier National Park and combined these with multiple leaf trait analyses and a set of manipulative feeding trials to explore how insect herbivory varies along a climatic gradient, and whether differences in plant or insect herbivore phenotypes that are influenced by a population’s climatic history can explain these patterns. We found a significant increase in herbivory with elevation that was related to both abiotic and biotic drivers, including snowmelt timing, leaf nutrition, and grasshopper feeding rates. Our results suggest that some high-elevation plant species may already be experiencing ecologically meaningful levels of insect herbivory that could intensify with climate warming. They also highlight the complexity of predicting how species interactions will change with warming in alpine and subalpine ecosystems, where environmental plasticity or local adaptation may lend tremendous complexity to ecological dynamics.

2.1 INTRODUCTION

Of the estimated 2.1 million extant species taxonomists have described to date, over 400,000 are land plants (IUCN Red List, 2020) and more than 1 million are insects or other invertebrates that make a living feeding on plant tissues (Fürstenberg-Hägg et al., 2013). Since virtually every plant species we know of hosts at least one insect herbivore (Strauss and Zangerl, 2002), the antagonistic relationship between a plant and an insect that feeds on it is arguably the most common type of pairwise species interaction on earth. It has been clear for decades that these relationships have a potential role to play in structuring plant dispersal and population dynamics across terrestrial ecosystems (Schultz et al., 2017; Agrawal et al., 2012; Maron and Crone, 2006; Fagan and Bishop, 1999; Crawley, 1989), and that they can strongly influence nutrient cycling (Metcalfe et al., 2013; Chapman et al., 2003; Belovsky and Slade, 2000).

Global climate change is likely to transform plant-insect herbivore interactions, because the direct positive effects of temperature on insect metabolism, development, reproductive potential, and voltinism are generally larger than its effect on primary production (deLucia et al., 2012; O'Connor et al., 2011; Bale et al., 2002). As a result, climate warming is projected to lead to marked increases in pest damage in agricultural settings (Deutsch et al., 2018). Forecasting shifts in plant-insect herbivore dynamics in natural settings has been an important focus of ecological research in recent years (Pincebourde et al., 2016; deLucia at al., 2012; Cornelissen, 2011). In general, theoretical predictions of increased consumption by insect herbivores in response to warming are supported by the results of experimental manipulations, including field experiments (Hamann et al., 2020; Birkemoe et al., 2016; Jamieson et al., 2014; Lemoine et al., 2014; Roy et al., 2004).

However, while temperature may be the dominant driver of herbivory under controlled or semi-controlled settings, in natural environments relationships between insects and their host plants are influenced by myriad interacting biotic and abiotic factors. The variety of these potential drivers is especially marked in mountain habitats, where characteristics including but not limited to local topography, precipitation, wind speeds, snow regimes, solar radiation, edaphic constraints, plant phenology, insect thermal requirements, and predation pressure operate at different scales and vary along spatial and temporal gradients, not necessarily monotonically (Tito et al., 2020; Moreira et al., 2018; Hodkinson, 2005). Such bedeviling conditions complicate the assumption that plants growing at higher elevations (and therefore cooler air temperatures) will experience lower rates of insect herbivory. Indeed, as described by Rassman et al., 2014, variable and even opposing elevational patterns have been observed in mountain ecosystems (e.g. Kristensen et al., 2020 vs. Pellissier et al., 2014).

The presence of multiple potentially relevant ecological variables also makes it more difficult to predict the future of montane plant-insect herbivore interactions under climate change, which itself could have both direct and indirect effects on each of these factors (Moreira et al., 2018). While many studies have used mountains as natural laboratories in which to investigate the effects of temperature on insect herbivory, relatively fewer have taken a more holistic approach that attempts to test the importance of multiple drivers of herbivory, such as those described above, along an elevational gradient. This is especially true when it comes to the net outcome of interactions between biotic and abiotic factors, which may manifest as elevational trait differences driven by phenotypic plasticity or local adaptation (but see for example Barrio et al., 2016, Haider et al., 2015, and Pellissier et al., 2012). We are particularly interested in understanding differences in both plant and insect herbivore phenotypes that may influence levels of consumption, as well as how these differences are influenced by a population's history within its environment. For example, cold-adapted plants may produce tougher leaves (Hagen et al., 2019), while the feeding rates of cold-adapted insects may show greater dependence on temperature (Buckley and Nufio, 2014). Work that connects these differences to observed patterns of herbivory could provide deeper insights into the effects of climate change on montane plant-insect interactions.

We used a combination of field observations, trait measurements, and experiments to characterize insect herbivory on subalpine lupines *Lupinus latifolius* var. *subalpinus* (Fabaceae, Piper & B.L. Robinson (C.P. Smith, 1907)), and Cascade timberline grasshoppers, *Prumnacris rainierensis* (Acrididae, Caudell, 1907), along an elevational gradient in a mountain range in western North America. We asked two primary questions: **1) How does grasshopper herbivory on lupines vary along an elevational and climatic gradient? 2) If differences in herbivory exist, what combination of abiotic (e.g. air temperature, snowmelt timing) and biotic drivers (e.g. leaf defenses, grasshopper feeding rates) influences these patterns?** We were interested in testing the hypothesis that air temperature is the main lever acting on the lupine-grasshopper interaction. If true, herbivory should be highest at warmer, lower-elevation sites, and climate warming will release the constraint that currently protects high-elevation plants from herbivory. In contrast, if historical contingencies of climate have influenced traits at a population level, they could produce alternate patterns. For example, if short growing seasons due to long periods of snow cover are associated with grasshopper populations that develop more quickly (Berner, 2004), high-elevation grasshoppers may impose more damage on lupines to sustain their higher metabolic rates and climate warming may exacerbate this at least temporarily.

Beyond our main questions, we felt it was important to understand whether grasshopper herbivory contributes to plant stress, given that it does not generally threaten survival in our system. And since plants experience many other types of insect herbivory that may also shift with climate change, we wanted to assess whether studying only one type of insect-inflicted damage—in this case chewing damage to leaves—might leave out an important piece of the picture. We therefore asked two secondary questions to help us interpret our results from a plant perspective: **3) Does herbivory damage affect lupine leaf photosynthetic capacity? 4) Do elevational patterns of foliar herbivory by insects correlate with patterns of insect seed predation in lupines?**

2.2 METHODS AND MATERIALS

2.2.1 *Study area*

We conducted our research in Mt. Rainier National Park, WA (46.8529° N, 121.7604° W). Standing at 4,392 m, Mt. Rainier (traditionally known as Mt. Tahoma) is the tallest volcanic peak in the rugged Cascade Mountain Range that runs parallel to the Pacific Coast, from California to Canada. Our two species of interest both occur in the subalpine, a narrow band of elevation between conifer forests below and rocky alpine communities above. This unique meadow ecosystem hosts a remarkable diversity of plants and wildlife that make their living under challenging environmental conditions, including relatively cool average temperatures, low summer rainfall, and up to 40 weeks a year of deep snow cover.

We collected data on two transects, one each on the South and East sides of the mountain, spanning the total range of subalpine meadow habitat: approximately 1,490—1900 m on the South and 1,660—2,020 m on the East side. Because the East side lies in the mountain's rain shadow, it has a different rainfall and snowfall regime, and plant community distributions are shifted elevationally by about 200 m. On each transect we had five sites, roughly evenly spaced along the elevational gradient, with nine permanently marked 1 m^2 plots at each site spaced at least 10 m apart. At each elevation, three plots were located on ridge tops, three along slopes, and three in depressions. While we did not necessarily consider such local topographic variation to be a direct driver of insect herbivory, it is strongly correlated with the timing of snowmelt (Ford et al., 2013), which could influence herbivory. We buried HOBO Pendant temperature sensors (Onset Computer Corporation) just below the soil surface at each plot to estimate snow disappearance dates (as in Sethi et. al, 2020).

2.2.2 *Study species*

L. lat. var. subalpinus (subalpine lupine) is a common herbaceous perennial of the northwestern United States, with a distribution range stretching from northern Oregon to Alaska. In the mountains of western Washington, most populations are found at 1200 m or higher, growing amid the forest understory as it approaches tree line and in large patches on open ridges and meadows. The dense foliage, charismatic blue-violet inflorescences, and bean-like seeds of this species support a rich array of invertebrate, mammal, and bird species, including bumble bee and hummingbird pollinators and foragers like hoary marmots and golden-mantled ground squirrels. As nitrogen-fixers, these plants increase soil nutrient availability for the high-elevation communities to which they belong, and are among the earliest colonizers of recently exposed soils left by deglaciation or permafrost disturbance (Cray and Pollard, 2015; Henderson, 1973).

P. ranierensis (Cascade timberline grasshopper) is a stout, short-winged (flightless), high-elevation grasshopper with yellow-green to coppery brown coloration, black lateral stripes, and legs marked with chevron-shaped ridges. It is endemic to the Cascade Mountains of Oregon and

Washington and extremely locally abundant within our study area. Eggs laid the previous season that have overwintered beneath a heavy layer of snow hatch at snowmelt, and mating adults are thick—in the thousands—in subalpine meadows on Mt. Rainier by the end of the summer. Although they are generalist forb-eaters that feed on many subalpine wildflower species, these grasshoppers are known to be particularly closely associated with *L. lat.* var *subalpinus* (Helfer, 1972; pers. obs.). Our identification of *P. rainierensis* was confirmed by Andrew Carmichael at the Systematic Entomology Laboratory (SEL), part of the Agriculture Research Service in the US Department of Agriculture, after we sent three collected specimens for inspection.

2.2.3 *Data collection and statistical analysis*

We outline each type of data collection in subsections below, along with descriptions of relevant statistical techniques. All analyses were carried out in R 4.0.3 (R Core Team, 2020).

2.2.3.1 *Observations of grasshopper herbivory*

We monitored grasshopper herbivory on *L. lat.* var. *subalpinus* between 2016 and 2018. Since lupines reproduce both by seed and clonally, through short rhizomes (Celis et al., 2017), we could not identify genetically unique plants non-destructively. Our analysis therefore treats whole plots, which were far enough apart (>10 m) to be extremely unlikely to contain the same individual, as replicates. There was clear variation in herbivory among "clumps"—clusters of smaller stems surrounding a main stem—so we monitored all lupine clumps in all study plots, except in a few plots with particularly high lupine populations where we monitored only the first 8 that leafed out (mean = 4.3 clumps per plot, sd = 1.5). Every two weeks during the growing season each year (approximately 12 weeks, from late June-September), we visited each plot and recorded % leaf area lost to chewing insect herbivory on 10-30 haphazardly selected, fully expanded, non-senescent lupine leaves per clump. We did this using simple visual estimation, classifying each leaf into one of eight damage classes (0%, 0-1%, 1-5%, 5-10%, 10-25%, 25-50%, 50-75%, and 75-100%). To test the accuracy of our observations, we collected 30 leaves showing a range of chewing damage and compared classifications made by our primary observer (Sethi) to estimates of leaf area lost calculated from scans of the same leaves in ImageJ (Schneider et al., 2012). We found they agreed well (weighted Cohen's Kappa of 0.86, calculated as described in Watson and Petrie, 2010, for ordinal data with >2 categories).

Visual estimation is commonly used to quantify invertebrate herbivory damage in the field (e.g., Scheidel and Bruelheide, 2001; Dostálek et al., 2018; Kristensen et al., 2020), and human observers are capable of precise and accurate estimates of herbivory comparable to those produced by digital methods (Johnson et al., 2016). This is especially true with chewing at leaf margins as opposed to interiors, which is the type of damage typically left by grasshoppers (Gangwere, 1966; Avanesyan and Culley, 2015). Damage patterns associated with chewing insects are unambiguous and easily diagnosable to functional group (Green and Maloy, 1990), and while other chewing insect herbivores that feed on lupines are present at our sites (including other orthopterans and several butterfly larvae), in our observations *P. rainierensis* outnumbered all of them combined by a factor of 4:1. We are confident that the vast majority of the damage recorded in our dataset was produced by *P. rainierensis.*

To understand potential drivers of the patterns of leaf area damage we observed, we created a linear mixed effects model (LMM) to test for the effects of elevation, snowmelt date, time since snowmelt, and canopy cover (continuous predictors) as well as of transect and year (categorical predictors) on herbivory (a continuous outcome variable). We estimated herbivory as follows: Using the observation data described above, we calculated apparent leaf damage (ALD) for each lupine clump at each observation as ∑(# leaves in each class * median %

damage in that class) / total leaves sampled). We used the median ALD observed across lupine clumps within each plot at each visit (substituting the mean instead did not substantially change the results). We included plot as a random effect, since each plot was sampled multiple times. Time itself was accounted for in the model via the *time since snowmelt* predictor, which we calculated as the Julian day of the observation date minus the Julian day of snowmelt at that plot in that year. Before running analyses, we centered and scaled all predictor variables so that we could directly compare their model-estimated coefficients and identify their relative importance.

We used the top-down variable selection process outlined in Zuur et al., 2009, preferring Bayesian Information Criterion (BIC) over Akaike Information Criterion (AIC) scores if the two differed because our analytical goal was hypothesis testing rather than predictive performance (Aho et al., 2014). We implemented our LMMs with R package "nlme" (Pinheiro et al., 2013), because it allowed us to account for heteroscedasticity in the residual errors driven by increasing variance with elevation. We did this by modifying the variance structure in our final model, passing the value "varIdent(form=~1|Elevation)" to the "weights" argument in the "lme" function to allow different residual variances by elevation.

2.2.3.2 *Pre-dispersal seed predation*

Because foliar damage is not the only form of insect herbivory plants experience, leaf damage observations do not capture the full picture of insect pressure on lupines. To assess whether pre-dispersal seed predation by insects poses an additional threat to lupine fitness, in 2018 and 2019 we collected 10 ripening seed pods from each of 5 haphazardly selected plants at each elevation where they were available. Due to restrictions on collecting, we measured seed predation at the site (elevation) level rather than the plot level. Seed pods were transported back to the lab inside moist paper towels in sealed plastic bags and examined under a microscope for signs of invasion and seed predation. Invertebrates found within seed pods were counted and identified to family. We used a simple linear regression to test for elevational patterns in seed predation, with proportion of predated seeds per pod as a continuous response and elevation as a continuous predictor.

2.2.3.3 *Lupine leaf traits: LMA, C:N, defense compounds*

To assess the role of leaf traits in explaining observed variation in herbivory rates at different sites, we measured three traits likely to affect food quality for arthropod herbivores: Leaf Mass per Area (LMA) as a proxy for leaf toughness (He and Sun, 2016), the ratio of carbon to nitrogen (C:N, Barbour et al., 2015), and constitutive defenses (in the form of quinolizidine alkaloid compounds, which are the main chemical deterrence used by lupines: see Wink, 1992, and Mithöfer and Boland, 2012). To calculate LMA and C:N ratio, we collected leaves from at least 10 replicate plants at each elevation and stored them in moist sealed plastic bags in a cool box until processing. Leaves were rehydrated overnight before being scanned. We calculated LMA using leaf area estimated with ImageJ and dry mass measurements taken after the leaves were dried in an oven at 45°C for one week. Finally, we ground and homogenized the leaves in a ball mill, encapsulated 2mg replicate samples in tin foil, and measured C:N concentrations using an elemental analyzer (CHN Analyzer 2400 Model, PERKIN ELMER Co). We used simple linear regressions to test for elevational patterns in leaf toughness and nutritional quality, with LMA and C:N ratio as continuous responses and elevation as a continuous predictor.

For chemical defense analysis, we collected leaves from 5 replicate plants at each elevation, flash froze them in liquid nitrogen in the field, and transferred them to an -80°C freezer until they were analyzed at the Murdock Metabolomics Laboratory at Washington State University (Pullman, WA). Appendix 2 contains details about alkaloid extraction and processing. We used simple linear regressions to test for elevational patterns in the concentrations of two major alkaloid defense compounds (sparteine and lupanine), with alkaloid concentration as a continuous response and elevation as a continuous predictor. We also used principal components analysis (PCA) with the "prcomp" function in the *stats* R package to visually assess whether the composition and concentration of alkaloids clusters among plants growing at the same elevation (this analysis included an additional three alkaloids for which standards were not available and for which relative concentrations were used).

2.2.3.4 *Herbivory and photosynthetic performance*

To explore the potential effects of herbivory on plant photosynthetic performance, we used a handheld device to measure a suite of photosynthetically relevant parameters (MultispeQ, PhotosynQ Inc; Kuhlgert et al., 2016) in light-adapted undamaged leaves (n=259) and leaves with at least 5% of area lost to herbivory (n=231), with measurements collected across a range of temperature and light conditions at each elevation to account for environmental variation. We used linear regressions to determine the slopes of the relationship between PAR (Photosynthetically Active Radiation) and three different photosynthetic parameters: Phi2 (the quantum yield of photosystem 2), PhiNPQ (non-photochemical quenching), and PhiNO (non-regulated, potentially photo-inhibitive processing of excess energy) for damaged and undamaged leaves separately. We then used ANOVA to test for significant differences between these slopes for damaged vs. undamaged leaves. Separately, we used a t-test to look for differences in relative chlorophyll levels between damaged and undamaged leaves.

2.2.3.5 *Experimental Feeding Trials*

Because our first year of data suggested greater grasshopper herbivory on lupines at higher-elevation sites vs. lower ones, we conducted experiments to disentangle the effects of feeding temperature, lupine population, and grasshopper population on grasshopper feeding rates. In late August/early September 2017 and 2018, when mature grasshoppers were present at all elevations, we visited our highest and lowest sites on each transect and collected adult *P. rainierensis* individuals, as well as fully unfurled, non-senescent, undamaged lupine leaves (stems attached). Field-collected specimens were transported back to the lab, held at room temperature, and used for feeding trials within 24 hours of collection. Until then, leaves were placed in individual floral tubes with stems submerged in water, and grasshoppers were placed in individual 16 oz. polyurethane cups with a damp paper towel.

We used a fully factorial design with eight treatment groups (average n=38 per treatment) comprising all combinations of grasshopper source (low vs. high elevation), and lupine source (low vs. high elevation), and temperature (25 °C vs. 32 °C). We selected these temperatures because they are representative of the mean and the mean plus 1.5 standard deviations, respectively, of 491 recordings of ambient daytime temperatures collected across all our sites by the MultiSpeQ during our photosynthesis measurements (see section *2.3.4*). After being fasted for 12 hours to ensure their stomachs and guts were empty, grasshoppers were placed in Percival I-36VL incubators and acclimated to their assigned test temperatures for 1 hour before being provided with 1-2 lupine leaves from their assigned population, depending on the size of the leaf. Trials lasted 8 hours to provide sufficient time for all grasshoppers to ingest, fill their crops, and clear their guts (after Buckley and Nufio, 2014, and Harrison and Fewell, 1995). Leaves were weighed before and after each trial to determine feeding rates. We placed control leaves from each population source inside empty polypropylene cups in each incubator for the duration of the experiments. Control leaves lost negligible mass (an average of <0.5% in 8 hours), ruling out confounding effects of changes in mass due to evapotranspiration.

We used a three-factor ANOVA to explore how grasshopper feeding rates responded to our experimental treatments: Temperature (fixed with two levels, 25°C and 32°C), Grasshopper source (fixed with two levels, Low and High), and Lupine source (fixed with two levels, Low and High). Our continuous outcome variable was leaf mass (g) consumed over the entire 8 hours, adjusted by grasshopper weight. Several grasshoppers showed signs of parasitism (emergence of fly larvae); we removed data from trials in which these individuals were involved.

2.3 RESULTS

2.3.1 *Observations of grasshopper herbivory*

There was a high incidence of grasshopper herbivory on lupines in the subalpine meadows we monitored, with 76.9% of our observations recording visible tissue loss associated with chewing damage. The magnitude of this damage per individual was generally low; an average individual lupine in our study plots lost 3.2% of the total leaf area it produced to grasshopper herbivory each season. There was, however, a long tail of higher losses; in one out of four of all plot-year combinations, at least one lupine clump lost over 10% of its total leaf area to herbivory, and some clumps experienced ten times that much tissue loss (maximum estimated mean ALD in a season: 36%). Our best-fitting mixed-effects model (Table 2.1) showed that grasshopper herbivory on lupines was significantly affected by the main effects of elevation, year, snowmelt date, time since snowmelt, and canopy cover, as well as by the pairwise interactions between elevation and transect, elevation and year, and elevation and time since snowmelt. For comparison, the results of our initial (global) model can be found in Table S2.1.

Elevation had the largest effect on herbivory, with plots at higher elevations experiencing greater losses. The slope of the elevation-herbivory relationship was steeper on the East transect in all years, and on both transects in 2018 (Fig. 2.1, Table 2.1). Snowmelt date and time since snowmelt both had positive relationships with herbivory, with plots that melted out later having higher herbivory, and herbivory increasing over time in plots after snowmelt. In addition, the increase in herbivory with time since snowmelt was greater in plots at higher elevations.

2.3.2 *Pre-dispersal seed predation*

We observed a high level of pre-dispersal seed predation. 46% of lupine pods (n=601) collected from the East transect and 39% of pods from the South transect (n=678) showed clear signs of invasion by specialized invertebrate herbivores—mainly fly, leaf beetle, and sawfly larvae. While most invaded pods contained some intact seeds at the time of collection, an average of 34% of seeds within invaded pods had been predated to the point where we would assume they were no longer viable (>25% tissue loss). We found a significant positive relationship between elevation and seed predation ($p<0.0001$), which matched the elevational pattern for leaf damage. However, this explained a small amount of the variation in seed predation ($R^2=0.02$) and is based on incomplete data because no lupine plants successfully set seed in some elevations in some years. (In 2018, lupines at the two highest elevations on both transects appeared to have failed to set seed, and in 2019 this was true of lupines at the second-highest elevation on the East transect.)

2.3.3 *Lupine leaf traits: LMA, C:N, defense compounds*

LMA, our proxy for leaf toughness, increased with elevation (Fig. 2.3a), as did C:N ratio, our (inverse) measure of nutritional value (Fig. 2.3b). In other words, high-elevation leaves are likely to be more difficult to chew and offer less nutritional value to grasshoppers for a given leaf mass. The concentration of sparteine, a major lupine alkaloid used as a defense chemical and the most abundant alkaloid found in our samples, also increased significantly with elevation (Fig. 2.3c).

Lupanine, another defensive alkaloid, did not differ significantly with elevation (p=0.69). PCA of all detected alkaloids collectively showed that the overall composition and concentration of alkaloid defense compounds did not cluster by site or elevation (Fig. S2.1).

2.3.4 *Herbivory and photosynthetic performance*

MultispeQ measurements showed no significant differences between damaged and undamaged leaves in terms of the efficiency of photosynthesis (defined as the slope of the relationship between PAR and the quantum yield of photosystem 2). However, leaves that had experienced herbivory showed significantly lower levels of non-photochemical quenching (PhiNPQ, p=<0.001) and significantly higher levels of non-regulated energy dissipation that can lead to photoinhibition (PhiNO, p=0.008) at a given light intensity (Fig. S2.2). Damaged leaves had a lower numerical mean level of relative chlorophyll than undamaged leaves, though this difference was not statistically significant (p=0.07).

2.3.5 *Experimental feeding trials*

We found significant effects of temperature, lupine population source, and grasshopper population source on weight-adjusted grasshopper feeding rates in our experimental trials (Fig. 2.2a). Consumption was higher at 32°C than 25°C (F value = 21.78, p<0.0001). Low-elevation lupine leaves were consumed more rapidly than high-elevation leaves (F value=10.72, p<0.001). Finally, high-elevation grasshoppers fed faster than low-elevation grasshoppers (F value =6.53, p<0.01). The interaction between temperature and grasshopper source was not significant (F value=2.78, p=0.09), but visualizing the interaction suggests high-elevation grasshoppers may be more sensitive to temperature than low-elevation counterparts (Fig. 2.2b). There were no significant differences in size between grasshoppers from different populations (p=0.09).

2.4 DISCUSSION

Consistent with resource limitation theory, which predicts higher resistance to herbivory among slow growing plants in climatically stressful habitats (Grime, 2006), montane lupines experience relatively low levels of herbivory overall. We estimate that chewing damage associated with grasshoppers resulted in an average loss per lupine clump of 3.2% of total leaf area annually, which is similar in magnitude to data on invertebrate herbivory collected in other open, high-elevation systems (e.g., Haider et al., 2015; Pellesier et al., 2014) and lower than losses attributed to invertebrates in temperate woodlands (e.g., 12% annually, Visakorpi et al., 2021) and pastures (e.g., 13% annually, Coupe and Cahill, 2003). This is not surprising, as bottom-up controls are likely to be relatively strong in montane meadows precisely because the potential impacts of losing expensive leaf tissue are high (Germino, 2014; Dostálek et al., 2018).

2.4.1 *Insect herbivory has meaningful impacts*

Despite being low in average intensity, we would argue that insect damage likely poses meaningful challenges to subalpine lupines on Mt. Rainier. Grasshopper herbivory. was a widespread phenomenon (Fig. 2.1), affecting 88% of all individual plants in our study. And for a substantial minority of individuals, recorded levels of leaf loss were considerably higher than the mean. In some seasons and at some elevations, about 20% of the population experienced the loss of 10% or more of their total leaf area (Fig. 2.1). There is also convincing evidence that even low intensities of insect damage can have important effects on individual plant performance and community dynamics (Visakorpi et al., 2021; Dostálek et al., 2018; Zvereva et al., 2012), including via depressed photosynthetic rates that can extend well beyond the area of removed leaf tissue and persist for days after damage (Zangerl et al., 2002). We did not find a significant relationship between herbivory and photosynthetic efficiency, but we do not know how much time passed between when leaves were attacked and when we took our measurements, so photosynthetic yields may have been temporarily reduced. We did find that lupine leaves with at least 5% area loss to insect herbivory had a reduced ability to harmlessly dissipate high levels of incoming radiation and directed a greater proportion of excess energy towards pathways that have the potential to cause photo-oxidative damage to chloroplasts (Fig. S2.2). Given the high levels of solar irradiation experienced by plants above the tree line, an increased susceptibility to photoinhibition could have important effects on lupine performance.

We therefore believe existing levels of grasshopper herbivory may contribute to increased stress and lower productivity for subalpine lupines, especially among plants growing at the very highest elevations, where both herbivory and abiotic stress are high. Addressing the potential fitness effects of herbivory on lupines is beyond the scope of our work, but in light of the high rates of pre-dispersal seed predation by invertebrates (affecting 42% of all the lupine pods we collected), achieving reproductive success for these plants likely requires intensive energetic investments. It is worth noting that the total impact of insect herbivory is greater than what we present, since lupines also experience losses that we did not monitor, including other types of leaf damage (e.g. skeletonizing and mining) and direct nutrient losses due to sap-sucking by aphids. We frequently observed large numbers of the latter on our study plants.

2.4.2 *Herbivory increases with elevation*

Mean and minimum daily air temperatures in subalpine meadows on Mt. Rainier decrease predictably with elevation (Fig. S2.4, Ford et al., 2013). Despite this, leaf area loss at our sites was significantly higher, rather than lower, at higher elevations (Fig. 2.1, Table 2.1). This result was consistent over both transects and all three years of data collection. The increase in herbivory with elevation we observed was in line with a sizable minority—22%—of the studies of elevational gradients and plant-insect herbivore interactions reviewed by Moreira et al., 2018. We offer three potential explanations for this pattern below.

2.4.2.1 *Snow cover and elevation interact to constrain grasshoppers*

We were struck by the fact that lupines growing in late-melting plots saw greater leaf area losses (Table 2.1). In an experimental study in an arctic habitat, delayed dates of snowmelt similarly led to increased insect herbivory on dwarf birch, and the authors speculated that longer insulation from snow encouraged winter soil microbial activity, increasing nitrogen availability and making leaves more palatable (Torp et al., 2010). It is possible that this is a factor in lupines as well (but see section *2.4.2.3*), and/or that delayed snowmelt in plots within an elevation causes plants with tender young leaves to emerge when grasshoppers at that elevation are already actively feeding. However, we were even more interested in the fact that there was an interaction between elevation and snowmelt timing such that the timing of snowmelt mattered more at higher elevations. On Mt. Rainier, the average time between snowmelt and the return of snow cover is a full 32 days shorter at our highest-elevation site vs. the lowest. This sets tight boundaries on the development time available to high-elevation grasshoppers.

Previous work has shown that alpine grasshoppers faced with time and temperature constraints may speed up development (Berner et al., 2004), for example via greater metabolic sensitivity to temperature and resulting increases in feeding rates. In our experiments, high-elevation *P. rainierensis* consumed at higher rates than their low-elevation conspecifics at all temperatures (Fig. 2.2a), despite being similar in body size, and might exhibit greater thermal dependence of feeding (Fig. 2.2b). These patterns match those found in Colorado populations of *Melanoplus boulderensis*, a cold-adapted grasshopper in the same subfamily as *P. rainierensis* in which high-elevation populations exhibited higher feeding rates than their low-elevation conspecifics at all temperatures and had increased thermal sensitivity of feeding rates (Buckley and Nufio, 2014). We believe high-elevation grasshoppers on Mt. Rainier may behave similarly, feeding more rapaciously because they are racing against time to complete their development and achieve reproductive maturity in the face of short, cool growing seasons.

2.4.2.2 *Solar radiation at high elevations may offset cool air temperatures*

In the field, behavioral thermoregulation can powerfully affect the body temperatures grasshoppers are able to achieve. Because of the seasonal constraints described above, high-elevation grasshoppers may be under especially strong selection for taking advantage of warm temperatures by basking for long periods of time in sunny areas, which can allow them to speed up digestion and facilitate higher rates of consumption (Buckley and Nufio, 2014). Therefore, air temperature alone—especially if measured in the shade—may be limited in its power to explain patterns of insect herbivory in open montane habitats. Not only does the intensity of solar radiation increase with elevation (Germino, 2014), but canopy cover decreases as well (Fig. S2.3). Higher-elevation sites are therefore less shaded and provide greater opportunities for thermal basking under higher irradiance. Canopy cover did not emerge as a significant predictor in our final herbivory model (Table 2.1), but that analysis was performed at the plot level, and grasshoppers may take advantage of sunny areas to raise their body temperatures before or after feeding (e.g., to speed digestion), even if their host plants are growing in shaded plots.

2.4.2.3 *Nitrogen limitation may lead to compensatory feeding*

We hypothesize that herbivory levels in our system may be partly driven by a type of compensatory feeding, in which herbivores consume larger amounts of relatively low-quality food to balance their nutritional needs (e.g., Berner et al., 2005). It is generally believed that nitrogen is the main limiting nutrient for insect herbivores. A meta-analysis of leaf functional traits along elevational gradients suggests that despite mixed results from individual studies, in general C:N ratios should increase with elevation (Read et al., 2013), leading to lower nutritional quality for invertebrates at higher elevations. This is in fact what we found (Fig. 2.3b). Since lupines are a nitrogen-fixing species, they are already among the most nitrogen-rich host plants available at our study sites, making it unlikely that grasshoppers can resolve their need for nitrogen by switching food sources. However, they may increase the total amount of lupine tissue they consume if the nutrition they gain from a given leaf mass decreases. This could be an important explanation for the high levels of herbivory we observed at higher elevations.

2.4.3 *Implications for climate change*

Montane plant species face challenging levels of abiotic stress. We found that subalpine lupines also encounter widespread insect herbivory, and that high-elevation plants lose more leaf area than low-elevation populations despite having better defended leaves (Figs. 2.3a and c). Given that insect feeding rates are an important driver of this interaction, and high-elevation grasshoppers feed faster at all temperatures, lupines at the range edge are likely to be even harder hit in the near term as summer temperatures warm and the number of heat waves in our region is expected to increase (Mauger et al., 2015). However, if climate change increases canopy cover and reduces opportunities for basking (as trees encroach into meadows), increases the length of growing seasons with earlier snowmelt, and/or increases soil microbial activity and thereby leaf nutritional quality, these may work to offset grasshopper appetites. One factor we did not measure is the strength of top-down controls of grasshopper populations like predation, pathogens, and parasitism; there is some evidence that these are generally stronger in warmer environments (Zhang and Adams, 2011). If this were true in our system, it would be consistent with the reduced leaf damage at lower elevations, and could mean that warming at higher elevations may eventually suppress insect herbivores via increased biotic controls.

Our results highlight the difficulty of predicting how montane plant-insect herbivore dynamics will respond to climate change in the long run, especially as we were unsuccessful in rearing our study species under laboratory conditions and were therefore unable to disentangle the relative proportion of either lupine or grasshopper phenotypes that could be attributed to local adaptation vs. environmental plasticity. We recommend that future work take on the challenge of conducting common garden experiments with multiple generations of field-collected plants and insects to better understand how climate change will shape these relationships in natural settings.

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2.6 STATEMENT OF DATA AVAILABILITY

Data and code associated with this study are available at:
https://github.com/mlsethi/lupine_grasshopper_interactions

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2.8 FIGURES AND TABLES

Fixed effects	**Estimate**	**CI**	**df**	**p**
(Intercept)	0.02299	0.01930 – 0.02669	650	<0.001*
Elevation	0.01955	0.01414 – 0.02496	86	<0.001*
Year [2017]	-0.00386	-0.00694 – -0.00078	650	0.015*
Year [2018]	0.00233	-0.00191 – 0.00657	650	0.284
Snow disappearance date	0.00362	0.00138 – 0.00586	86	0.002*
Time since snow disappearance	0.00779	0.00655 – 0.00902	650	<0.001*
Elevation:Transect[South]	-0.01660	-0.02223 – -0.01096	650	<0.001*
Elevation:Year [2017]	0.00227	-0.00104 – 0.00558	650	0.182
Elevation:Year[2018]	0.00719	0.00349 – 0.01088	650	<0.001*
Elevation:Time since snow disappearance	0.00344	0.00223 – 0.00464	650	<0.001*

Table 2.1: Estimated coefficients, confidence intervals, estimated degrees of freedom, and significance values for our final (best-fitting) herbivory model, which had the following formula: Herbivory ~ Elevation + Transect + Year + Snow disappearance date + Time since snow disappearance + Elevation:Transect + Elevation:Year + Elevation:Time since snow disappearance. Plot ID was included as a random effect. The marginal R^2 was 0.739 and the conditional R^2 was 0.920. The BIC and log-likelihood for this model were -3397.39 and 1768.15 respectively, compared to -2829.49 and 1477.57 for the global model (Appendix A: Table A1).

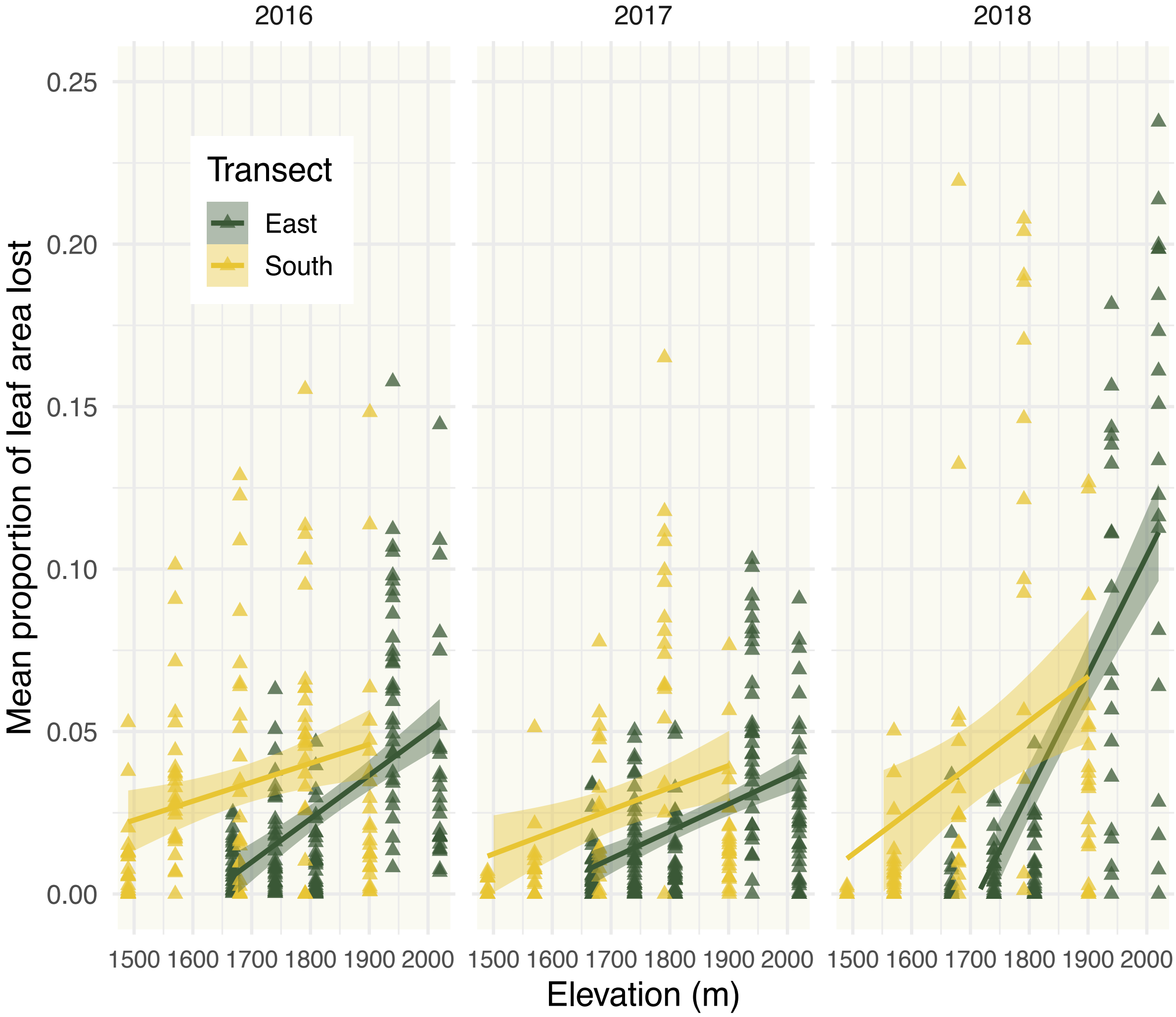

Figure 2.1: Estimated mean proportion of leaf area lost to grasshopper herbivory. Each data point represents the median leaf area lost across all lupine clumps in a given plot on a given day of observation.

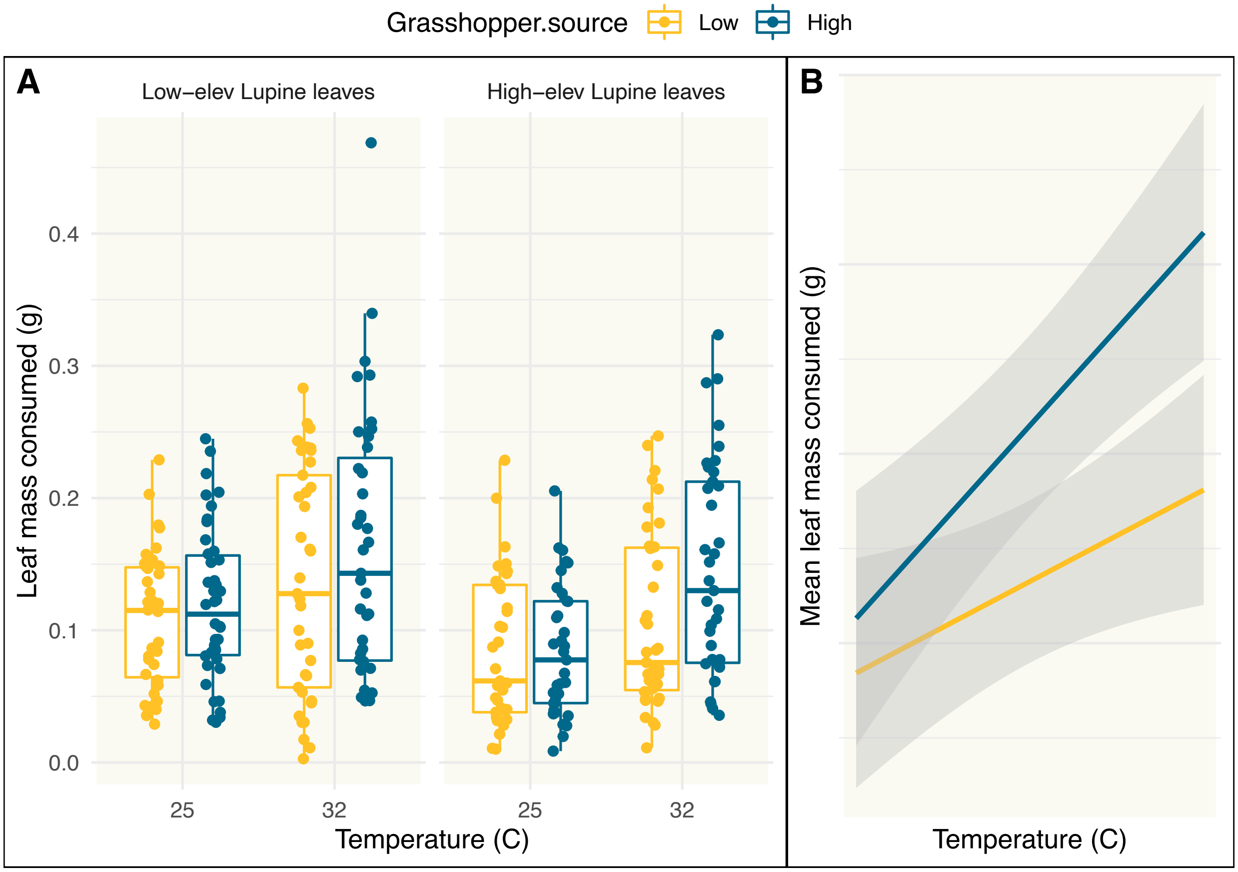

Figure 2.2a: Results of our experimental feeding trials; boxplots show means and quartiles. **2.2b**: Interaction plot showing mean leaf mass consumed by high vs. low elevation grasshopper populations at different temperatures.

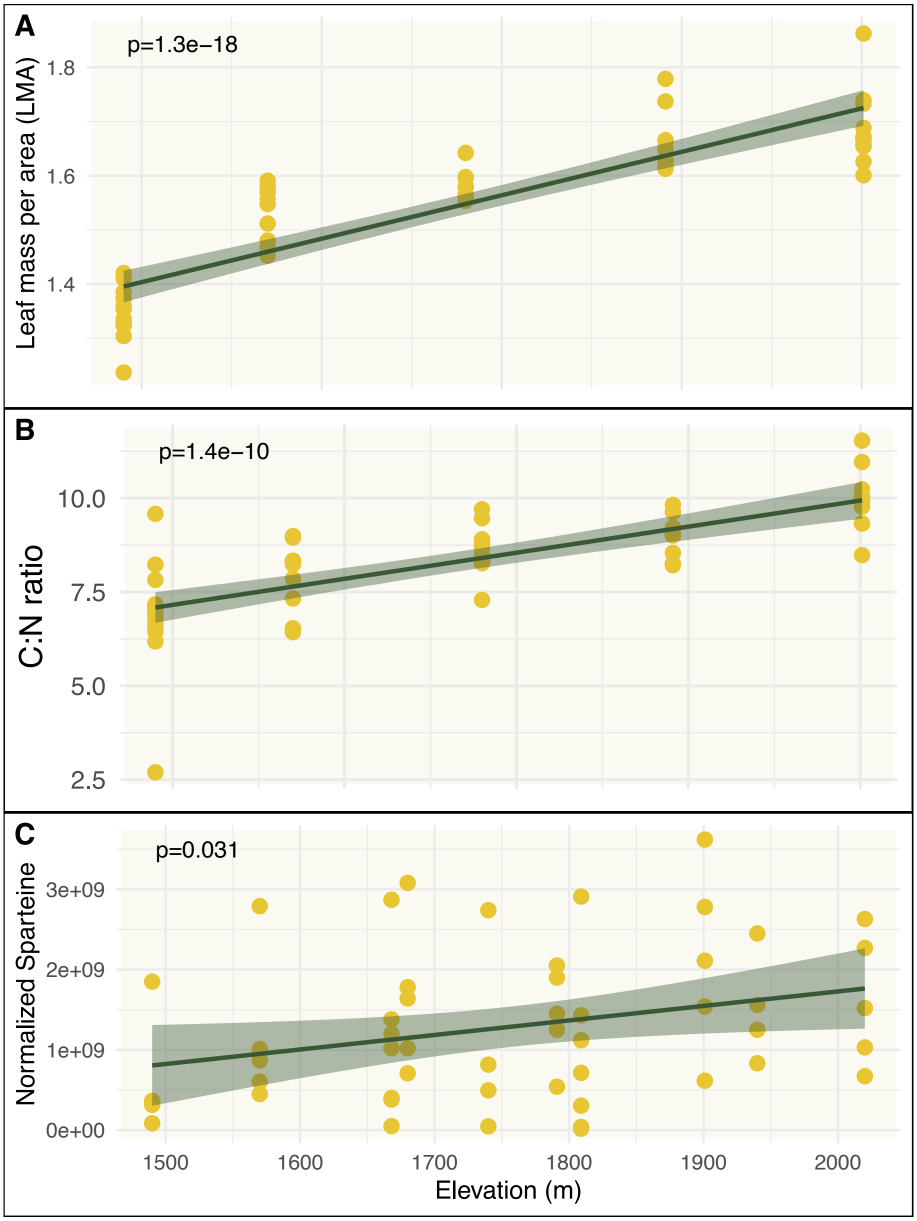

Figure 2.3: Elevational patterns in leaf traits: A (LMA), B (C:N ratio), C (Normalized sparteine concentration).

2.9 SUPPLEMENTARY MATERIALS

Fixed effects	**Estimate**	**CI**	**df**	**p**
(Intercept)	0.02183	0.01327 – 0.03039	647	<0.001*
Elevation	0.01687	0.00734 –0.02641	82	0.001*
Transect	0.00824	-0.00521 –0.02170	647	0.232
Year [2017]	-0.00821	-0.01549 – -0.00092	647	0.029*
Year [2018]	0.02402	0.01404 –0.03400	647	<0.001*
Snow disappearance date	0.00902	0.00481 –0.01323	647	<0.001*
Time since snow disappearance	0.01452	0.01202 –0.01703	647	<0.001*
Canopy cover	0.00319	-0.00470 –0.01107	82	0.429
Elevation:Transect[South]	-0.01505	-0.02670 –-0.00340	647	0.013*
Elevation:Year [2017]	-0.00379	-0.00950 –0.00191	82	0.197
Elevation:Year[2018]	0.02828	0.02131 –0.03526	82	<0.001*
Elevation: Snow disappearance date	0.00088	-0.00224 –0.00400	647	0.585
Elevation:Time since snow disappearance	0.00326	0.00089 –0.00563	647	0.008*
Elevation:Canopy cover	-0.00075	-0.00646 –0.00496	647	0.796
Transect[South]:Canopy cover	-0.00070	-0.01275 –0.01134	647	0.090
Transect[South]:Year[2017]	-0.00254	-0.01559 –0.01051	82	0.706
Transect[South]:Year[2018]	0.00313	-0.01091 – -0.01718	82	0.665

Table S2.1: Estimated coefficients, confidence intervals, estimated degrees of freedom, and significance values for our global (most complex) model, which had the following formula: Herbivory ~ Elevation + Transect + Year + Snow disappearance date + Time since snow disappearance + Canopy cover + Elevation:Transect + Elevation:Year + Elevation:Snow disappearance date+ Elevation:Time since snow disappearance + Elevation:Canopy cover + Transect: Canopy cover +Transect:Year. The marginal R^2 was 0.405 and the conditional R^2 was 0.530.

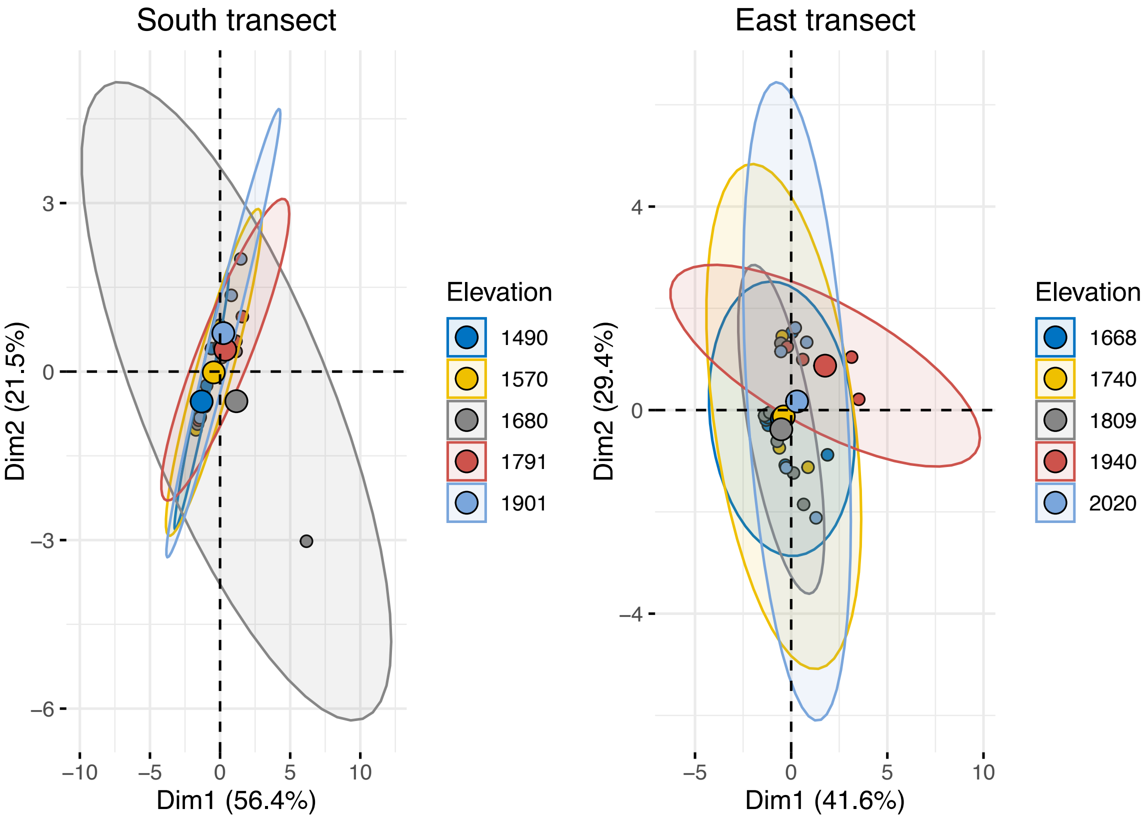

Figure S2.1: Principal Component Analysis (PCA) plot of alkaloid composition by elevation and transect, showing the first two dimensions of the analysis. There was no obvious clustering of the overall shape of chemical defenses by elevation on either transect.

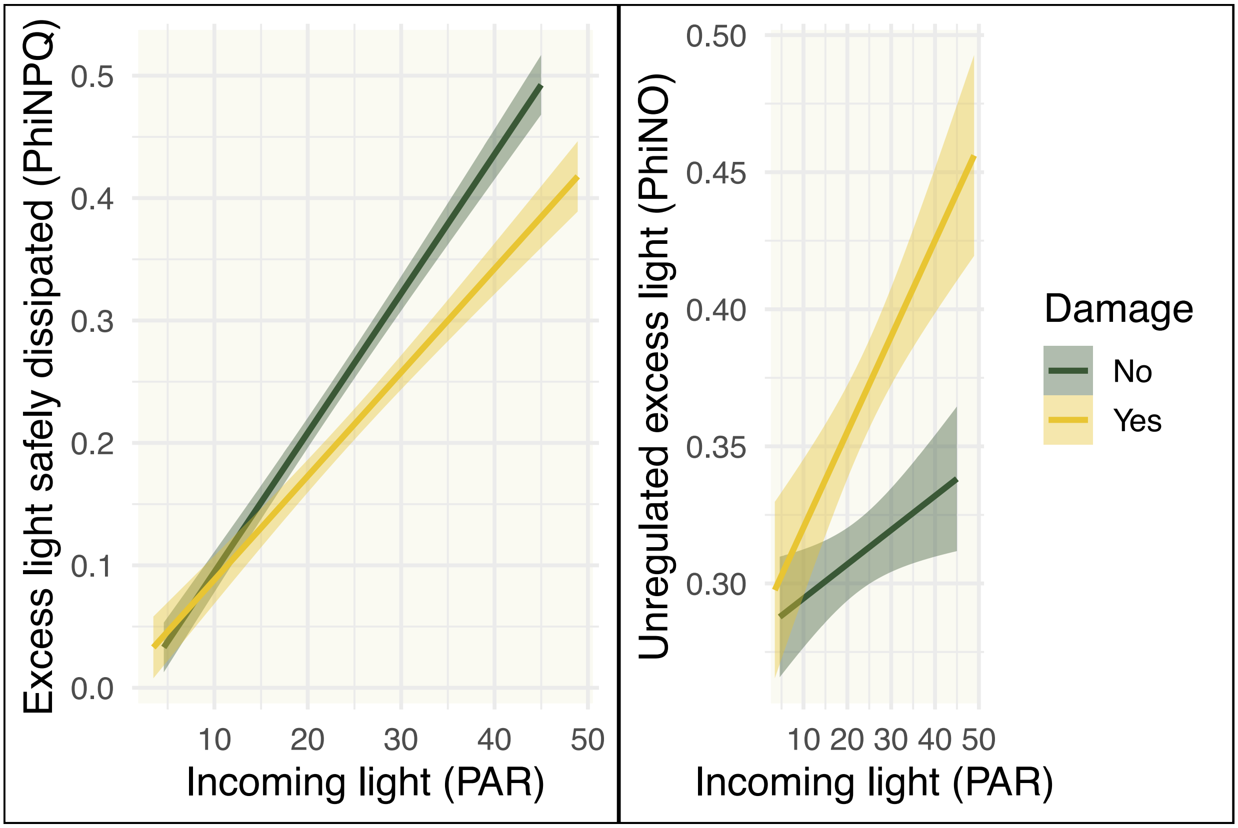

Figure S2.2: Effect of herbivory damage on the amount of excess photosynthetically available radiation (PAR) that is safely dissipated through nonphotochemical quenching (A) vs. that is passed to nonregulated, potentially harmful photoinhibitive processes.

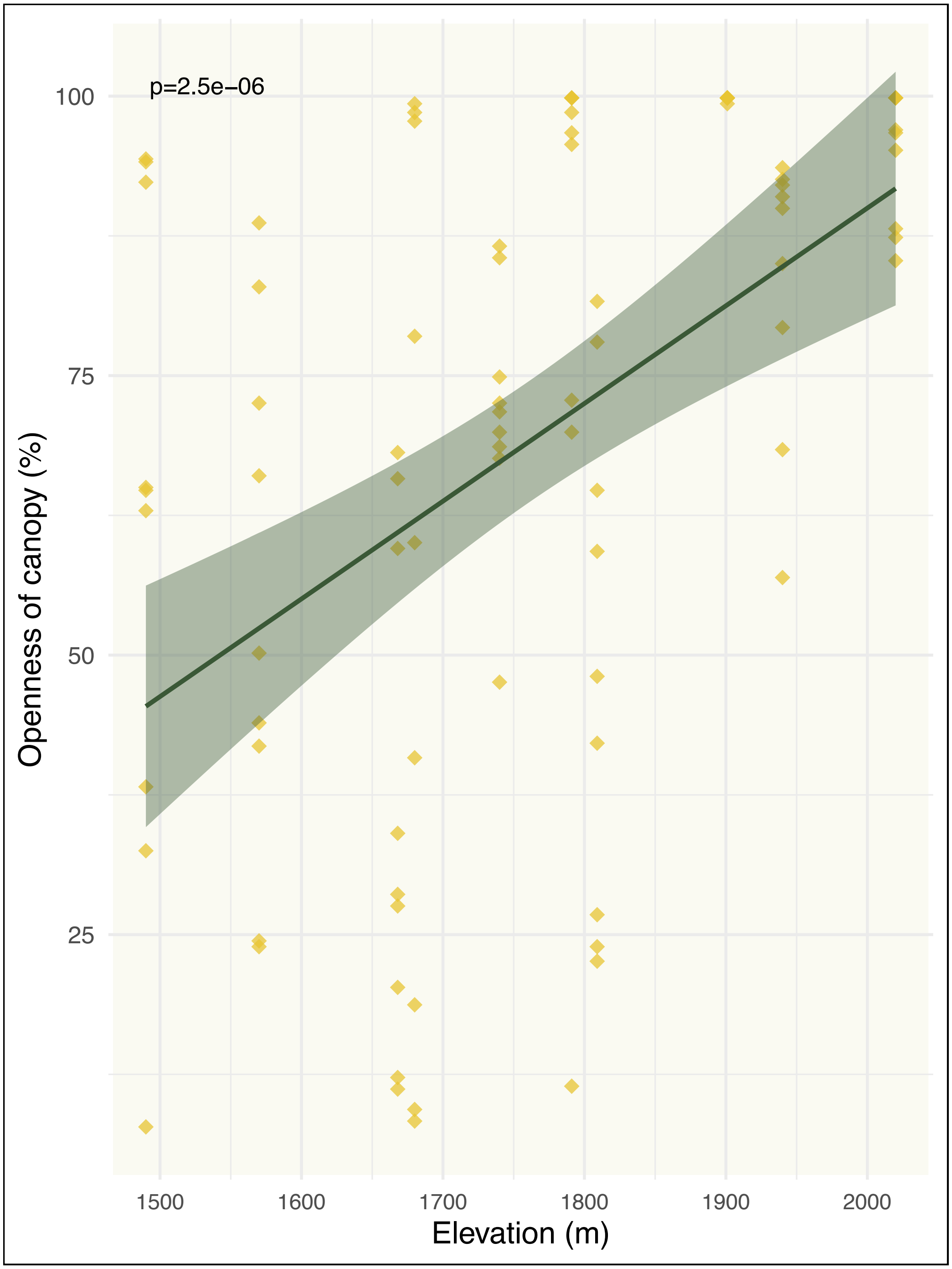

Figure S2.3: Openness of the canopy (%) above study plots by elevation.

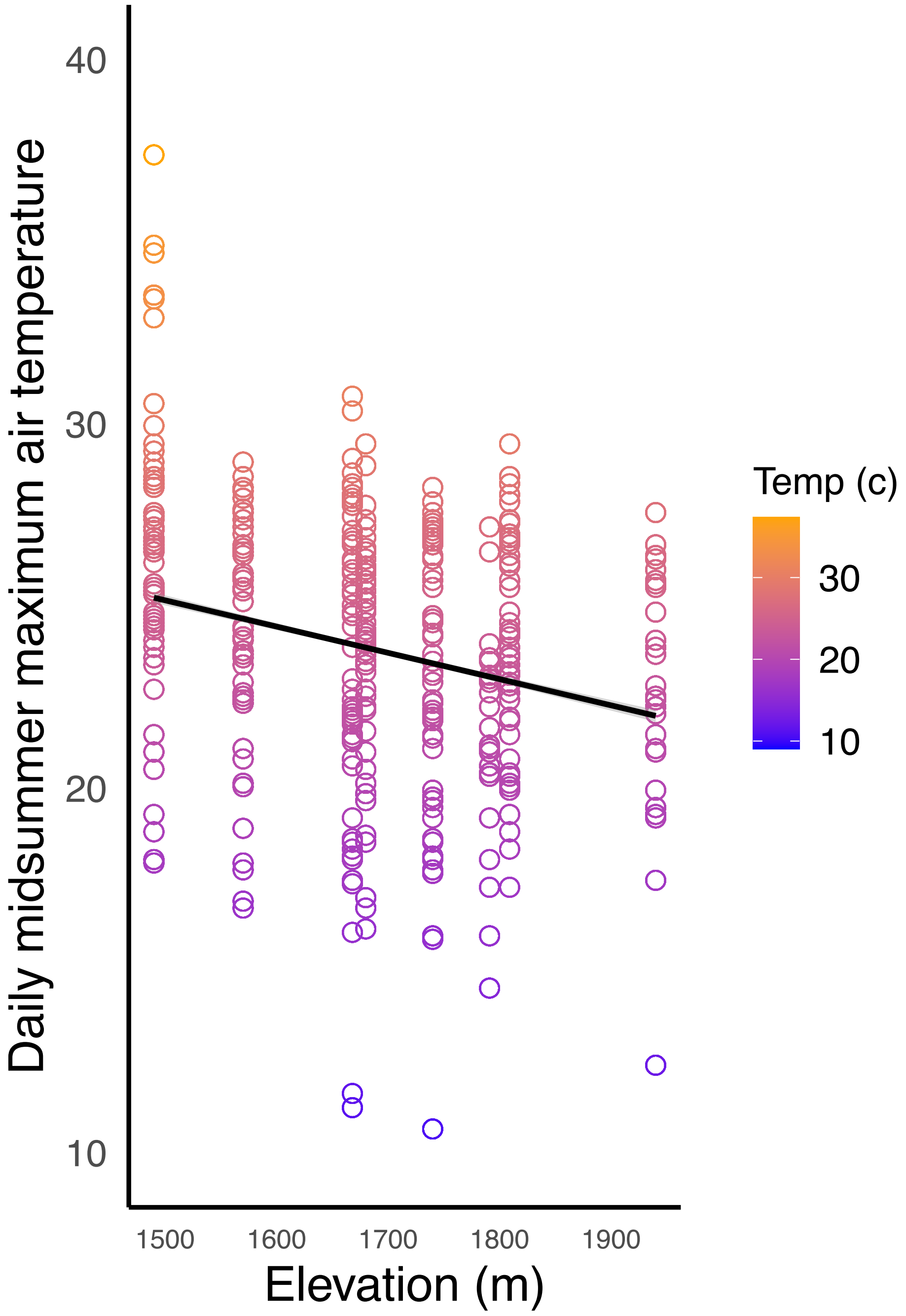

Figure S2.4: Daily midsummer maximum temperatures by elevation.

Appendix 2: Lupine alkaloid analysis

Fresh, undamaged lupine leaves were collected at field sites, immediately flash frozen in liquid nitrogen, brought back to the lab and stored at -80°C until further processing. For analysis, exactly 30 mg of leaf material was weighed out into a 2 ml Eppendorf vial and homogenized in a ball mill (Retsch) under liquid nitrogen. The homogenate was transferred to an 8 ml glass vial, to which 1 ml of chloroform (containing 0.05 mg caffeine as an internal standard) and 1.5 ml of 1N HCl were added. The vial was capped, the contents mixed vigorously for 15 min in a multi-tube vortexer (ThermoScientific), and the mixture centrifuged at 3,000 x g (Eppendorf 5810R). The upper aqueous phase was removed to a new glass vial. An additional 1.5 ml 1N HCl were added to the vial with the precipitated plant material, and the mixture re-extracted and centrifuged as before. Again, the upper phase was removed to a new glass vial. The combined acidic aqueous phases were titrated to pH 9 by stepwise addition of 14.8 M ammonium hydroxide. The slightly alkaline aqueous extract was extracted against 1 ml chloroform for 15 min on a multi-tube vortexer and centrifuged at 3,000 x g. Finally, the lower chloroform phase was carefully removed and transferred to a 2 ml screw-cap glass vial. A 1 µl aliquot of the resulting extract was injected onto a gas chromatograph (Agilent 6890N) connected to a mass selective detector (Agilent 5973inert) and processed under the following conditions: injector, 250°C, split ratio 1:1, split flow rate 1 ml/min; column, HP-5MS (Agilent), 30 m x 0.25 mm x 0.25 µm, flow rate 1 ml/min; oven, 150 – 180°C at 3°/min, 180 – 275°C at 40°/min, 5 min final hold; transfer line, 250°C; source temperature, 230; quadrupole temperature, 150; electron ionization mode; acquisition of data for m/z 50 – 550 at 2.9 scans/s; data acquisition software Agilent HassHunter 10.0.368; data processing software, Agilent ChemStation F.01.03.2357. Peak annotation was performed by comparing retention times and mass spectra with those of authentic standards or database records (tandem mass spectrometry library of the National Institute of Standards and Technologies, version 2.3). We performed two gas chromatograph runs; the first involved a direct injection to capture alkaloids with smaller peak areas, while we split the sample for the second run to analyze sparteine (the most abundant peak). Before statistical analysis, peak abundances were normalized to a consistent response factor based on the intensity of the internal standard peak (caffeine).

Chapter 3. Impacts of Habitat Restoration on Invertebrates: A Global, Pan-Taxonomic Meta-Analysis

ABSTRACT

Long-term datasets are providing increasing evidence of precipitous declines in the populations of many of the earth's estimated 10 million species of invertebrates. Human land use changes—especially the intensive conversion of natural habitat for agricultural purposes—are generally implicated as by far the most important driver of what some consider an insect biodiversity crisis. The value of habitat restoration efforts to invertebrates rests on the assumption that recovering degraded plant communities will increase the availability of foraging, nesting, or other resources for their animal interactors, reconnect isolated populations, and ultimately facilitate the return of complex ecological assemblages. Yet while there has been an increasing interest in assessing invertebrate responses to vegetation restoration, either for their own sake or as biological indicators of recovery, no study that we know of to date integrates global data on restoration impacts across invertebrate taxa. We therefore conducted a meta-analysis to assess the impact of habitat restoration on invertebrate populations. Results from 59 studies reporting measures of abundance, species richness, diversity and/or evenness in response to restoration suggest overall positive impacts on invertebrate fauna, with latitude, habitat type, restoration approach, and taxa of interest contributing to determining the success of these efforts. However, high among-study heterogeneity remained even after conducting meta-regressions with explanatory variables as moderators, making general conclusions difficult.

3.1 INTRODUCTION

In insects, argued the British naturalists William Kirby and William Spence in their 600-page 19th-century introduction to entomology, “nature...has combined and concentrated almost all that is either beautiful and graceful, interesting and alluring, or curious and singular in every other class and order of her children.” (Kirby & Spence 1856). Apart from the close attention it pays to their intrinsic charm and complex natural histories, the volume also devotes two chapters to what we would now describe as the essential roles played by invertebrates in many ecosystem functions. These include “promoting the fertilization of plants” (pollination), “serving as food for other animals” (trophic transfer), and “removing nuisances and deformities” (decomposition)—all of which, along with other services, have been well documented by modern ecologists (Noriega et al. 2018; Yang & Gratton 2014). It is truly old news, then, that threats to insect biodiversity and abundance are likely to have important impacts on trophic networks and the overall stability of ecosystems (Soliveres et al. 2016; Stout and Finn, 2015). Over the past several decades, a series of long-term datasets have been providing increasing evidence that we are in fact witnessing precipitous declines in the populations and distribution ranges of many of the earth’s estimated 14 million species (Stork 2018) of terrestrial invertebrates (Wagner 2020; Sánchez-Bayo & Wyckhuys 2019; Hallmann et. al. 2017).

What has sometimes been described as a worldwide “insect apocalypse” in the popular media (e.g. Jarvis, 2018) is a more complex phenomenon than that term implies, with some taxa clearly experiencing stunning losses (van Strien et al. 2019; Brooks et al. 2012) and others apparently increasing in abundance over time (van Klink et al. 2020; Herrera 2019). There have also been serious disagreements within the research community over how existing data should be analyzed and interpreted (Welti et al. 2021; Mupupele et al. 2019), and in general our understanding of the consistency of global patterns is stymied by gaps in insect biodiversity datasets (Rocha-Ortega et al. 2021). Montgomery et al. 2020 and Didham et al. 2020 both provide excellent reviews of the inherent research challenges in this area and offer clear roadmaps for addressing them in the future. But even the incomplete view we have now suggests that for many ecologically, economically, and culturally important invertebrate species—not limited to a single taxon, habitat type, or region of the world—the declines are real, and will continue if no actions are taken (e.g. Guo et al. 2019; Powney et al. 2019).

We already know that a multitude of hazards are acting in concert that demand different conservation interventions. Among other threats, for example, terrestrial arthropods are vulnerable to the direct and indirect effects of climate change (Harris et al. 2019; Warren et al. 2018); increasing levels of light pollution (Owens et al. 2020); antagonistic interactions with introduced species (Tallamy et al. 2020); and even, despite their small size, the risk of roadkill (Martin et al. 2018). But the leading cause of invertebrate biodiversity declines by far is habitat loss and degradation, especially through the conversion of natural plant communities to serve human purposes such as agriculture or development (e.g. Theng et al. 2020; Seibold et al. 2029; Bommarco et al. 2012; Fattorini 2011; reviewed in Samways 2017).

Habitat restoration—that is, activities that activate or expedite the recovery of degraded ecosystems, usually with a focus on restoring native vegetation—is therefore an important component of invertebrate conservation (Samways et al. 2020; Shuey 2013). The use of restoration as a tool for protecting wildlife in general is predicated on the assumption that helping plant communities recover via one or more of the following pathways: increasing the availability of foraging, nesting, or other resources, reconnecting populations that had been isolated through fragmentation, and facilitating the formation of more complex networks of interacting organisms (Winterhalder et al. 2004; Shuey 2013). And we typically expect restoration to have positive impacts on invertebrate populations even when such projects are not specifically undertaken to benefit them, so much so that ants and other easily collected terrestrial arthropods are regularly used as presumptive indicators of restoration success (e.g. Casimiro et al. 2019; Riggins et al. 2009; reviewed in Borges et al. 2021 and Majer, 2009). In fact, a recent report of 301 papers evaluating the success of ecological restoration projects found that 48 used the abundance or richness of terrestrial arthropods as an outcome measure, vs. only 34 that used vertebrates (Wortley et al., 2013).

A number of attempts to synthesize this growing body of data via meta-analyses have been made from the perspective of specific insect taxa. For example, Tonietto & Larkin 2017 found that restoration generally increased the abundance and richness of wild bees across the board, while Casimiro et al. 2019 found more complex responses among ant communities, with functional group richness recovering more quickly than species richness and “natural” (i.e. passive) regeneration having a more positive effect than active restoration efforts. However, we are aware of no study to date with a global, pan-taxonomic scope that integrates all available data from across the world about restoration impacts on any terrestrial arthropod taxa. Here we describe the results of such a meta-analysis in which we asked three main research questions:

1) Are invertebrate biodiversity measures higher in restored sites than unrestored sites?

2) Are invertebrate biodiversity measures similar in restored sites and reference sites?

3) Do either of these outcomes differ based on either intrinsic factors (e.g. latitude, habitat type) or factors specific to the restoration itself or its assessment (e.g. restoration approach, time since restoration, invertebrate sampling methods)?

We were interested in these questions because their answers could provide important insights about how to plan restoration efforts in order to yield the largest returns on investment when it comes to invertebrate conservation, and potentially highlight instances where restoration is unlikely to have a significant rescue effect. In addition, we wanted to identify potential coverage inconsistencies (e.g. geographic and taxonomic biases) and make recommendations that we hope will improve study design and reporting in future empirical evaluations of restoration success involving invertebrate outcomes.

3.2 METHODS

3.2.1 *Data collection*

We carried out an extensive search of the literature using Web of Science (Clarivate Analytics, 2015) on 21 October 2019 using the following topic search: (("habitat restoration" OR restor* OR rehabilitat* OR reforest* OR afforest* OR "seed addition" OR abandon* OR "old fields" OR fallow) NEAR (habitat OR grassland OR meadow OR forest OR prairie) AND (invertebrat* OR insect* OR arthropod* OR pollinat* OR butterfl*) NOT (marine OR benthic OR river* OR insectivor*)). This search yielded 1,345 papers. Additionally, we scanned the first 35 pages of Google Scholar search results using the same combination of terms, and searched amongst dissertations, theses, conference papers and proceedings, and government records in the Agricultural & Environmental Science Collection. These searches yielded 19 additional results that were not duplicates. Finally, we put out a call for unpublished data on social media and directly emailed several researchers whom we thought would be likely to have relevant unpublished data, resulting in an additional 8 datasets. We read the titles and abstracts (or reviewed metadata in the case of unpublished datasets) for all 1,372 studies returned from the literature search and excluded any that were not clearly assessments of restoration outcomes with a scope that included invertebrates. This left us with 201 studies for which we read the full text.

To be retained for our analyses, studies had to report at least one quantitative metric of invertebrate population or community health that was collected in **restored vs. unrestored** (disturbed) sites, or in **restored vs. reference** (pristine) sites. In order of most to least commonly reported, these measures included abundance (or proxies for abundance, such as activity density or signs of insect herbivory), species (or morphospecies) richness, species diversity, and species evenness. With dismay, we excluded a number of otherwise relevant studies reporting an array of more complex measures of community similarity or turnover between sites, such as Bray-Curtis similarities, Renkoken similarities, Morisita's similarities, or Jaccard dissimilarities, because it would not have been meaningful to average these diverse metrics (Hekkala and Roberge 2018). Finally, we excluded studies that reported only modeled (i.e. predicted) effects on biodiversity, or where some elements required for a weighted analysis were not clearly reported in the paper (most commonly, sample sizes or variances were missing) and the authors failed to respond to our requests to provide them. We were able to include a total of 59 studies published between 1996 and 2019 in 30 scientific journals (see Appendix 3.1). The PRISMA diagram in Figure 3.1 summarizes our study assessment and selection process, and Appendix 3.1 lists the citations of all included studies.

3.2.2 *Statistical analysis*

For our effect size metric we selected Hedge's g, a standardized mean difference that is commonly used in ecological meta-analyses of continuous outcomes. This measure has several desirable characteristics, including that it is able to deal with mean effect sizes of 0, and that it corrects for small sample sizes, which was common in our dataset (mean n=8). When Hedge's g was calculated as the mean difference between restored and unrestored sites, positive values indicated presumptive improvements in invertebrate population metrics as a result of restoration. When it was calculated as the difference between restored and reference sites, values close to zero indicated presumptive success in terms of restoration bringing sites closer to their "original" or desired state. Hedge's g requires means, sample sizes, and standard deviations, so we extracted these values for each outcome measure (invertebrate abundance, richness, diversity, or evenness) associated with each treatment (restored, unrestored, or reference) from the main and/or supplementary text and/or tables of each study. When such information was only available visually, we digitally analyzed figures using the WebPlotDigitizer tool (Rohatgi, 2019). Where necessary, we treated medians as means, and converted standard errors, interquartile ranges, or coefficients of variation to standard deviations. We calculated Hedge's g using the package "metafor" 2.4-0 (Viechtbauer, 2010) in R version 4.0.3 (R Development Core Team, 2020). Because many studies included multiple comparisons (e.g. both restored vs. unrestored and restored vs. reference sites, multiple restoration techniques, sampling types, or taxonomic groups of interest), we calculated a total of 535 individual effect sizes.

For each effect size, we also recorded the following attributes: Latitude; time since restoration in months; habitat type; restoration approach; invertebrate sampling type. When data from multiple time points were reported, we used only the values collected the longest after the time of restoration, given that our interest is ultimately in long-term outcomes. Habitat types were broadly categorized as either forests, grasslands, or wetlands, and sampling types as either passive (e.g. traps), active (e.g. hand-searching, transect counts), or a combination of both. We used the definitions proposed by Atkinson and Bonser 2020 for restoration approach, such that any restoration that included the reintroduction of multiple native species of vegetation via planting or seeding was considered “reconstructive,” any that consisted only of removing the source of disturbance (e.g. ending mining) and allowing natural succession to take over was considered “natural,” and any that did not involve planting or seeding but did include active interventions such as amending substrate or removing invasive species was considered “assisted.” Finally, we recorded the invertebrate taxon of interest at the level of order whenever possible, and at higher taxonomic levels if necessary (e.g. class Diplopoda for millipedes, subclass Acari for mites). Figure 3.2 shows various summary characteristics of the included studies.

3.2.3 *Overall effects of restoration on invertebrates*

In our first analysis, we used the *rma.uni()* function in “metafor” to estimate the “grand mean” effect size of all restored vs. unrestored comparisons, and of all restored vs. reference comparisons. Our goal here was to obtain a high-level assessment of the overall sign and size of the impact of habitat restoration on invertebrates across all studies and response types; i.e., summary answers to questions 1 and 2 above. However, we stress two important limitations of this analysis. First, it included 218 effect sizes extracted from 21 pseudoreplicated studies. In these studies, replicates consisted of multiple samples—traps, hand-searches, transect counts, etc.—that were collected from within the same restored, unrestored, or reference site, rather than samples collected from multiple sites within each treatment. This is a common problem in ecological studies in general and studies of restoration in particular, since many restoration projects only involve a single site. We did not want to lose the information contained in these studies, but because pseudoreplicated samples are not independent, they are likely to have lower variances, giving them higher weights (defined as the inverse of the variance) when effect sizes are pooled (Spake and Doncaster, 2017). To prevent these studies from having an outsize effect on the grand mean estimation, we used unweighted models for this analysis.

3.2.4 *Factors influencing the effects of restoration on invertebrates*

The second limitation of the "grand mean" estimation is that it did not take into account heterogeneity among studies, which can often be at least partially accounted for by conducting subanalyses and/or creating mixed-effects models that include explanatory variables. We therefore conducted eight subanalyses, one for each unique combination of biodiversity metric (abundance; richness; diversity; evenness) and comparison type (restored vs. unrestored; restored vs. reference). Since in this case we wanted to use weighted models, we limited our dataset to the 38 studies and 317 effect sizes with true replication. For each subanalysis we created a weighted mixed-effects meta-regression model using the *rma.mv()* function. We included as moderators (predictors) as many of the study attributes mentioned in section 3.2.2 above as possible, with effect sizes nested in studies as a random effect where possible. Our goal here was to assess whether the overall outcomes of restoration for invertebrates are moderated by any included study attributes (i.e., to answer question 3 above). For ease of interpretation, we re-coded the levels of each categorical predictor such that the level that was most common in the overall dataset was treated as the reference (baseline); thus, the reference level for habitat type was "forest," the reference level for restoration approach was "reconstructive," the reference level for sampling type was "passive," and the reference level for taxon was "multiple taxa."

For robust variance estimation and to avoid overfitting, the number of moderators we were able to include in each model was limited by the sample size of the subgroup. For example, when there were fewer than 5 studies (levels) represented in a subgroup, we did not include effect sizes nested in studies as random effects, and in all cases we ensured that the ratio of effect sizes to moderators was no lower than 3:1.

Since we knew heterogeneity was likely to be high even in the subanalyses, we chose to avoid model selection and present the results of the full model in each case, allowing for greater interpretability of the confidence intervals. After fitting each model, we examined it for remaining among-study heterogeneity. When significant effects were found, we also tested models for publication bias. We did this using visual inspection of funnel plots in which effect sizes were plotted against variance, as well as by calculating Rosenberg's weighted fail-safe number (Rosenberg, 2005), which is an estimate of the number of null findings that would have to be added to the analysis to change the results from significant to non-significant.

3.3 RESULTS

3.3.1 *Overall effects of restoration on invertebrates*

The "grand mean" difference in invertebrate outcomes when comparing restored to unrestored sites, considering all types of biodiversity responses collectively, was significantly positive (Figure 3.3a, mean Hedge's g: 1.539, 95% CI: 1.160 — 1.918, $p < 0.0001$). The "grand mean" difference in invertebrate outcomes when comparing restored to reference sites, considering all types of biodiversity responses collectively, was significantly negative (Figure 3.3b, mean Hedge's g: -1.089, 95% CI: -1.370 — -0.807, $p < 0.0001$). In other words, on a summary level, invertebrate biodiversity measures did appear to be measurably higher in restored sites than unrestored sites, but they also remained measurably lower in restored sites than reference sites. Tests of heterogeneity after both analyses showed significant dispersion between studies in terms of the scale of their effect sizes (Tau2 =5.52, Cochran's T =2945.16, and $p<0.001$ for restored vs. reference comparisons; Tau2 =6.78, Cochran's T =1994.16, $p<0.001$ for restored vs. reference comparisons). This was not surprising, given that in both cases there were substantial numbers of effect sizes with signs that were the opposite of the mean (i.e., restored sites with poorer biodiversity than unrestored sites, and restored sites with higher biodiversity than reference sites). Most of this was due to among-study heterogeneity (I2 = 93% and 95% respectively); that is, variance in "true" effect sizes from study to study.

3.3.2 *Factors influencing the effects of restoration on invertebrates*

Subgroup analyses supported the finding from our grand mean analysis that there was an overall positive effect of restoration on invertebrate outcomes when **restored sites were compared with unrestored sites**. Considering each type of biodiversity response separately, we continued to find significant positive mean effect sizes for abundance, diversity, and richness, but there was no significant effect of restoration on species evenness (Table S3.1, Figure 3.4a). Our mixed-effects models suggested that a few moderating factors might influence these effects (Table S3.1). Most notably, effect sizes from restoration projects in grassland habitats, as well as those focused on Coloeptera and Lepidoptera, were negatively associated with species richness. Other significant findings are unlikely to be interpretable given the low representation of the relevant factor level in the subgroup. For example, wetland habitats and studies of Diptera appeared to have significant effects on both abundance and richness, but these effect sizes were each associated with only two studies in the subgroup being analyzed.

The results of subgroup analyses in which **restored sites were compared with reference sites** complicated the suggestion from our grand mean analysis that on average, restored sites were significantly impoverished compared to reference sites in terms of invertebrate populations. When considering each biodiversity response separately, this was true only for evenness; we found no significant differences between restored and unrestored sites when it came to abundance, richness, and diversity (Table S3.2, Figure 3.4b). Again, our mixed-effects models suggested that moderating factors might influence these effects (Table S3.2); here, higher latitudes and the use of active sampling were both associated with higher abundance in restored sites. Higher latitudes were also associated with higher richness in restored sites, while grassland habitats and natural restoration approaches were associated with lower richness in restored sites. Again, some significant predictors were unlikely to be interpretable due to their low representation.

3.3.3 *Among-study heterogeneity*

Even after splitting our dataset into subgroups by response type and including both fixed and random effects as moderators, all of which we hoped would allow us to account for potential sources of variation in effect sizes, we continued to find significant among-study heterogeneity in every mixed-effects meta-regression model we ran ($p<0.0001$ for Cochran's T in all models).

3.3.4 *Assessment of publication bias*

We visually examined funnel plots from all subgroup analyses. These appeared generally symmetrical with no obvious signs of publication bias (e.g. "missing" studies with large standard errors and low effect sizes). However, while commonly used, this means of assessing the potential for publication bias is not considered reliable especially when sample sizes are small (Simmonds 2015). We therefore calculated Rosenthal's fail-safe number for each analysis that produced a significant mean effect, and used the one-sided 95% confidence limit suggested by Fragkos et al. 2014 as our cutoff point. This revealed that the comparisons of species richness and abundance between restored and unrestored sites were robust to potential publication bias (3,748 and 3,890 studies with null results respectively would be required to refute the finding of significant benefits of restoration to invertebrates). In contrast, the significantly higher species diversity in restored sites over unrestored sites, and the significantly lower evenness in restored sites vs. reference sites, were not robust to bias. In both cases, the number of studies (3 and 233) respectively—required to refute these findings were far below the 95% confidence limit.

3.4 DISCUSSION

Because we were unable to satisfactorily account for among-study heterogeneity in any of our analyses, we hesitate to present our results as meaningful general indicators of how successful habitat restoration is as a conservation strategy for invertebrates. Below, we offer high-level interpretations of our main study findings as they stand, and offer several recommendations for future research.

3.4.1 *Impacts of restoration on invertebrates*

Both our grand mean and subgroup analyses (Figures 3.3a, 3.4a) suggested that habitat restoration may work to "rescue" invertebrate populations from the degraded states they often enter after major disturbances to native vegetation have occurred, as theory predicts (Samways et al. 2020; Shuey 2013). The "grand mean" increase we found between restored and unrestored sites (estimated standardized mean difference = 1.54) was on the same scale as those found in a study of restoration on wild bees which also selected Hedge's g as its effect size (Tonietto and

Larkin, 2018). Meta-regressions confirmed significantly better outcomes in restored sites across three summary measures of biodiversity: abundance, richness, and diversity. We were interested in the finding that grassland habitat emerged as a significant negative predictor of species richness compared to forests, something which we also saw in restored vs. reference comparisons of richness and abundance. If these results reflect the reality on the ground, it may be due as much to the relative sophistication of our scientific understanding regarding these ecosystems as anything else. Trimble and van Aarde 2012 found a much higher number of studies per million km2 of forest biomes compared to other biomes in a systematic review of research on biodiversity in human-modified landscapes; if restoration ecologists lack knowledge about the responses of biodiversity to landscape change in grasslands, that could certainly explain part of this negative association. The fact that studies of Coleoptera and Lepidoptera were also negative predictors of species richness after restoration compared to studies involving multiple taxa is harder to parse, but may simply be connected to the fact that a setting a broader taxonomic scope could make it easier to achieve larger increases in richness.

Our grand mean analysis suggested that restoration efforts may not be attaining the ultimate goal of reconstructing the size and ecological richness of lost insect communities (Figure 3.3b), with restored sites failing on average to match the biodiversity outcomes measured in pristine, undisturbed sites. Our subgroup analyses resulted in more optimistic findings (Figure 3.4b). Here there was a shortfall only in terms of species evenness, but no significant differences in abundance, richness, and diversity between restored and reference sites. We were struck by the fact that higher latitude was positively associated with abundance and richness in these analyses, which stands in contrast to the finding from a meta-analysis of ecosystem restoration in general that found recovery was generally more complete and rapid in the tropics than at higher latitudes (Jones et al. 2018). Our finding that natural restoration negatively predicted richness in restored vs. reference sites is also inconsistent with previous findings that found such approaches equaled or bested reconstructive approaches (Shimamoto et al. 2018; Crouzeilles et al. 2017). Again, given the heterogeneity in our dataset, it is difficult to say whether this is actually associated with differential invertebrate responses compared to ecosystem health in general.

3.4.2 *Challenges and future opportunities*

3.4.2.1 *Data availability*

Given the high number of otherwise relevant studies that we were unable to include because the necessary data were inaccessible, perhaps the biggest recommendation we can make is that all parties involved in assessing restoration effects on invertebrates make their raw data publicly available. We are far from the first to make this call to restoration ecologists in particular (e.g. Majer, 2009), but add that we think it would be especially valuable for non-academic researchers (e.g. government agencies and non-profit conservation organizations) to deposit their observations in a repository such as Dryad. This shift alone would more than double the number of studies we would have been able to include in our present analysis. It would also provide for perfect standardization of research outcomes, as meta-analysts could compute their own response variables from the raw data. This would prevent erroneous comparisons such as confusion between species richness and species density (as pointed out by Spake and Doncaster, 2017). In addition, it would allow us to move beyond what Hekkala and Roberge 2018 call “low-informative” measures of biodiversity (e.g. richness, abundance), which remain the most highly reported and therefore the most likely to be synthesized, and toward “high-informative” measures like compositional or functional changes.

Besides the raw data on outcomes, we also call on researchers to increase the level of detail with which they describe basic characteristics of restoration efforts; in 28 out of 59 studies, the size of the restored area was not reported; in 13, it was not clear what entity was responsible for the restoration. These were both factors we were extremely interested in because of their relevance to conservation practice. In addition, key environmental covariates associated with study sites (e.g. elevation) were rarely, if ever, reported. The more variables authors share about their study sites, the more likely it is that among-study heterogeneity can be accounted for.

3.4.2.2 *Study design*

The problem of pseudoreplication cannot always be avoided, especially if a post-hoc study is being conducted on a single restoration site. However, wherever possible, we urge researchers to compare multiple restored, unrestored, and/or reference sites to avoid spatial autocorrelation between samples.

3.4.2.3 *Conclusions*

We originally entered into this meta-analysis because we believed it had great potential to improve our understanding of the impacts of habitat restoration on invertebrate populations, and to provide practical insight into the value of restoration efforts at a time when conservation interest in insects and other arthropods is perhaps higher than ever. To some extent, our findings are hopeful; they suggest that many restoration projects are, in fact, improving invertebrate biodiversity, and in some cases may bring degraded sites closer to their undisturbed states. Ultimately, though, the results of this work make it clear that given the state of the literature today, taking a global, pan-taxonomic scope may be too broad to allow us to compare across studies and draw reliable conclusions. This is especially true given as among-study heterogeneity increases, so does the minimum sample size required to include a robust suite of predictors to account for multiple sources of variation. Therefore, apart from the recommendations above, we urge that more attention be paid by restoration scientists to underrepresented taxa, geographic locations, and habitat types (Figure 3.2), so that the few valuable studies that do already exist can be placed into their proper context.

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3.6 FIGURES

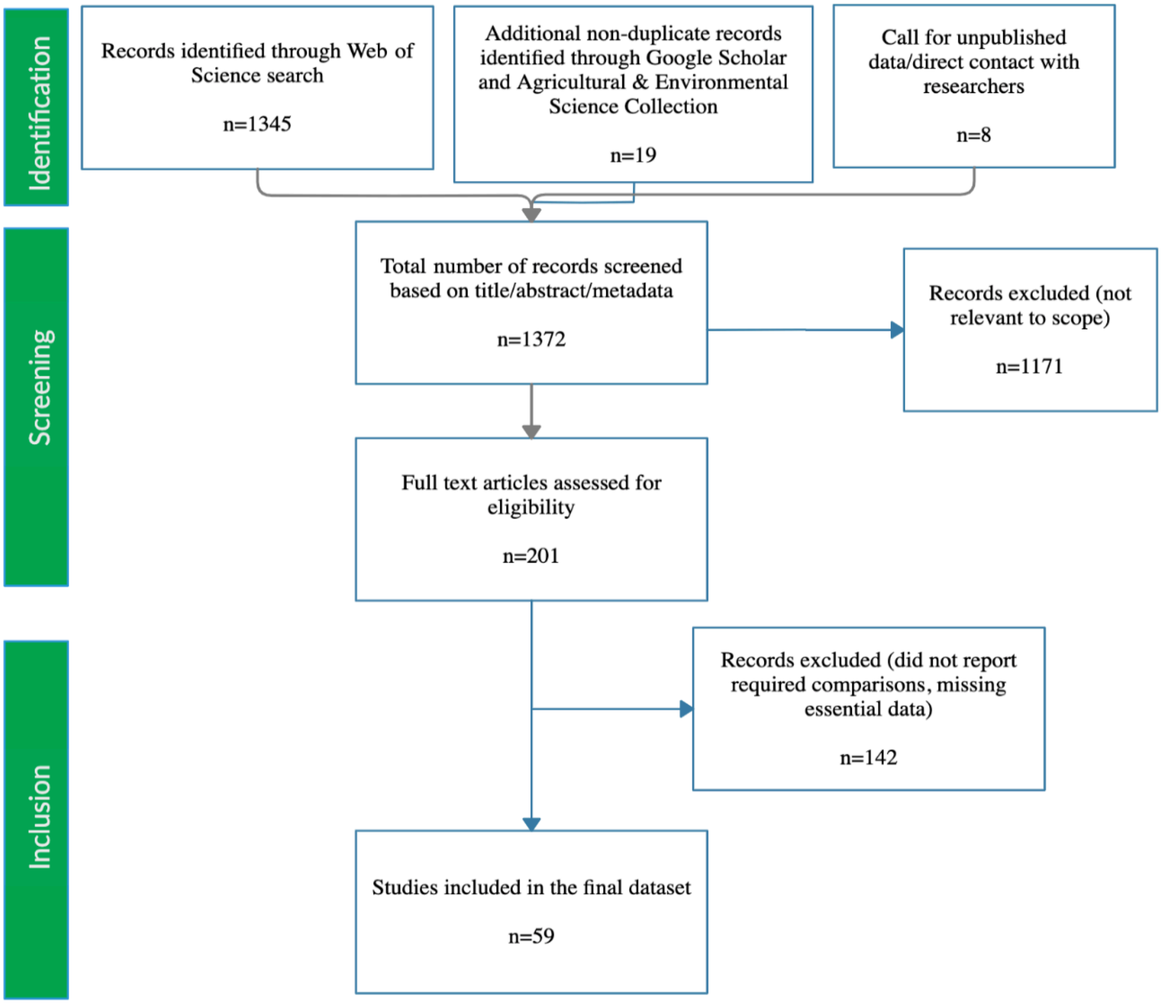

Figure 3.1: PRISMA diagram showing the flow of our literature search and study selection process.

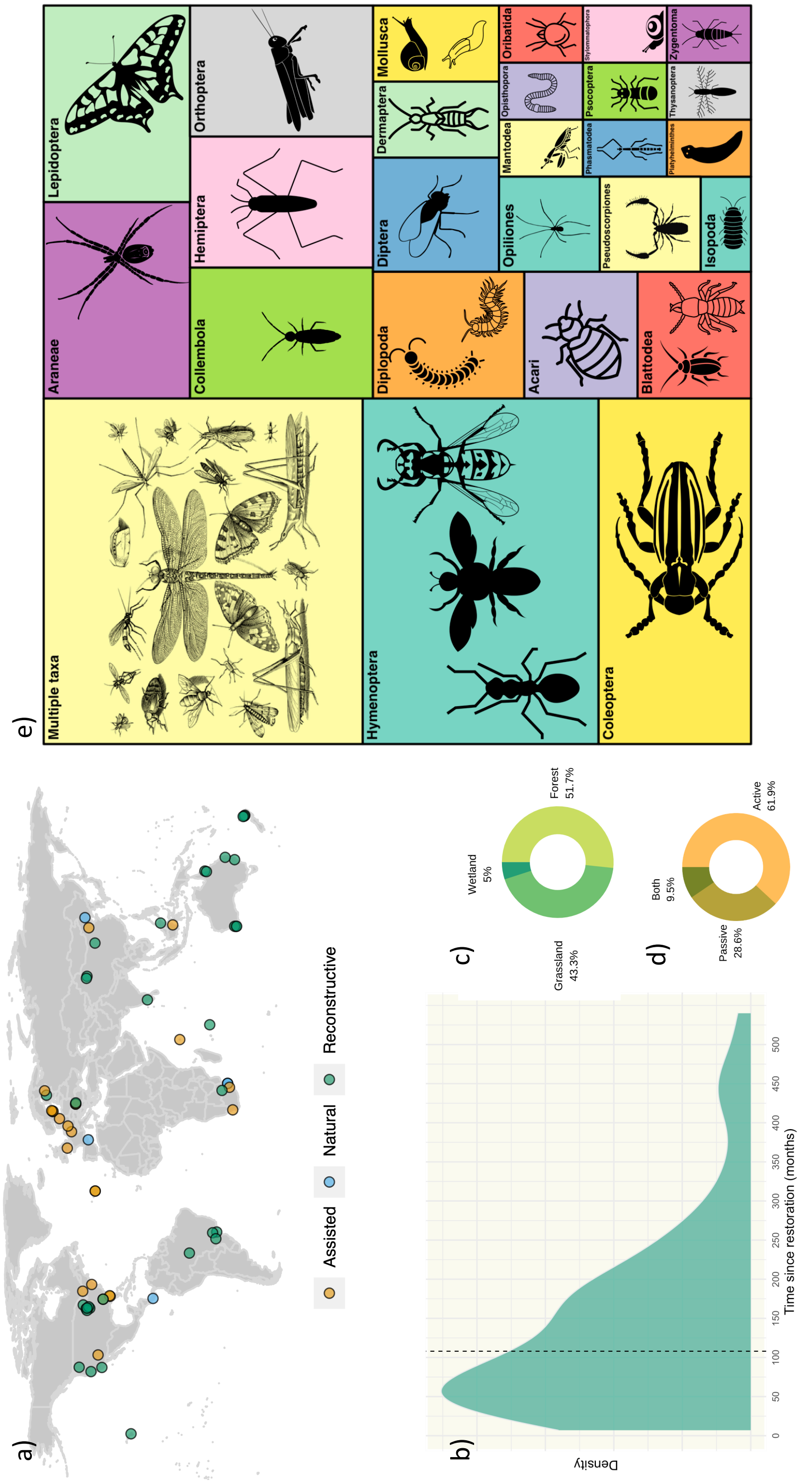

Figure 3.2: Summary characteristics of included studies, including a) geographic locations and restoration approaches; b) time since restoration, with the dotted line showing the median of 108 months (9 years); c) habitat types; d) sampling types; and e) taxonomic groups; the size of boxes in the tree map correspond to the number of effect sizes relating to the taxa in question. "Multiple taxa" includes effect sizes focusing on functional groups.

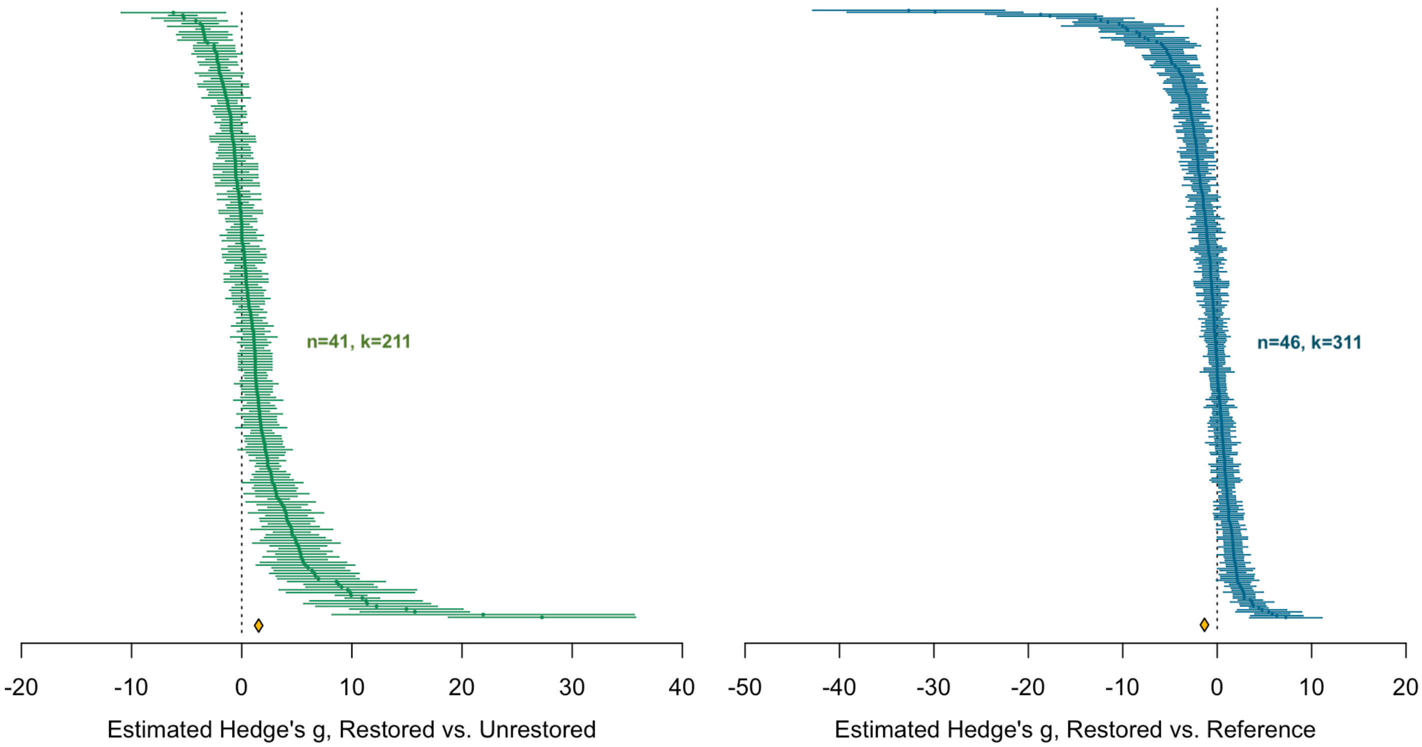

Figure 3.3: Forest plot showing the results of an analysis of a) restored vs. unrestored sites, and b) restored vs. reference sites, including pseudoreplicated studies. Golden diamonds represent the “grand mean” effect sizes.

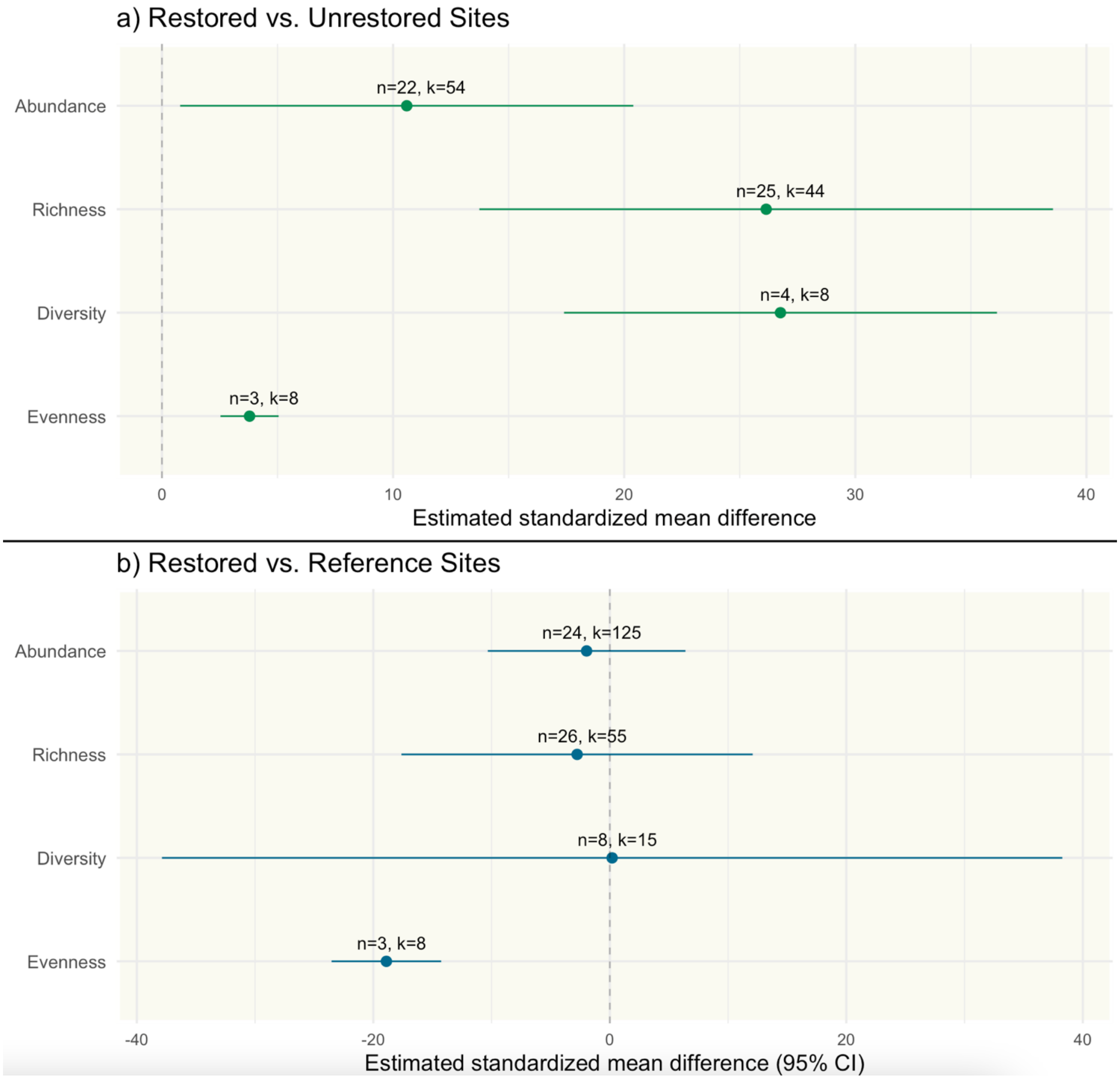

Figure 3.4: Estimated Hedge's g and 96% CI from subanalyses (mixed-effects meta-regression models) of a) restored vs. unrestored sites; b) restored vs. reference sites.

3.7 SUPPLEMENTARY MATERIALS

Tables

Table S3.1: Estimated coefficients, confidence intervals, and significance values (bolded values are significant) for our mixed-effects meta-regression models comparing abundance, richness, diversity, and evenness between restored and unrestored sites. p-values for Cochran's T (test for heterogeneity) were <0.05 for all models.

Fixed effects	**Estimate**	**CI**	**p**
Abundance			
(Intercept)	9.08	0.51 – 17.64	**0.038**
Order: Araneae	1.90	-5.46 – 9.25	0.613
Order: Blattodea	-8.22	-23.43 – 7.00	0.290
Order: Coleoptera	-4.25	-12.82 – 4.33	0.331
Order: Diptera	-13.66	-25.78 – -1.53	**0.027**
Order: Hymenoptera	-3.03	-11.54 – 5.49	0.486
Order: Lepidoptera	-4.09	-14.37 – 6.20	0.436
Order: Orthoptera	3.44	-11.14 – 18.02	0.644
Time since restoration	-0.01	-0.03 – 0.02	0.668
Latitude	-0.11	-0.25 – 0.03	0.115
Habitat type: Grassland	-0.88	-8.17 – 6.41	0.813
Habitat type: Wetland	14.57	3.12 – 26.02	**0.013**
Restoration approach: Assisted	1.89	-5.86 – 9.64	0.633
Restoration approach: Natural	-4.59	-9.78 – 0.60	0.083
Sampling type: Active	1.47	-3.10 – 6.03	0.529
Richness			
(Intercept)	16.06	5.21 – 26.91	**0.004**
Order: Araneae	0.19	-13.90 – 14.29	0.979
Order: Coleoptera	-12.93	-22.98 – -2.87	**0.012**
Order: Diptera	-16.01	-27.82 – -4.19	**0.008**
Order: Hymenoptera	-9.12	-19.55 – 1.30	0.086
Order: Lepidoptera	-17.05	-31.16 – -2.95	**0.018**
Order: Orthoptera	10.20	-4.73 – 25.14	0.181
Time since restoration	-0.00	-0.02 – 0.02	0.982
Latitude	-0.05	-0.17 – 0.07	0.379
Habitat type: Grassland	-7.13	-14.01 – -0.25	**0.042**
Habitat type: Wetland	12.39	0.43 – 24.35	**0.042**
Restoration approach: Assisted	3.73	-4.05 – 11.50	0.348
Restoration approach: Natural	-7.52	-13.52 – -1.52	**0.014**
Sampling type: Active	7.26	-1.62 – 16.13	0.109
Diversity			
(Intercept)	26.54	17.49 – 35.59	**<0.001**
Order: Blattodea	0.22	-1.68 – 2.13	0.819
Order: Hymenoptera	-14.34	-23.21 – -5.47	**0.002**
Order: Orthoptera	-23.04	-30.75 – -15.33	**<0.001**
Time since restoration	-0.11	-0.15 – -0.07	**<0.001**
Evenness			
(Intercept)	-4.76	-21.98 – 12.47	0.588
Order: Diptera	8.27	-5.26 – 21.79	0.231
Order: Hymenoptera	-0.09	-14.08 – 13.90	0.990
Order: Orthoptera	-7.06	-21.68 – 7.55	0.343
Time since restoration	0.01	-0.07 – 0.08	0.886

Table S3.2: Estimated coefficients, confidence intervals, and significance values (bolded values are significant) for our mixed-effects meta-regression models comparing abundance, richness, diversity, and evenness between restored and reference sites. p-values for Cochran's T (test for heterogeneity) were <0.05 for all models.

Fixed effects	**Estimate**	**CI**	**p**
Abundance			
(Intercept)	-0.20	-7.80 – 7.40	0.959
Order: Acari	-5.11	-11.82 – 1.59	0.135
Order: Araneae	-3.30	-9.05 – 2.45	0.261
Order: Blattodea	2.50	-3.43 – 8.42	0.409
Order: Coleoptera	-2.40	-7.39 – 2.58	0.345
Order: Collembola	2.67	-4.07 – 9.40	0.437
Order: Dermaptera	2.68	-5.87 – 11.24	0.539
Order: Diplopoda	0.89	-5.78 – 7.56	0.795
Order: Diptera	-2.58	-8.98 – 3.82	0.429
Order: Hemiptera	-3.15	-9.63 – 3.33	0.341
Order: Hymenoptera	-1.38	-7.31 – 4.54	0.647
Order: Isopoda	-2.31	-10.76 – 6.14	0.592
Order: Lepidoptera	-14.80	-22.85 – -6.76	**<0.001**
Order: Mantodea	-12.71	-21.34 – -4.08	**0.004**
Order: Mollusca	-11.06	-19.64 – -2.48	**0.012**
Order: Opiliones	-4.38	-12.85 – 4.09	0.311
Order: Orthoptera	1.22	-5.44 – 7.88	0.719
Order: Phasmatodea	-11.27	-19.85 – -2.70	**0.010**
Order: Pseudoscorpiones	1.36	-5.30 – 8.03	0.689
Order: Psocoptera	4.83	-1.88 – 11.54	0.158
Order: Thysanoptera	3.29	-5.28 – 11.85	0.452
Order: Zygentoma	-3.87	-12.31 – 4.58	0.369
Time since restoration	-0.01	-0.04 – 0.02	0.564
Latitude	0.26	0.10 – 0.41	**0.002**
Habitat type: Grassland	-6.89	-17.78 – 4.00	0.215
Habitat type: Wetland	-26.03	-41.40 – -10.67	**0.001**
Restoration approach: Assisted	-1.01	-11.80 – 9.77	0.854
Restoration approach: Natural	-1.36	-3.52 – 0.79	0.215
Sampling type: Active	3.48	0.96 – 6.00	**0.007**
Sampling type: Both	20.26	-1.21 – 41.73	0.064
Richness			
Overall	3.65	-5.50 – 12.80	0.434
Order: Araneae	-10.90	-26.45 – 4.66	0.170
Order: Coleoptera	-6.58	-17.16 – 4.00	0.223
Order: Diptera	-10.97	-22.23 – 0.29	0.056
Order: Hemiptera	-0.41	-14.20 – 13.37	0.953
Order: Hymenoptera	-6.52	-16.68 – 3.64	0.208
Order: Lepidoptera	-14.14	-33.28 – 5.00	0.148
Order: Orthoptera	0.11	-25.18 – 25.41	0.993
Time since restoration	-0.00	-0.02 – 0.02	0.931
Latitude	0.22	0.07 – 0.37	**0.004**
Habitat type: Grassland	-9.82	-19.60 – -0.03	**0.049**
Habitat type: Wetland	-20.60	-34.46 – -6.73	**0.004**
Restoration approach: Assisted	3.74	-8.94 – 16.42	0.563
Restoration approach: Natural	-6.01	-9.25 – -2.77	**<0.001**
Sampling type: Active	-0.11	-10.99 – 10.77	0.984
Sampling type: Both	-4.12	-22.94 – 14.70	0.668

Diversity			
(Intercept)	2.58	-23.89 – 29.06	0.848
Order: Blattodea	-2.39	-27.25 – 22.47	0.851
Order: Collembola	1.88	-35.36 – 39.12	0.921
Order: Hemiptera	-1.13	-19.93 – 17.67	0.906
Order: Hymenoptera	-3.82	-27.79 – 20.16	0.755
Order: Orthoptera	-23.30	-50.44 – 3.83	0.092
Time since restoration	-0.01	-0.15 – 0.14	0.911
Evenness			
(Intercept)	-53.50	-73.20 – -33.79	**<0.001**
Order: Diptera	34.60	19.22 – 49.98	**<0.001**
Order: Hymenoptera	42.48	26.99 – 57.98	**<0.001**
Order: Orthoptera	-25.92	-47.22 – -4.63	**0.017**
Time since restoration	0.22	0.14 – 0.30	**<0.001**

Appendix 3.1: List of included studies

Amazonas, N. T., Viani, R. A. G. G., Rego, M. G. A. A., Camargo, F. F., Fujihara, R. T., & Valsechi, O. A. (2018). Soil macrofauna density and diversity across a chronosequence of tropical forest restoration in southeastern Brazil. Brazilian Journal of Biology, 78(3), 449–456.

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