

DISSERTATION

RANGELAND MANAGEMENT IMPACTS ON NATIVE BEE DIVERSITY AND
POLLINATION SERVICES IN THE SHORTGRASS STEPPE OF COLORADO'S FRONT
RANGE

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Khum Bahadur Thapa-Magar

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Doctoral Committee:

Advisor: Thomas Seth Davis

María E. Fernández-Giménez

Daniel Laughlin

Punya Nachappa

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ABSTRACT

RANGELAND MANAGEMENT IMPACTS ON NATIVEs BEE DIVERSITY AND POLLINATION SERVICES IN THE SHORTGRASS STEPPE OF COLORADO’S FRONT RANGE

Rangelands are a globally abundant ecosystem type that provides key ecosystem services and supports the livelihoods of millions of people. In western North America, rangelands are widely used for livestock grazing; in some cases, livestock grazing is associated with conversion to novel cover types with high frequencies of non-native grasses and forbs. Despite these potential effects, it is not well known how direct livestock grazing or indirect grazing-associated effects affect the biodiversity of arthropods, especially wild pollinators. However, both pollinators and insects in general are reported to be in global decline. Therefore, it is important to understand the impacts of widespread land uses as grazing on these communities. In addition, few studies address behaviors of pollinators in rangelands and how functional variation in pollinator assemblages affect yields of commercially valuable crops. In this dissertation, I address (1) how grazing management affects pollinator biodiversity in general; (2) whether grazing interacts with invasive grass species abundance to affect pollinator functional diversity in the shortgrass steppe of Colorado’s Front Range; and (3) how a specific behavioral trait (visitation time to flowers) interacts with species richness to drive pollination services.

Several theoretical models have been developed to predict grazing effects on biodiversity. Among the best-known is Milchunas, Sala, and Lauenroth’s (1988) model (the ‘MSL’ model), which interprets grazing effects within the framework of interactions between relative ecosystem

aridity and evolutionary history of grazing. In the first chapter, I conducted a meta-analysis of 60 studies published in the primary literature to analyze the effects of grazing on pollinator communities (native bees and butterflies) within the context of the MSL model. I characterized the response of pollinator communities to grazing intensity, interactions between habitat aridity and evolutionary history, and grazing management from a global perspective. Three key findings emerged: (1) in mesic habitats high grazing intensities generally had negative impacts on pollinator abundance and richness, but these effects were weaker in arid habitats where effects on species richness were nil; (2) grazing effects were mediated by evolutionary history and pollinator communities in arid habitats with long histories of grazing were not affected by livestock; however, this was not true for arid habitats with short grazing histories or mesic habitats with long grazing histories, where negative effects were detected; (3) both livestock species and pollinator life history mediated effects on pollinator communities: cattle tended to have more deleterious effects on pollinators than sheep or mixed livestock grazing, and social bees and butterflies were more negatively impacted than solitary bees. I conclude partial support for the MSL model in terms of the impacts of interactions between habitat aridity and evolutionary history of grazing on pollinators: pollinator biodiversity in arid habitats responded to grazing as predicted by the MSL model, but there were too few studies in mesic habitats with short grazing histories to test a full factorial. These findings have implications for livestock grazing management across landscapes.

In the second chapter, I tested how cattle grazing and invasive cheatgrasses (*Bromus tectorum* and *B. japonicus*) affect bee foraging and nesting habitats and the biodiversity of wild bee communities in a regional shortgrass steppe system. Bee nesting habitats were improved in non-grazed sites with low cheatgrass cover, though foraging habitat did not differ among cattle-

grazed sites or non-grazed sites with low or high cheatgrass cover. Floral cover was a good predictor of bee abundance and functional dispersion. Mean bee abundance, richness, diversity and functional diversity were significantly lower in cattle-grazed habitats than in non-grazed habitats. Differences in bee diversity among habitats were pronounced early in the growing season (May) but by late-season (August) these differences were not detectable. Sites with high floral cover tended to support large, social, polylectic bees; sites with high grass cover tended to support oligolectic solitary bees. Both cattle-grazed sites and sites with high cheatgrass cover were associated with lower abundances of above-ground nesting bees but higher abundance of below-ground nesters than non-grazed sites with low cheatgrass cover. I conclude that high cheatgrass cover is not associated with reduced bee biodiversity or abundance, but cattle grazing was negatively associated with bee abundances and affected bee species composition. Although floral cover is an important predictor of bee assemblages, this was not affected by cattle grazing and our study suggests that cattle likely impact bee communities through effects other than those mediated by forbs, including soil disturbance or nest destruction.

In the final chapter, I tested how variation in bee richness and specific functional behaviors (floral visitation time) interact to affect pollination services using a regionally abundant, naturalized crop species (sunflower) as a study system. Understanding whether pollinator behaviors and species richness drive crop yields is a key area of investigation in pollination ecology. I describe variation in mean floral visitation times among bee taxa and test how interactions between bee richness and the proportion of bumblebees in localized communities impact seed yields. Seven bee genera commonly visited sunflower including *Agapostemon*, *Bombus*, *Halictus*, *Lasioglossum*, *Megachile*, *Melissodes*, and *Svastra*. Mean visitation times to sunflower varied across genera and *Bombus* and *Halictus* spp. spent the most

time foraging on inflorescences, but the number of visits by *Bombus* spp. was the only parameter associated with increased yields. Experimental pollination deficit reduced seed development and yields, and these effects were stronger in stands of wild-type sunflower in the field compared to a confection variety grown in the greenhouse. Relationships between bee richness and pollination services differed for potted and wild sunflower: when bees had short-term access to potted sunflower, bee richness and relative *Bombus* abundances were not associated with pollination quotients. When bees had long-term access to wild sunflower, relative *Bombus* abundances predicted pollination services but were modified by site-level bee richness: as richness increased, the effects of *Bombus* abundance decreased. This study demonstrate that bee species richness is not always a clear predictor of pollination services; instead, my results underscore the importance of specific taxa when species richness is low (here, bumblebees), and show that the effects of bee functional groups important for pollination may be modified by changes in site-level species richness.

Collectively, my findings suggest several key points: (1) theoretical models that describe how livestock grazing intensity affects biodiversity of native plants are only somewhat transferable to pollinating arthropods and a knowledge gap exists in humid ecoregions. However, (2) meta-analysis reveals that grazing intensity likely interacts with both habitat aridity and relative history of grazing to impact pollinator biodiversity. (3) Within the Front Range region of Colorado, intensive grazing by cattle was associated with a reduction in pollinator diversity early in the growing season, but these effects were not mediated by abundance of forbs, and (4) the abundance of invasive grasses were not negatively associated with pollinator diversity. (5) Evaluation of the relationship between pollinator diversity effects and pollination services suggested that bee taxa with specific behaviors ('long visitors') were important for pollination

services when pollinator richness was low, but as richness increased the importance of these taxa was reduced.

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PREFACE

Chapter 1 is under revision at the journal *Ecological Solutions and Evidence* at the time of dissertation completion; Chapter 2 and 3 are published in *PLOS ONE* and *Environmental Entomology* respectively. Accordingly, some minor formatting differences in references may exist between chapters.

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CHAPTER 1: A META-ANALYSIS OF THE EFFECTS OF HABITAT ARIDITY, EVOLUTIONARY HISTORY OF GRAZING, AND GRAZING INTENSITY ON POLLINATOR COMMUNITIES WORLDWIDE

1. Introduction

Rangelands are a globally abundant ecosystem type covering an estimated 54% of terrestrial ecosystems (Estell et al., 2012; Reynolds et al., 2007). Rangelands provide key ecosystem services and support the livelihoods of millions of people by providing subsistence and pastoral opportunities (Derner et al., 2017). In addition to benefitting human well-being, rangelands provide critical habitats and foraging resources to a variety of wildlife, including insects. Insects, especially bees (Hymenoptera) and butterflies (Lepidoptera), are among the most important taxa driving key ecological interactions such as pollination. An estimated 90% of the world's plant species are pollinated by insects, and bees and butterflies are perhaps the most common flower visitors, providing the foundation for pollination services across many ecosystems (Ollerton, Winfree, & Tarrant, 2011). Pollination plays a vital role in ecosystem function and is important for maintaining genetic diversity in wild and managed plant populations, promoting biodiversity, and enhancing the economic value of many agricultural crops (Chaplin-Kramer et al., 2011). However, there is evidence that insect pollinators are declining in rangelands due to anthropogenic disturbances, environmental stressors, and ecosystem management practices including grazing by livestock (Potts et al., 2010; Rafferty, 2017). Accordingly, there is a broad need to understand how grazing intensity, duration and type of grazing animal (livestock species) affect pollinators across different rangeland environments.

Livestock grazing is a common land use practice in rangeland ecosystems, and is an important process for converting plant biomass into animal protein for human consumption (Alkemade et al., 2013; Asner et al., 2004). Grazing practices on rangelands are regarded both critically and favorably by natural resource managers due to their dual nature as potential disturbances and as management tools, respectively, and it is increasingly recognized that appropriate management of rangelands is needed to conserve ecosystem services across large spatial scales. In some cases, grazing practices may conflict with conservation goals and several recent studies demonstrate direct or indirect negative effects of livestock grazing on pollinator biodiversity. For example, direct effects of livestock on pollinators include destroying nesting and foraging habitats through soil compaction or consumption of floral resources (Glaum & Kessler, 2017; Moreira, Castagneyrol, Abdala-Roberts, & Traveset, 2019) and the trampling of adult bees and their larvae (Sugden, 1985; Sjödin, 2007). In contrast, indirect effects tend to be mediated by the plant community and generally include altering plant assemblages and floral species composition (Smallidge & Leopold, 1997; Carvell, 2002; Hatfield & LeBuhn, 2007; Roulston & Goodell, 2011). On the other hand, several studies have also demonstrated positive effect of livestock grazing on pollinator diversity (Kruess and Tscharntke, 2002; Sjödin, 2007).

However, the effects of grazing on plant communities are mediated by a variety of site-specific as well as global factors. These factors were integrated into a conceptual model developed by Milchunas, Sala, and Lauenroth (1988) (hereafter, the ‘MSL model’), which attempts to reconcile interactions between grazing and plant richness as a function of grazing intensity, evolutionary history of grazing, and relative ecosystem aridity. The MSL model predicts that effects of grazing intensity on plant diversity in arid ecosystems are controlled by evolutionary history. Areas with long evolutionary histories of grazing experience little-to-no

reduction in plant biodiversity with increased grazing intensity; however, in areas with short evolutionary histories of grazing, increased grazing intensity is associated with rapid decline in plant biodiversity. In contrast, in humid or mesic regions with long evolutionary histories of grazing, grazing intensity tends to maximize plant diversity at intermediate levels, and this relationship is similar but truncated in humid regions with short evolutionary histories of grazing. Recently, meta-analyses (see Gao and Carmel, 2020; Herrero-Jáuregui and Oesterheld, 2018) have tested these principles using a broad array of studies and found general empirical support for the MSL model in plant communities. However, it remains unknown whether these effects cascade to impact associated pollinator assemblages (Hanberry et al., 2020). An understanding of how relationships between grazing intensity, evolutionary history of grazing, and relative ecosystem aridity affect pollinator assemblages carries implications for global pollinator conservation efforts.

Using meta-analysis, the goal of the present study is to evaluate variation in pollinator community abundance and richness within the framework of the MSL model extended to include effects on pollinators (Figure 1.1), by describing the response of pollinators to livestock grazing under various grazing intensities, coevolutionary histories, and regional-scale environmental factors (relative aridity). Specifically, I address the following questions to address our goal: (1) Does ecosystem aridity mediate effect of grazing intensity on pollinator diversity?; (2) Is there an interaction between relative aridity and evolutionary history of grazing on pollinator communities?; (3) Do grazing effects on pollinator communities differ with livestock species or pollinator groups?; and (4) Do grazing management practices, including rotation or livestock species composition, have implications for pollinator conservation? Our results provide new insights into the dynamics of grazing-mediated effects on pollinator assemblages at a worldwide

scale, with importance for interpretation of factors driving diversity and distribution of insect communities providing key ecosystem services.

2. Materials and methods

2.1. Data collection

I collated primary literature that meets our research objectives from online database (ISI web of Science, hereafter ‘WOS’) using the following word combinations: (graz* OR grazing OR grazing intensity OR livestock OR cattle OR sheep OR goat) AND (pollina* OR hymenoptera OR wild bee OR bee OR solitary bee OR bumblebees OR butterfly OR lepidopteran) (see PRISMA flow chart, Appendix 1, Figure A.1.1). I only included articles published in English in the field of ecology, plant sciences, environmental sciences, biodiversity conservation, and entomology to discard unrelated research to our study and reduce redundancy in the WOS article retrieving process. The literature searches were performed at two different times and results were pooled: a first search was made in June 2020 and second in December 2020. In addition, I used literature cited in previous research syntheses and meta-analyses about the response of pollinators to grazing (e.g., Filazzola et al., 2020; Tonietto and Larkin, 2018; Wang and Tang, 2019; Winfree et al., 2009). In total, our search yielded 1,478 papers and this number was subsequently reduced to 106 paper for full text assessment by manually screening the titles and abstracts.

In our meta-analysis, I included those studies that reported the effect of livestock grazing on pollinator (wild bees or butterfly) abundance and richness and provided statistical parameters that included means, standard deviations or standard errors and sample sizes under conditions of livestock grazing and grazing exclusion. Grazing treatments could include several intensities (low, moderate and heavy livestock grazing). For studies that compared intensive versus

extensive grazing practices, intensive grazing was considered as high intensity and extensive grazing was considered as low intensity. I excluded articles that did not report geographic coordinates of study locations or enough details to reliably extract coordinates. Coordinates were applied to a global aridity index to derive relative aridity of study locations. Further, studies conducted completely in agriculture landscapes (i.e., planted pastures) were discarded.

After applying the above criteria, 174 study cases (97 reporting pollinator abundance, and 77 reporting richness) were incorporated from 60 articles published in the primary literature (i.e., 60 papers were filtered from the initial 106 possibilities) (see list of references in Appendix 1, List A.1.1). Most studies were conducted in Europe (n=32) and North America (n= 17 United States, n=2 Canada), followed by Asia (n=3), the Middle East (n=2), South America (n=2), and Australia and Africa each with a single study. Each study area was subsequently characterized based on relative aridity and estimated evolutionary history of grazing.

To characterize relative aridity in each study area I used provided coordinates to extract Aridity Index Values (UNEP, 1997) from the Global Aridity Index and Potential Evapotranspiration Climate Database v2 (Trabucco & Zomer, 2018). An aridity index value of ≤ 0.5 was used to assign study areas as semi-arid—arid, and areas with an aridity index value of > 0.5 were characterized as sub-humid—humid (UNEP, 1997) (Figure 1.2). To characterize the evolutionary history of grazing I follow with slight modification the recent approach of Gao and Carmel (2020) for the classification of study sites. Studies conducted in Asia, Europe, Africa and the Great Plains of North America including Eastern part of America were classified as having a ‘long’ evolutionary history, while western North America, South America, and Australia were considered as having comparatively ‘short’ evolutionary histories of grazing. Gao and Carmel (2020) classified all of North America as having a short evolutionary history of grazing, but the

Great Plains evolved with heavy grazing (Mack and Thompson, 1982). Thus, our classification differs slightly from theirs. Although I recognize the limitations of this approach in assigning grazing history to incorporated studies, it was necessary to make some generalizations about evolutionary histories to test predictions of the MSL model.

2.2. Meta-analysis

In our meta-analysis, I used standardized mean difference (hereafter, ‘Hedge’s d ’) as the statistic to compare effect sizes between livestock grazing (treatment) and grazing exclusion (control) (Gurevitch et al., 2001). Due to the diverse nature of statistical parameters reported in the primary literature, I calculated Hedge’s d in three ways. First, I calculated effect size directly from the statistical parameter of mean, standard deviation, and sample size. These were reported in most included studies. Second, I calculated effect size from correlation coefficient (r) and sample size for studies that reported correlations between continuous grazing intensities and pollinator diversity by transforming correlation coefficient into Fischer’s z and then to Hedge’s d . Third, I calculated effect size from t and F statistical scores and reported sample sizes.

I used a random effects model to estimate the effect size and significance of grazing intensity or aridity effects on each pollinator-related response variable (abundance and richness) using the “*rma*” function in “*metaphor*” R packages (Viechtbauer, 2010). The effect of livestock grazing over grazing exclusion was considered significant if the 90% CI did not overlap with zero (Koricheva et al., 2013). I also tested if there is variability of effect size among the studies for pollinator abundance and richness using Q statistics. If the Q statistic is significant (i.e., $P < 0.05$), it indicates high heterogeneity between the selected studies. Further, I also re-applied the abovementioned model with the moderator variables of relative habitat aridity (arid vs humid), evolutionary history of grazing (short vs long), pollinator life history (solitary bees vs

social bees [bumblebees] vs butterflies) and livestock type (cattle vs sheep vs mixed, where ‘mixed’ livestock grazing indicates any combination of two or more livestock species) to test if effect sizes differed between groupings. I used *QM* and *P*-values of model results to test if the effect of moderating variables were statistically significant. All calculations and analyses were conducted in R version 4.0.2 (R core Team, 2020). I also performed several standard analyses to eliminate the possibility of publication bias and confirmed minimal evidence of publication bias (Appendix 1, Figure A.1.2).

3. Results

Overall, livestock grazing had significant negative effects on pollinator abundance ($d=-1.121$, 95% CI -1.591 to -0.652 , $P<0.001$) and species richness ($d=-1.051$, 95% CI -1.577 to -0.525 , $P<0.001$) in humid areas, while in arid areas effects of grazing on pollinator abundance were negative ($d=-0.217$, 95% CI -0.433 to -0.001 , $P=0.001$) but effects on richness ($d=-0.116$, 95% CI -0.445 to 0.212 , $P=0.212$) did not differ significantly from zero (Figure 1.3). There was a marginally significant effect size of grazing on pollinator species richness ($QM=2.743$, $df=1$, $P=0.097$) in humid compared to arid habitats, but no detectable effects on pollinator abundance ($QM=2.030$, $df=1$, $P=0.154$). The effect size of grazing on pollinator abundance (Humid: $Q=1314.129$, $df=74$, $P<0.001$; Arid: $Q=35.063$, $df=21$, $P=0.027$) and richness (Humid: $Q=945.775$, $df=54$, $P<0.001$; Arid: $Q=89.252$, $df=21$, $P<0.001$) was heterogeneous among the studies.

3.1. Relative aridity mediates effects of grazing on pollinator communities

In humid habitats, moderate ($d=-1.891$, 95% CI -3.382 to -0.400 , $P=0.013$), heavy ($d=-1.158$, 95% CI -1.737 to -0.578 , $P<0.001$), and undefined ($d=-1.045$, 95% CI -1.758 to -0.333 , $P=0.004$) grazing intensity had significant negative effects on pollinator abundance

compared to no grazing, but light grazing did not impact pollinator abundances ($d=-0.538$, 95% CI -1.226 to 0.150 , $P=0.125$). However, patterns for species richness were variable and suggested that light ($d=-1.244$, 95% CI -2.046 to -0.552 , $P=0.002$), moderate ($d=-1.915$, 95% CI -3.531 to -0.299 , $P=0.020$), and undefined levels of grazing intensity are likely to have negative impacts on pollinator richness ($d=-0.895$, 95% CI -1.597 to -0.193 , $P=0.012$) but I did not detect a significant negative impact of heavy grazing on pollinator richness ($d=-0.474$, 95% CI -1.148 to 0.200 , $P=0.168$). In arid habitats, grazing intensity was marginally negatively associated with pollinator abundances under heavy grazing pressure ($d=-0.267$, 95% CI -0.562 to -0.027 , $P=0.075$) and undefined grazing intensity ($d=-0.358$, 95% CI -0.717 to 0.001 , $P=0.058$), but light and moderate grazing intensity had no effect on pollinator abundances ($d=0.263$, 95% CI -0.159 to 1.685 , $P=0.222$ and $d=-0.423$, 95% CI -1.293 to 0.447 , $P=0.340$, respectively). Effect sizes were homogenous among the grazing intensity levels on pollinator abundance ($QM=5.758$, $df=3$, $P=0.124$) and pollinator species richness ($QM=2.878$, $df=3$, $P=0.410$).

3.2. Interactions between aridity and evolutionary history of grazing on pollinator communities

In humid habitats with a long evolutionary history of grazing, grazing significantly negatively affected pollinator abundance ($d=-1.172$, 95% CI -1.648 to -0.697 , $P<0.001$) and species richness ($d=-1.058$, 95% CI -1.592 to -0.529 , $P<0.001$). However, this pattern was not observed in humid habitats with a short evolutionary history of grazing. A small sample size in this category ($n=2$) and high variability in effect size make a clear interpretation challenging.

In arid habitats with a long evolutionary history of grazing, grazing had no detectable effects on pollinator abundance or richness. In arid habitats with a short evolutionary history of

grazing, however, grazing was associated with significant negative effects on both pollinator abundance ($d=-0.945$, 95% CI -1.862 to -0.027 , $P=0.043$) and species richness ($d=-0.337$, 95% CI -0.463 to -0.210 , $P<0.001$; Figure 1.4).

3.3. Impacts of grazing on pollinator communities differ by livestock and pollinator type

Cattle grazing was associated with negative effects on both pollinator abundance ($d=-1.143$, 95% CI -1.591 to -0.695 , $P<0.001$) and species richness ($d=-0.886$, 95% CI -1.281 to -0.490 , $P<0.001$); however, there was no evidence for negative effects of sheep grazing or mixed animal grazing on pollinator abundance or richness. The effect size did not differ between livestock types (QM = 1.2064, df = 2, $P = 0.547$).

For all classified pollinator types, grazing was negatively associated with abundance (Solitary bees: $d=-1.202$, 95% CI -1.826 to -0.577 , $P<0.001$; Bumblebees: $d=-0.474$, 95% CI -1.332 to -0.162 , $P=0.012$; Butterflies: $d=-0.862$, 95% CI -1.589 to -0.135 , $P=0.020$). The same was true for species richness for butterflies ($d=-1.061$, 95% CI -1.661 to -0.462 , $P<0.001$) and bumblebees ($d=-0.788$, 95% CI -1.279 to -0.297 , $P=0.001$), but not for solitary bees: ($d=-0.542$, 95% CI -1.128 to 0.195 , $P=0.149$; Figure 1.5). Effect sizes were similar across pollinator types (QM= 0.7034, df = 2, $P = 0.703$).

4. Discussion

Few syntheses attempt to describe grazing effects on pollinator communities, and existing analyses that address this subject focus on grazing as a general category in the larger context of anthropogenic impacts (Winfree et al., 2009) and habitat restoration (Tonietto and Larkin, 2018), or consider pollinators (i.e., Hymenopterans) only as a general group within multi-trophic systems (Filazzola et al., 2020; Wang and Tang, 2019). Accordingly, these earlier studies do not parse out effects of different grazing practices or their interactions with habitat variables on

pollinators. Our meta-analysis expands on these previous works by directly interpreting such effects at a worldwide scale and within a well-described framework (i.e., the MSL model) to assess the relative contributions of habitat aridity and grazing practices on pollinators, with specific focus on native bee and butterfly communities.

Our results indicate that aridity strongly mediates effects of grazing intensity on pollinator abundance and species richness. In humid/mesic habitats increased grazing intensity generally had negative effects on pollinator abundance and species richness, whereas in arid habitats impacts of grazing on pollinator abundance were considerably reduced and there were no detectable impacts of grazing on pollinator richness (Figure 1.3). This finding has important implications for grazing management and suggests that grazing intensity \times aridity relationships are likely to have effects on ecosystem services provided by native pollinators.

Effects of relative aridity on pollinators are likely indirectly controlled by adaptations of rangeland forb species to grazing (Evju et al., 2009), and raising livestock in arid habitats with short evolutionary histories of grazing is likely to have negative consequences for pollinator community richness and abundance. The same was not true for pollinators in arid habitats with comparatively long evolutionary histories of grazing by large herbivores, where I detected no effects of grazing on pollinator assemblages (Figure 1.4). This contrasted with grazing effects on pollinators in humid habitats, where pollinator abundances and richness generally declined under grazing pressure even in areas with long evolutionary histories of natural and managed grazing. However, it is difficult to make conclusions about relationships between grazing practices and pollinator responses in humid/mesic habitats with short evolutionary histories of grazing, as I could only identify two studies meeting these criteria. The limited literature investigating grazing effects on pollinators in such habitats indicates a knowledge gap that should be addressed.

Accordingly, I am only able to conclude partial support for the MSL model in the framework of grazing-mediated effects on pollinators, as additional studies are needed to adequately test the complete aridity \times evolutionary history factorial. However, our results follow some patterns predicted by Milchunas, Sala, and Lauenroth (1988) for pollinators in arid areas.

In humid habitats, meta-analysis detected the strongest negative effects on bee abundance and species richness in areas experiencing moderate-intensity grazing. This result runs contrast to predictions of the MSL model, which generally predicts that the strongest negative impacts on species richness will result from heavy grazing. Multiple factors could explain this outcome. First, the MSL model was developed to predict grazing effects on plant species richness, and the same principles may not extend to higher trophic levels (insects). For example, grazing effects on plant diversity may be driven by consumption of abundant or highly competitive plant species, or creating disturbances and growing space that favor ruderal plants; in contrast, grazing effects on pollinator diversity may be more related to soil compaction and destruction of nesting habitats, or altering flowering phenologies of important forbs. Second, there were small sample sizes in some of the grazing intensity categories; with a larger sample the pattern could change to more closely resemble or more strongly contradict the predictions of the MSL model. Third, different studies incorporated in the meta-analysis may use different definitions of grazing intensity, which would complicate the interpretation of grazing intensity across studies or regions.

Grazing effects on communities are complex and estimation of a single parameter (intensity) may not be sufficient for describing ecological outcomes. Use of other variables including stocking would help to standardize grazing intensity estimates across studies, but currently most of the available studies describing grazing effects on pollinator communities do not report this variable, indicating a general deficiency in the literature and a need for greater

standardization across studies. In addition, incorporation of stocking rates alone may not be informative without some estimate of primary productivity (e.g., plant biomass production per unit area); for example, similar stocking rates may result in differential grazing pressures when compared between high- and low-productivity sites. Physiographic factors including light intensity (as predicted by aspect), elevation and temperature, and heat load index could interact with primary productivity to mediate effects of stocking rates and alter relative grazing pressure across landscapes. Therefore, incorporating a more complex set of interacting predictors could help to inform further studies investigating grazing effects on pollinator communities.

Our results for bee and butterfly pollinators match those from empirical studies examining grazing-mediated impacts on arthropod communities in arid landscapes with long evolutionary histories of grazing and suggest a general convergence of findings across an array of studies, taxa, and regions. For example (Newbold et al., 2014) found that arthropod communities were relatively insensitive to variable grazing intensities in short grass steppe, which has a long evolutionary history of grazing by bison (and more recently, cattle). Similarly, pioneering work by Coyner (1939), Weese (1939), and Smith (1940) reported positive-to-null effects of high-intensity cattle grazing on various insect communities in tallgrass prairie systems of western North America. Likewise, in eastern Mongolia, heavy grazing pressure was found to be associated with an increase in the number of plant-pollinator interactions, even though overall forb diversity was reduced (Yoshihara et al., 2008). However, a study from the Inner Mongolia region of China (Ma et al., 2017) showed that over-grazing can reduce abundances of primary and secondary (arthropod) consumers over time. So, although a growing body of evidence suggests insect communities tolerate grazing effects in arid rangelands, there likely exist thresholds of grazing intensity that should not be exceeded in the interest of insect conservation.

The larger overall effect size of grazing on pollinators in humid habitats is potentially attributable to multiple, non-mutually exclusive factors. First, there is general evidence for a higher diversity and more widespread distribution of pollinators in arid habitats (e.g., Michener, 2007). This could suggest that pollinator communities in arid regions may be able to withstand greater absolute losses before proportionally similar effects on biodiversity (i.e., as compared to humid areas) are detected. Differences in native bee life history strategies between arid and humid habitats could contribute to this pattern. In humid habitats social bees are more abundant and solitary bees were relatively infrequent by comparison to bee assemblages in arid habitats, where solitary bee taxa comprise a large portion of the overall biodiversity. Landscape factors could also drive differences in grazing effects between arid and humid habitat. Many pollinator species are adapted to nest in conditions with exposed bare ground, crevices, sandy soils, and with a significant litter and wood component. Such ground cover characteristics may be more common in arid rangelands. In addition, higher overall soil moisture content and precipitation inputs in humid rangelands might contribute to more readily compacted soil (El-Swaify et al., 1985) with potentially deleterious effects on ground-nesting pollinators (e.g., Xie et al., 2013).

I also report that both livestock and pollinator types are important when considering grazing-mediated impacts on pollinator assemblages. I found that grazing by cattle is more likely to be associated with negative effects (reduced species abundance and richness) on pollinator assemblages than grazing by either sheep or mixed livestock/multiple species (Figure 1.5). Depending on the specific comparison made, there were ~5-10-fold more studies available for cattle than sheep or mixed livestock, indicating a large disparity in research emphasis on livestock-mediated grazing effects and suggesting that there remains relatively little known about impacts of sheep or mixed livestock grazing on pollinator ecology. Sheep consume a more even

leaf:stem ratio than cattle (which tend to consume primarily leaf material, Poppi et al., 1981); however, cattle consume much more biomass per animal unit than sheep. Sheep also tend to forage more selectively than cattle (Tóth et al., 2018). These dynamic differences in livestock feeding behaviors could cascade to associated forb and pollinator communities, and these potential effects merit further investigation. Grazing was more likely to negatively impact the abundance and species richness of butterflies and social bees (i.e., *Bombus* spp.) than solitary bee taxa, for which there was no evidence of declining richness under grazing. However, effect sizes were relatively similar across pollinator types. This finding may partially explain the contrasting results of grazing effects between arid and humid systems (Figure 1.3): in arid habitats, bee taxa with a solitary life history are typically considerably more species-rich than social bee taxa (Michener, 2007).

Recent studies suggest that cattle grazing has varying impacts on pollinators in arid ecosystems, but these effects are not necessarily driven by effects of cattle on forb communities and instead may be associated with impacts on cover and soil properties. For instance, Thapa-Magar et al. (2020) found evidence for a seasonal reduction in functional dispersion of native bee communities in mid-grass prairie sites of eastern Colorado (USA) grazed by cattle, but concluded that these shifts were due to effects on bee nesting habitats rather than foraging resources. Kimoto et al., (2012) reported similar results for bumblebee (*Bombus* spp.) communities in an arid prairie system in Oregon (USA), and showed that cattle presence caused soil compaction and a reduction in herbaceous litter. In contrast, tests of cattle-mediated effects on bee communities in arid Mediterranean habitats reported null effects on wild bee abundance and richness, despite impacts on forb communities and bee foraging preferences (Shapira et al., 2019). Interpreted in the context of the MSL model, these collective findings and our own meta-

analysis indicate that cattle grazing impacts on bee biodiversity are likely habitat-mediated rather than food-mediated, which may suggest an overall limited role of evolutionary history of grazing in predicting bee community responses to livestock disturbances.

5. Implications and Conclusions

I report partial support for the MSL model as a predictive framework to interpret the effects of livestock grazing on pollinator communities. However, our analysis also identified a key gap in current knowledge of how pastoral practices in mesic habitats with short grazing histories impact pollinators. The respective interactions I outline here are likely to have consequences for conservation efforts and the maintenance of ecosystem services, and it is probable that cattle and other large livestock affect pollinator assemblages primarily via impacts on nesting resources rather than foraging resources. These effects should be considered in grazing management practices. Solitary bees are potentially more tolerant of livestock impacts than social bees, but the mechanisms underlying this pattern remain undescribed. In addition, further empirical work is needed to develop a clearer understanding of how different livestock species (e.g., cattle, sheep, bison, goats, etc.) affect pollinator communities, as very few direct comparative studies are available for making conclusions. However, it seems clear that increasing grazing intensity beyond low or moderate intensities has generally negative effects on pollinator abundance and species richness across a global collection of studies, and these effects are more pronounced in mesic habitats than arid habitats. Although evolutionary history of ungulate grazing is difficult to ascribe with certainty, this is a useful concept for identifying which ecoregions and rangelands may be more likely to suffer negative consequences from pastoral practices and can help to prioritize conservation efforts.

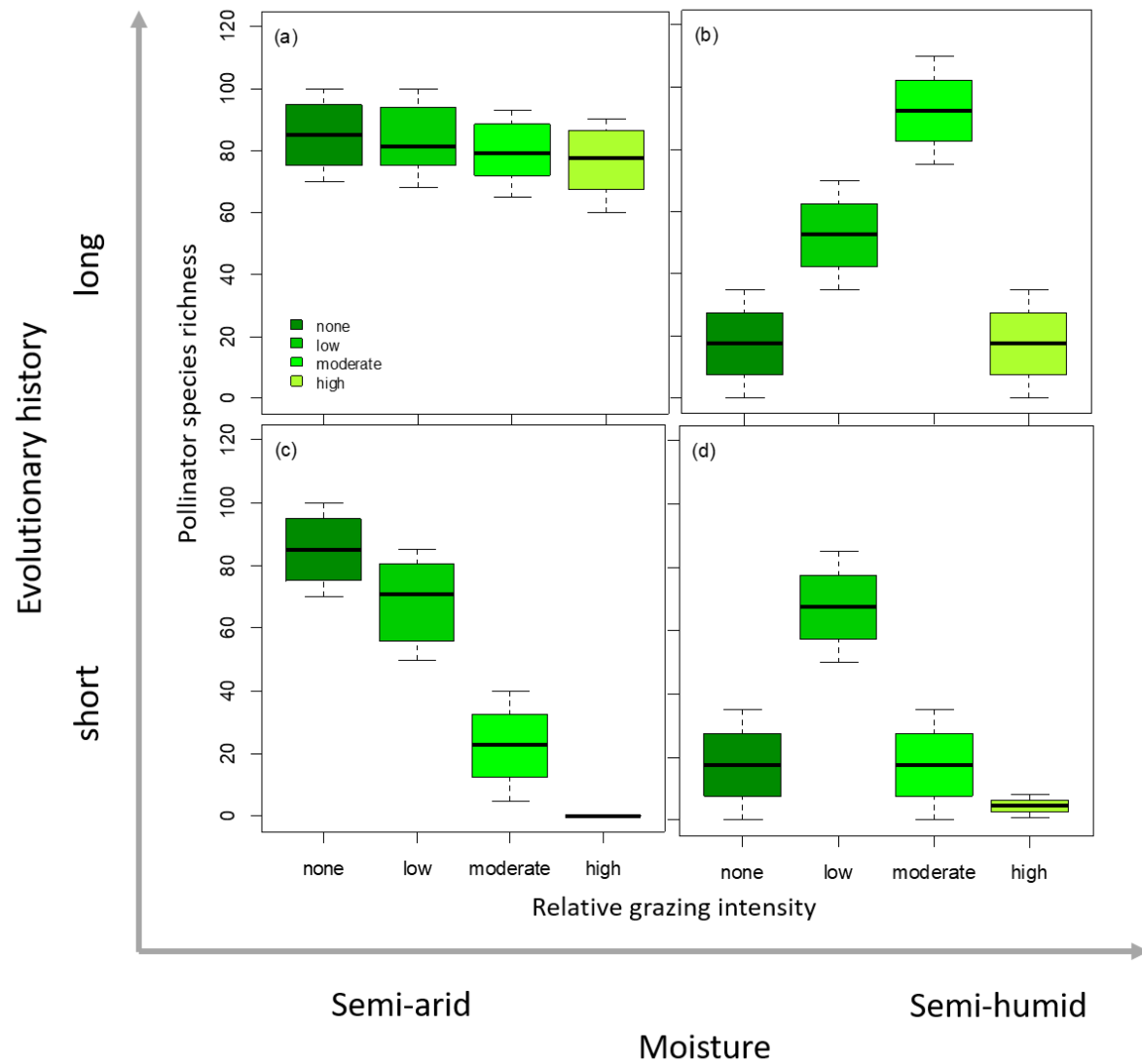


Figure 1.1. MSL model predicts that response of biodiversity to grazing intensity area mediated by state factors of moisture and evolutionary history. Comparisons are made across ecoclimatic regions; here I evaluate response of pollinator abundance and richness to grazing intensity (represented categorically rather than continuously) in semi-arid system with (a) long and (c) short grazing histories and compare these to response in semi-humid systems with (b) long and (d) short grazing histories. Adapted from Milchunas et al. (1988).

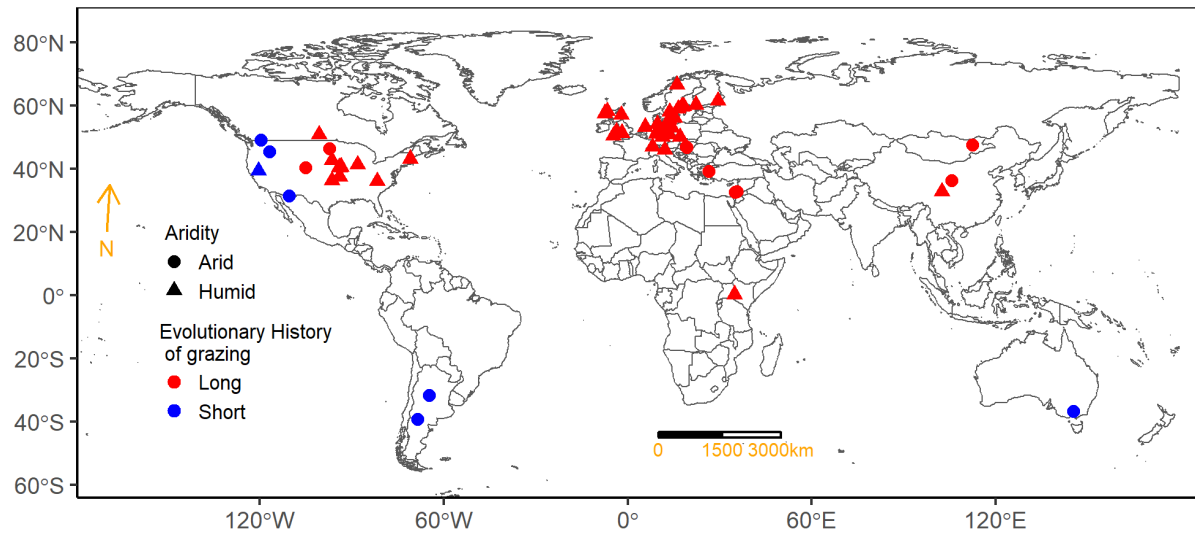


Figure 1.2. Global distribution of livestock grazing study sites incorporated in the present meta-analysis, shown relative to estimated aridity and evolutionary history of grazing.

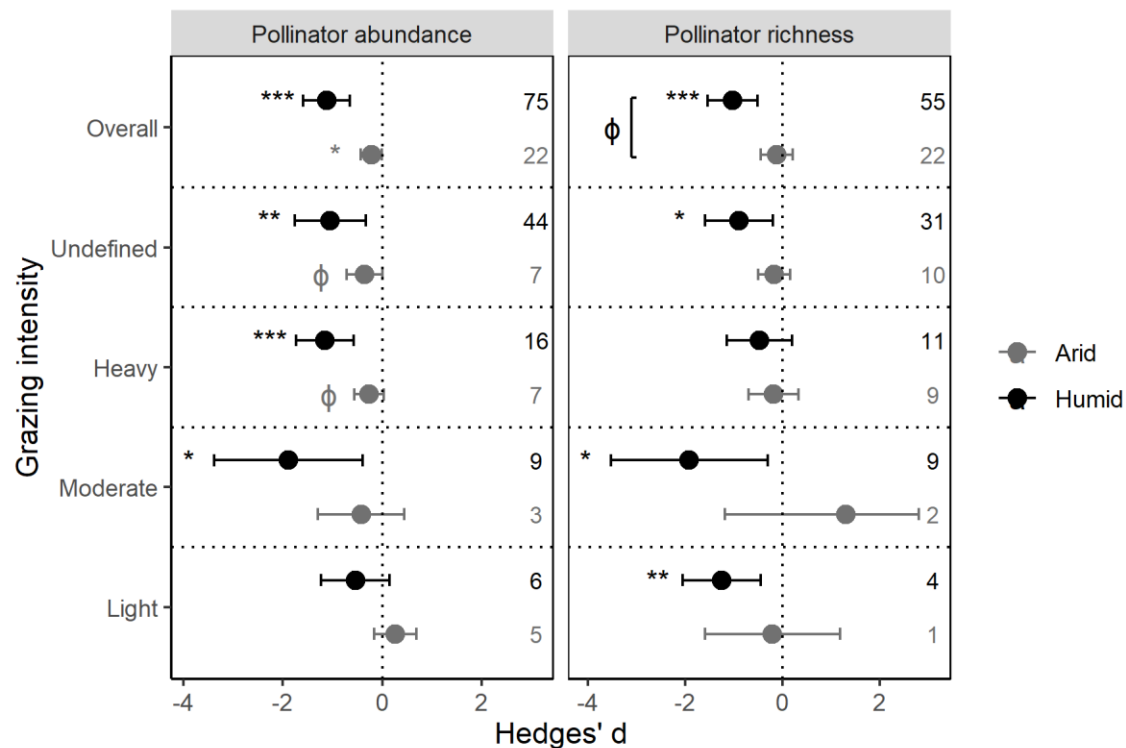


Figure 1.3. Relative aridity mediates the effects of grazing intensity on pollinator abundance and richness. Comparisons are of grazing effects are made against non-grazed control sites, and symbols denote significant deviation from the 'no effect' line shown at zero; ϕ $P < 0.10$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. The number of studies incorporated in the analysis are shown at right in each panel.

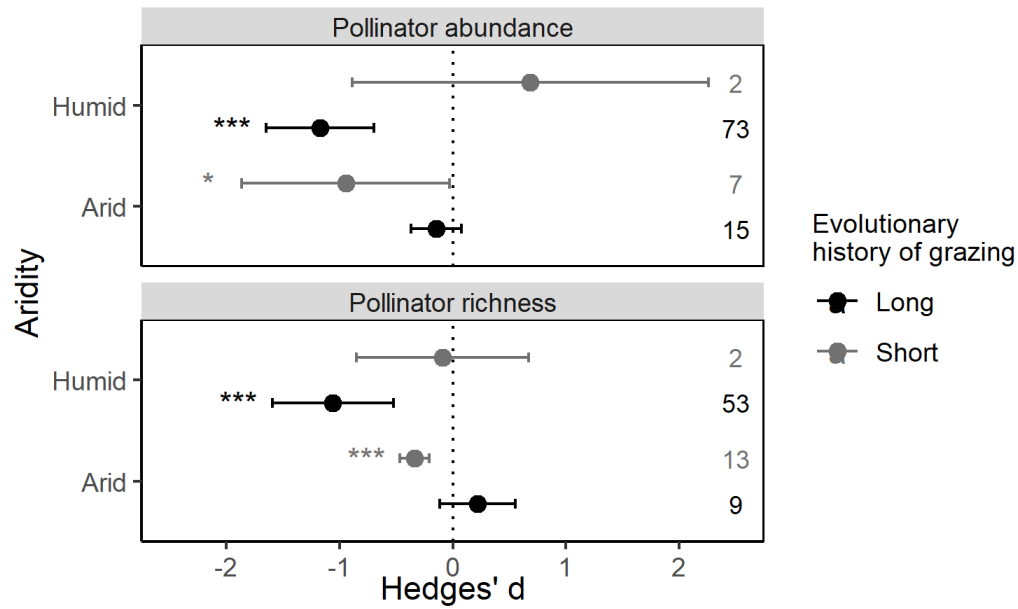


Figure 1.4. Interaction between aridity and evolutionary history of grazing on pollinator abundance and richness. Non-grazed sites are treated as controls for the comparison. The number of studies incorporated in the analysis are shown at right, and asterisks denote significance deviation from the 'no effect' line shown at zero; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

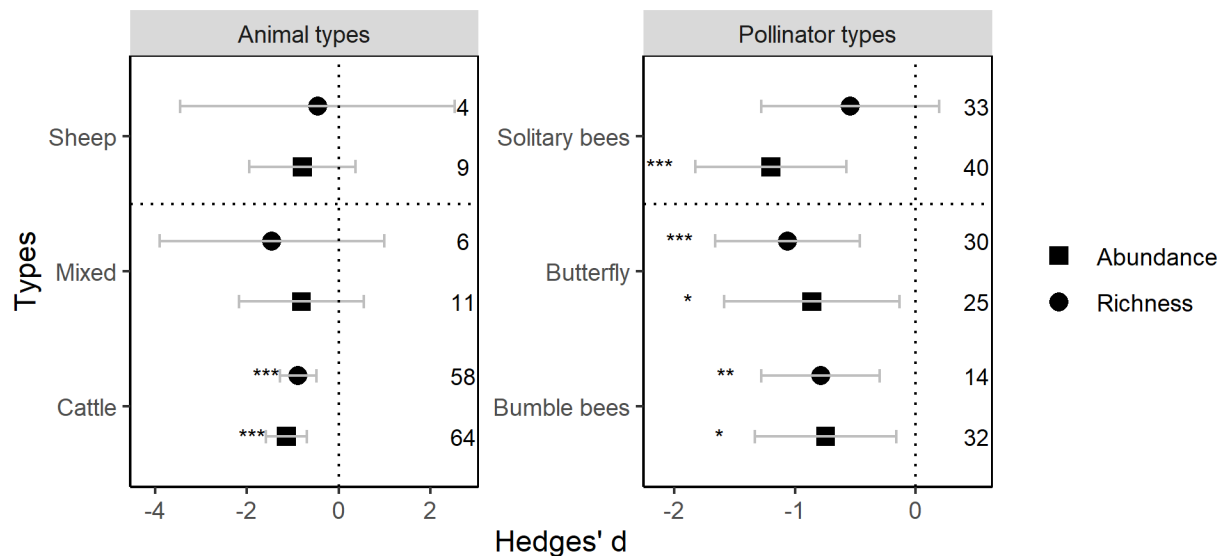


Figure 1.5. Effect of grazing by different types of animals on pollinator abundance and richness, and effect of grazing on different types of pollinators. Asterisks denote significant deviation from the 'no effect' line shown at zero; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. The number of studies incorporated in the analysis are shown at right.

REFERENCES

- Alkemade, R., Reid, R.S., van den Berg, M., de Leeuw, J., Jeuken, M., 2013. Assessing the impacts of livestock production on biodiversity in rangeland ecosystems. *Proc. Natl. Acad. Sci.* 110, 20900–20905.
- Asner, G.P., Elmore, A.J., Olander, L.P., Martin, R.E., Harris, A.T., 2004. Grazing system, ecosystem response, and global change. *Annu. Rev. Environ. Resour.* 29, 261–299.
- Bawa, K.S., 1990. Plant-Pollinator Interactions in Tropical Rain Forests. *Annu. Rev. Ecol. Syst.* 21, 399–422.
- Briske, D.D., Derner, J.D., Brown, J.R., Fuhlendorf, S.D., Teague, W.R., Havstad, K.M., Gillen, R.L., Ash, A.J., Willms, W.D., 2008. Rotational grazing on rangelands: Reconciliation of perception and experimental evidence. *Rangel. Ecol. Manag.* 61, 3–17.
- Carvell, C., 2002. Habitat use and conservation of bumblebees (*Bombus* spp.) under different grassland management regimes. *Biol. Conserv.* 103, 33–49.
- Chaplin-Kramer, R., Tuxen-Bettman, K., Kremen, C., 2011. Value of wildland habitat for supplying pollination services to californian agriculture. *Rangelands*, 33, 33–41.
- Coyner W.R. 1939. A report of the effect of overgrazing on the Acrididae. *Proc. the Oklahoma Acad. Sci.* 19: 83–85.
- Danforth, B.N., Minckley, R.L., Neff, J.L., 2019. *The Solitary Bees*. Princeton University Press, New Jersey, USA.
- Derner J.D., Hunt L., Filho K.E., Ritten J., Capper J., Han G. 2017. Livestock Production Systems, in: Briske D. (Eds.), *Rangeland Systems*. Springer Series on Environmental Management, Springer, New York, pp. 347–372.
- El-Swaify, S. A., Pathak, P., Rego, T. J., & Singh, S., 1985. Soil management for optimized productivity under rainfed conditions in the semi-tropics, in: Stewart, B. A., ed. *Advances in Soil Science*. Springer-Verlag, New York.
- Estell, R.E., Havstad, K.M., Cibils, A.F., Fredrickson, E.L., Anderson, D.M., Schrader, T.S., James, D.K., 2012. Increasing shrub use by livestock in a world with less grass. *Rangel. Ecol. Manag.* 65, 553–562.
- Evju, M., Austrheim, G., Halvorsen, R., Myrsterud, A., 2009. Grazing responses in herbs in relation to herbivore selectivity and plant traits in an alpine ecosystem. *Oecologia* 161, 77–85.
- Farruggia, A., Dumont, B., Scohier, A., Leroy, T., Pradel, P., Garel, J.-P., 2012. An alternative rotational stocking management designed to favour butterflies in permanent grasslands. *Grass Forage Sci.* 67, 136–149.
- Filazzola, A., Brown, C., Dettlaff, M.A., Batbaatar, A., Grenke, J., Bao, T., Peetoom Heida, I., Cahill, J.F., 2020. The effects of livestock grazing on biodiversity are multi-trophic: a meta-analysis. *Ecol. Lett.* 23, 1298–1309.
- Gao, J., Carmel, Y., 2020. A global meta-analysis of grazing effects on plant richness. *Agric. Ecosyst. Environ.* 302, 107072.
- Gilbert, L.E., 1972. Pollen Feeding and Reproductive Biology of *Heliconius* Butterflies. *Proc. Natl. Acad. Sci.* 69, 1403–1407.

- Glaum, P., Kessler, A., 2017. Functional reduction in pollination through herbivore-induced pollinator limitation and its potential in mutualist communities. *Nat. Commun.* 8, 2031.
- Gurevitch, J., Curtis, P.S., Jones, M.H., 2001. Meta-analysis in ecology. *Adv. Ecol. Res.* 32, 205–207.
- Hanberry, B.B., DeBano, S.J., Kaye, T.N., Rowland, M.M., Hartway, C.R., Shorrock, D., 2020. Pollinators of the Great Plains: Disturbances, stressors, management, and research needs. *Rangel. Ecol. Manag.*
- Hatfield, R.G., LeBuhn, G., 2007. Patch and landscape factors shape community assemblage of bumble bees, *Bombus* spp. (Hymenoptera: Apidae), in montane meadows. *Biol. Conserv.* 139, 150–158.
- Herrero-Jáuregui, C., Oesterheld, M., 2018. Effects of grazing intensity on plant richness and diversity: a meta-analysis. *Oikos* 127, 757–766.
- Kimoto, C., DeBano, S.J., Thorp, R.W., Taylor, R. V, Schmalz, H., DelCurto, T., Johnson, T., Kennedy, P.L., Rao, S., 2012. Short-term responses of native bees to livestock and implications for managing ecosystem services in grasslands. *Ecosphere* 3 art88.
- Koricheva, J., Gurevitch, J., Mengersen, K., 2013. Handbook of meta-analysis in ecology and evolution. Princeton University Press, New Jersey, USA.
- Kruess, A., Tscharncke, T., 2002. Grazing Intensity and the Diversity of Grasshoppers, Butterflies, and Trap-Nesting Bees and Wasps. *Conserv. Biol.* 16, 1570–1580.
- Ma, J., Huang, X., Qin, X., Ding, Y., Hong, J., Du, G., Li, X., Gao, W., Zhang, Zhuoran, Wang, G., Wang, N., Zhang, Zehua, 2017. Large manipulative experiments revealed variations of insect abundance and trophic levels in response to the cumulative effects of sheep grazing. *Sci. Rep.* 7, 11297.
- Mack, R.N., Thompson, J.N., 1982. Evolution in Steppe with Few Large, Hooved Mammals. *Am. Nat.* 119, 757–773.
- Michener, C.D., 2007. The bees of the world, 2nd edition. The Johns Hopkins University Press, Baltimore, USA.
- Milchunas, D.G., 2006. Responses of plant communities to grazing in the southwestern United States. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station.
- Milchunas, D.G., Sala, O.E., Lauenroth, W.K., 1988. A generalized model of the effects of grazing by large herbivores on grassland community structure. *Am. Nat.* 132, 87–106.
- Moreira, X., Castagneyrol, B., Abdala-Roberts, L., Traveset, A., 2019. A meta-analysis of herbivore effects on plant attractiveness to pollinators. *Ecology* e02707.
- Newbold, T.A.S., Stapp, P., Levensailor, K.E., Derner, J.D., Lauenroth, W.K., 2014. Community responses of arthropods to a range of traditional and manipulated grazing in shortgrass steppe. *Environ. Entomol.* 43, 556–68.
- Ollerton, J., Winfree, R., Tarrant, S., 2011. How many flowering plants are pollinated by animals? *Oikos* 120, 321–326.
- Poppi, D., Minson, D., Ternouth, J., 1981. Studies of cattle and sheep eating leaf and stem fractions of grasses. 2. Factors controlling the retention of feed in the reticulo-rumen. *Aust. J. Agric. Res.* 32, 109.
- Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E., 2010. Global pollinator declines: trends, impacts and drivers. *Trends Ecol. Evol.* 25, 345–353.
- R Core Team. 2020. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

- Rafferty, N.E., 2017. Effects of global change on insect pollinators: multiple drivers lead to novel communities. *Curr. Opin. Insect Sci.* 23, 22–27.
- Reynolds, J.F., Stafford Smith, D.M., Lambin, E.F., Turner, B.L., Mortimore, M., Batterbury, S.P.J., et al. 2007. Ecology: Global desertification: Building a science for dryland development. *Science* 316, 847–851
- Rosenberg, M. S., Rothstein, H. R., & Gurevitch, J., 2013. Effect sizes: Conventional choices and calculations, in: Koricheva, J., Gurevitch, J. & Mengersen, K. (Eds), *Handbook of meta-analysis in ecology and evolution*. Princeton University Press, New Jersey, pp. 61–71.
- Roulston, T.H., Goodell, K., 2011. The Role of Resources and Risks in Regulating Wild Bee Populations. *Annu. Rev. Entomol.* 56, 293–312.
- Scohier, A., Ouin, A., Farruggia, A., Dumont, B., 2013. Is there a benefit of excluding sheep from pastures at flowering peak on flower-visiting insect diversity? *J. insect Conserv.* 17, 287–294.
- Shapira, T., Henkin, Z., Dag, A., Mandelik, Y., 2019. Rangeland sharing by cattle and bees: moderate grazing does not impair bee communities and resource availability. *Ecol. Appl.* 30, e02066.
- Sjödin, N.E., 2007. Pollinator behavioural responses to grazing intensity. *Biodivers. Conserv.* 16, 2103–2121.
- Smallidge, P.J., Leopold, D.J., 1997. Vegetation management for the maintenance and conservation of butterfly habitats in temperate human-dominated landscapes. *Landsc. Urban Plan.* 38, 259–280.
- Smith, C.C., 1940. The Effect of Overgrazing and Erosion Upon the Biota of the Mixed-Grass Prairie of Oklahoma. *Ecology* 21, 381–397.
- Sugden, E. a, 1985. Pollinators of *Astragalus monoensis* Barneby (Fabaceae): new host records: potential impact of sheep grazing. *Gt. Basin Nat.* 45, 299–312.
- Thapa-Magar, K.B., Davis, T.S., Kondratieff, B., 2020. Livestock grazing is associated with seasonal reduction in pollinator biodiversity and functional dispersion but cheatgrass invasion is not: Variation in bee assemblages in a multiuse shortgrass prairie. *PLoS One* 15, e0237484.
- Tonietto, R.K., Larkin, D.J., 2018. Habitat restoration benefits wild bees: A meta-analysis. *J. Appl. Ecol.* 55, 582–590.
- Tóth, E., Deák, B., Valkó, O., Kelemen, A., Migléc, T., Tóthmérész, B., Török, P., 2018. Livestock Type is More Crucial Than Grazing Intensity: Traditional Cattle and Sheep Grazing in Short-Grass Steppes. *L. Degrad. Dev.* 29, 231–239.
- Trabucco, A., & Zomer, R. J. 2018. Global Aridity Index and Potential Evapo-Transpiration (ET0) Climate Database v2. CGIAR Consortium for Spatial Information (CGIAR-CSI).
- United Nations Environment Programme (UNEP). 1997. *World Atlas of Desertification*, 2nd edition. Middleton, N., & David, T., eds. Arnold, London & Wiley, New York.
- Viechtbauer, W., 2010. Conducting Meta-Analyses in R with the metafor Package. *J. Stat. Softw.* 36, 1–48.
- Wang, C., Tang, Y., 2019. A global meta-analysis of the response of multi-taxa diversity to grazing intensity in grasslands. *Environ. Res. Lett.* 14, 114003.
- Weese A.O. 1939. The effect of overgrazing on insect populations. *Proc. the Oklahoma Acad. Sci.* 19: 95–99.

- Winfree, R., Aguilar, R., Vazquez, D.P., LeBuhn, G., Aizen, M.A., 2009. A meta-analysis of bees' responses to anthropogenic disturbance. *Ecology* 90, 2068–2076.
- Xie, Z., Qiu, J., Chen, X., 2013. Decline of nest site availability and nest density of underground bees along a distance gradient from human settlements. *Entomol. Sci.* 16, 170–178.
- Yoshihara, Y., Chimeddorj, B., Buuveibaatar, B., Lhagvasuren, B., Takatsuki, S., 2008. Effects of livestock grazing on pollination on a steppe in eastern Mongolia. *Biol. Conserv.* 141, 2376–2386.

CHAPTER 2: LIVESTOCK GRAZING IS ASSOCIATED WITH SEASONAL REDUCTION IN POLLINATOR BIODIVERSITY AND FUNCTIONAL DISPERSION BUT CHEATGRASS INVASION IS NOT: VARIATION IN BEE ASSEMBLAGES IN A MULTI-USE SHORTGRASS STEPPE

1. Introduction

Wild bees play key functional roles in natural landscapes including the pollination of wild plants and crops and are vital for maintaining biodiversity and ecosystem function (Kearns et al., 1998; Kremen et al., 2007). Roughly 90% of the world's plant species are pollinated by animals, in which bees are the dominant flower visitors for pollination services (Ollerton et al., 2011). However, wild bees are declining globally, with serious implications for human food security and ecosystem function (Potts et al., 2010; Vanbergen and Pollinators Initiative, 2013). Most authors now agree that wild bees are vital for pollination services in agricultural systems and can exceed the services provided by honey bees (*Apis mellifera* L.) (MacInnis and Forrest, 2019, Greenleaf and Kremen, 2006). Accordingly, conservation of wild bee communities is important to maintain pollination services in both agricultural areas and natural landscapes.

Habitat alteration and exotic species introduction are hypothesized to be among the major contemporary drivers directly and indirectly affecting bee communities (Rafferty, 2017). In rangeland ecosystems, managed livestock grazing is a dominant process by which habitat alteration occurs (Alkemade et al., 2013). Livestock grazing can impact wild bees directly or indirectly through various mechanisms, including effects on bee nesting and foraging habitats (Moreira et al., 2019) and behaviors (Sjödin, 2007). For example, soil compaction due to livestock activity can damage potential or existing ground nesting sites crucial for ground- and

cavity-dwelling wild bee species (Murray et al., 2012) or livestock may consume or alter composition of forb species that wild bees rely on for foraging resources (Carvell, 2002; Roulston and Goodell, 2011). In addition, livestock may directly kill adult bees as well as their larvae via trampling (Sugden' 1985; Sjödin et al., 2007). Since ground-nesting solitary bee species comprise a substantial proportion of many wild bee communities, these effects are a serious concern for rangeland managers concerned with maintenance of ecosystem services and may ultimately affect rangeland productivity. In addition, repeated pressure on plant communities from livestock grazing can also impact plant growth, architecture (Kruess and Tschardt, 2002a, b), floral traits, plant attractiveness to pollinators, plant reproductive success (Jones and Agrawal, 2017; Bauer et al., 2017), and soil characteristics (Potts et al., 2005). An understanding of these collective effects on wild bee pollinators in rangelands remains nascent but could be related to functional variation among bee species. For example, it is possible that bee life-history traits (such as below- or aboveground nesting habits) explain the distribution of bee species in grazed- and non-grazed habitats.

In addition to managed livestock grazing, biological invasion is another ecological process driving habitat alteration in rangeland systems and may also have consequences for wild bee communities (Kearns et al., 1998). Both invasive forbs and grasses affect wild bee communities indirectly through impacts on native plant community composition and abundance. Invasive plants may outcompete native forbs for nutrients, light, space and water (Levine et al., 2003; Parkinson et al., 2013). Invasive grasses, particularly *Bromus* species including *B. tectorum* L. and *B. japonicus* Thunb. (hereafter, 'cheatgrasses') have extensively occupied many rangeland ecosystems in western North America (Goergen et al., 2011). Invasion of rangeland habitats by cheatgrasses may impact wild bee communities via multiple mechanisms, but these

interactions have not yet been examined. For instance, cheatgrass does not provide food or useful nest-site structures for bees and may gradually replace native forbs by altering disturbance patterns, especially fire cycles (Balch, Bradley, D'Antonio, & Gómez-Dans, 2013)

To provide new information on the interactions between pastoral land use, habitat degradation via invasive species, and wild bee communities, I ask the question “Does livestock grazing (cattle) or site occupancy by cheatgrass impact bee biodiversity relative to non-grazed, non-invaded sites?” To answer this question, our objectives were to (1) compare wild bee nesting and foraging resources in rangeland habitats used for cattle grazing, invaded by cheatgrass, and non-grazed, non-invaded habitats; (2) analyze the relationships between these three habitat types and seasonal variation in bee assemblages and functional dispersion, and (3) characterize associations between foraging and nesting resources and bee functional traits. Our studies provide new insights into the relationship between wild bee communities and dominant ecological processes affecting their habitats in a shortgrass steppe ecosystem, with implications for the management of rangelands and maintenance of pollination services.

2. Materials and methods

2.1. Description of study area and site selection

Study sites were selected in semiarid shortgrass-steppe habitats in the Front Range region of central- and northern-Colorado (Figure 2.1). Sites were typically predominated by blue gramma (*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths) and buffalo grass (*B. dactyloides* (Nutt.); Burke et al. 2008). The shortgrass-steppe has an evolutionary history of ungulate grazing by bison and elk that predates European settlement. Following European settlement, these rangelands have been managed primarily for cattle grazing (Cook and Redente 1993). However, thousands of acres of public domain rangeland areas have been conserved as

natural areas, recreational open spaces, or wildlife refuges by state and federal governments. Many of these areas have also become heavily colonized by invasive species, including cheatgrass (Banks and Baker, 2011). Both public land management agencies and private ranches in the region typically use fenced enclosures to control cattle grazing, and I took advantage of existing enclosures to select rangeland study sites that were actively managed for cattle (hereafter referred to as ‘grazed’ sites, $n = 10$) and sites where cattle were excluded (‘non-grazed’ sites, $n = 20$). Actual stocking rates (AUM/ha) were not accessible at the time of the study. The cattle grazed sites were low in cheatgrass cover (average cheatgrass cover=5.5% per site). Non cattle-grazed sites (wild ungulates including elk and pronghorn antelope are not excluded from cattle enclosures) were further subdivided to represent locations with low ($n = 10$, average cheatgrass cover=2.1% per site) or high ($n = 10$, average cheatgrass cover=26.3% per site, Table 2.1) cheatgrass cover. Sites selected to represent ‘high’ cheatgrass cover were chosen based on the visual criteria of evident and expansive cheatgrass presence over several hectares. All study sites were separated by a minimum distance of 1 km. Permits and permissions for accessing study sites were obtained from multiple agencies including Boulder County Parks and Open Spaces, City of Boulder Open Space and Mountain Parks, City of Fort Collins, and local ranchers.

Ground cover was classified using line point-intercept transects and used to characterize differences among selected study sites (Goodall, 1952; Brady et al., 1995). At each study site, a single central point was established from which an array of five equidistant 50 m transects extended; transects were oriented to 0° , 72° , 144° , 216° and 288° and along each transect an intercept was taken at one meter intervals (250 total intercepts per site). Intercepts at each sample point were recorded as one of six possible categories: rock, bare ground (exposed soil), wood or

litter material, non-*Bromus* grass, cheatgrass (*Bromus* spp. including *B. tectorum*, *B. japonicus*, and *B. inermis*), or actively flowering forbs (floral cover). Only the first intercept contacted by pin drop was counted; therefore, only a single cover category was assigned per intercept.

Intercepts that contacted forbs not actively in flower or woody plants were assigned as ‘litter and wood’ cover, intercepts contacting forbs with active floral displays were assigned as ‘floral cover’. Forbs actively flowering at the time of sampling were also identified in the field to the lowest possible taxonomic level to estimate richness of floral cover. To further account for seasonal variation in bee foraging habitat (floral cover and richness), floral cover sampling was repeated four times during the 2018 growing season in May, June, July, and August with each sampling occurring mid-month.

From this sampling effort I verified that non-grazed sites were reliably grouped into two categories representing areas of variable cheatgrass cover, and that both cattle-grazed and ungrazed sites had similar forb cover and floral richness (Table 2.1). Ground cover data and records of seasonal variation in floral cover and richness were subsequently used to evaluate relationships between bee assemblages and habitat factors (described below in Data analysis section).

2.2. Bee collection procedures

Bees were collected from each study site using a passive trapping method (‘blue vane’ traps). Traps consisted of an ultra-violet reflective blue vane fixed to a yellow collection bucket (SpringStar, Woodinville, WA, USA). Although previous research suggests that bee sampling method may affect detection of habitat factors influencing bee communities (Rhoades et al., 2017), blue vane traps are well suited for collecting across large landscapes as they are easily deployed and are

not biased to observer skill or abilities (Kimoto et al., 2012, Hall 2018 ; Predergast et al., 2020).

Traps were placed at the previously established

central location at each site to sample bee assemblages over four separate periods (May, June,

July, and August) that corresponded with the assessments of floral cover. In each trapping

period, traps were hung from wooden stakes at a height of 1 m, and trap contents were collected

after 48 h. Bees were collected into plastic bags, placed on dry ice, and immediately

returned to the laboratory for curation.

All collected bee specimens were pinned, mounted, sorted to morphospecies and were subsequently identified to the lowest taxonomic level possible, in most cases this was to genus and species. Specimen identifications were confirmed by insect taxonomists external to the study (Scott et al., 2011). Vouchers of identified bee specimens are curated at the C.P. Gillette Museum of Arthropod Diversity at Colorado State University (Fort Collins, Colorado).

2.3. Bee functional traits

Bee qualitative and quantitative functional traits were compiled for the purposes of calculating functional dispersion, a metric that describes the relative diversity of functional traits in a species assemblage (Laliberté and Legendre, 2010). I considered multiple ecological traits related to wild bee life history, behavior, and foraging ranges including diet breadth (lecty), nesting habit and nest locations, pollen carrying structures, sociality, and body size (Michener, 2007).

Traits including intertegular distance (ITD, a proxy for body size) and tibial hair density were resolved using high-resolution photographic methods as follows: photographs were taken for ten replicate specimens (5 male, 5 female) per species from three orientations (head, dorsal and ventral views) for each of 49 species using Canon-EOS Rebel T7i DSLR (49 species \times 3

orientations $\times 10$ specimens per species = 1470 photograph images). For rare species (i.e., those that were represented by less than 5 males and/or 5 females) supplementary specimens were acquired from museum collections (C.P. Gillette Museum of Arthropod Diversity) for photography and trait characterization. ITD was measured from photograph layers using the image J program (Schneider et al. 2012) to generate an average value for each species. For categorical life history traits, I used scientific literature, online databases, books and field observations for traits classification (Appendix 2, Table A.2.1 and Table A.2.2; Michener, 1999; 2007; Scott et al., 2011; Cariveau et al., 2016; Danforth et al., 2019; Hall et al., 2019). Individuals that were not positively identified to species, but able to be identified to genus, were assigned trait values from the closest congener considered to have a similar life history (Michener, 2007). Flight phenology (early, middle, or late-season) was assigned based on the collection period in which abundances were maximized for a given species (Appendix 2, Table A.2.3).

2.4. Data analysis

All analyses were implemented in R version 3.6.2 and, unless otherwise stated, incorporate a Type I error rate of $\alpha=0.05$ for assigning statistical significance. However, modeled effects were interpreted as marginally significant at the $\alpha=0.10$ level. In parametric analyses using continuous variables, response and predictor variables were standardized to meet assumptions of normality and homogeneity.

2.5. Computation of bee diversity indices and functional dispersion.

A bee species abundance matrix was used to derive species-level abundances as well as bee species richness and α -diversity (Shannon's H') for each site \times collection date combination. I computed functional dispersion (FDis) for bee assemblages at each site \times collection date

combination using the methods of Laliberté and Legendre (2010) and the metrics shown in Table S1; FDis was computed using the R add-on package ‘FD’ (Laliberté et al. 2015) and applying the Cailliez correction for non-Euclidean distances generated by inclusion of categorical traits. The metrics of bee species abundance, species richness, diversity, and FDis were used as response variables in the analyses described below.

2.5. Objective 1: analyze the relationships between habitat types and seasonal variation in bee assemblages and functional dispersion.

I examined how cattle grazing or cheatgrass colonization affect bee diversity using several statistical approaches. First, I tested the fixed effects of site classification (n=3) and collection period (n=4; May, June, July, and August) and the site classification \times collection period interaction on the responses of mean bee abundance, richness, diversity, and FDis using a two-way ANOVA model.

Sampling curves were generated to estimate and compare rates of species detection across the three different site classifications and was implemented using the R add-on package ‘iNEXT’ (Hsieh et al., 2016). To quantify β -diversity and turnover in genera across collection periods and sample locations, I used nonmetric multidimensional scaling (NMDS) of Bray-Curtis dissimilarities to evaluate variability in bee community assemblages across habitats and sample month.

2.6. Objective 2: characterize associations between foraging and nesting resources and bee functional traits.

I also examined how variation in foraging and nesting resources affected bee community metrics to determine whether efforts to manage cover would have potential impacts on bee assemblages. I used a generalized linear model with an identity link function to analyze variation

in bee assemblage abundance, richness, diversity, and FDis due to variation in cover composition (rock, bare ground, wood/litter, non-*Bromus* grasses, cheatgrass, and floral cover) and floral richness.

To analyze the associations between specific bee functional traits and local habitat factors I used fourth-corner analysis (Legendre et al., 1997; Brown et al., 2014) implemented in the R add-on package ‘mvabund’ (Wang et al., 2020). Generalized linear models of fourth-corner statistics were fit for bee species abundances as a function of a matrix of species traits and environmental variables (and their 2-way interaction) using a Least Absolute Shrinkage and Selection Operator’s (LASSO) penalty which restricts influences of interactions that do not add to the Bayesian Information Criteria (BIC). Analysis of model deviance was estimated using a Monte-Carlo resampling procedure (9,999 resamples) to evaluate the global significance of trait-environment relationships.

3. Results

3.1. Objective 1: Analyze the relationships between habitat types and seasonal variation in bee assemblages and functional dispersion

A total of 4,368 bees representing four families (Apidae, Colletidae, Halictidae, and Megachilidae) were captured in blue vane traps. The four families were represented by 18 genera and 49 species. The European honeybee, *Apis mellifera*, represented only ~2% of the total collection, indicating that cultured bees had relatively little impact on the study. Three genera including bumble bees (*Bombus* spp.), long-horned bees (*Melissodes* spp.), and furrow bees (*Halictus* spp.) collectively comprised about 63% of the sample (Table 2.2). Rarefaction analysis indicated that rates of species detections were similar among the three habitat classifications (Appendix 2, Figure A.2.1).

There were significant differences in bee community metrics due to site classification, month of collection, and their interaction. Bee abundance varied significantly due to the main effects of site classification ($F_{2, 109} = 3.437$, $P = 0.035$) and collection period ($F_{3,109} = 15.785$, $P < 0.001$), but there was no evidence of an interaction between these terms ($F_{6, 109} = 0.655$, $P = 0.685$; Figure 2.2A). On average, bee trap captures were 18 and 29% higher in sites with high cheatgrass cover than in sites with low cheatgrass cover or sites that were cattle-grazed, respectively. Posthoc tests revealed that this difference was statistically significant and mean bee abundances differed between sites with high cheatgrass cover and cattle-grazed sites, but bee abundances in sites with low cheatgrass cover were intermediate and not statistically different from either category. Average bee captures in June and July were similar and were 66 and 19% higher than captures in May and August, respectively (Table 2.3A).

Bee species richness also varied due to the main effects of site classification ($F_{2, 109} = 8.431$, $P < 0.001$) and collection period ($F_{3,109} = 21.072$, $P < 0.001$), but not their interaction ($F_{6,109} = 0.858$, $P = 0.528$; Figure 2.2B). Post-hoc analysis revealed that captured bee species richness was on average 22% higher in sites with high cheatgrass cover than sites with low cheatgrass cover and cattle-grazed sites (which did not differ from one another). Similar to patterns found for bee abundance, species richness in June and July were similar and were on average 53 and 14% higher than in May and August, respectively (Table 2.3B).

Bee diversity (as measured by Shannon's H' statistic) also varied significantly due to the main effects of site classification ($F_{2, 103} = 10.805$, $P < 0.001$), collection period ($F_{3,103} = 21.485$, $P < 0.001$), as well as their interaction ($F_{6,103} = 2.529$, $P = 0.025$). Early in the growing season sites with high cheatgrass cover had significantly higher diversity than either cattle-grazed or non-grazed sites (which did not significantly differ from one another), but by later in the growing

season, cheatgrass-colonized and non-grazed sites were similar in terms of diversity but diversity significantly declined in cattle-grazed sites (Figure 2.3C; Table 2.3C).

Functional dispersion (Fdis) of bee assemblages varied due to the main effect of site classification ($F_{2,109} = 18.266$, $P < 0.001$) and varied marginally across collection periods ($F_{3,109} = 2.539$, $P = 0.060$), but did not vary due to an interaction between collection period and site classification ($F_{6,109} = 2.048$, $P = 0.158$, Figure 2.2D). Bee FDis was significantly reduced in cattle grazed sites and was on average 28% lower than in non-grazed sites; there was no difference in mean FDis between sites with low- and high cheatgrass cover (Table 2.3D). Post-hoc tests did not reveal clear pairwise differences in FDis across seasons, though Fdis was on average 14% lower in May than in other summer months (Jun-Aug).

Analysis of bee community composition with NMDS indicated distinct differences in species assemblages between cattle-grazed and sites with high cheatgrass cover, but species assemblages in sites with low cheatgrass cover were similar to both cattle-grazed and high-cheatgrass cover sites (Figure 2.3A). Differences in species assemblages between cattle-grazed sites and sites with high cheatgrass cover were generally reflected by a turnover in the ratio of *Bombus*:*Melissodes* species; however, abundances of multiple genera were consistent across site classification (Table 2.2). There were also distinct seasonal differences in the genera composition of bee assemblages with both *Bombus* and *Melissodes* becoming more abundant throughout the season and all other species generally becoming less prevalent (Figure 2.3B), though some genera such as *Agapostemon* were consistent in their abundances throughout the growing season (Appendix 2, Table A.2.3).

3.2. Objective 2: Characterize associations between foraging and nesting resources and bee functional traits.

Linear model analysis testing ability of habitat components (cover) to predict variation in bee assemblages revealed that, although elements of foraging or nesting habitat were not strongly differentiated by site classifications, some were nonetheless good predictors of bee community metrics (Appendix 2, Table A.2.4). Specifically, there was significant positive association between bee abundances and floral cover ($\beta = 0.549$, $P = 0.037$, Figure 2.4A), although the species richness of bee assemblages was not associated with any cover factor or floral richness. Similarly, diversity of bee assemblages was not significantly associated with any cover factors. However, the FDis of bee communities was significantly negatively associated with increasing bare ground cover ($\beta = -0.673$, $P = 0.007$, Figure 2.4B), and FDis was also marginally negatively associated with increasing grass cover ($\beta = -0.848$, $P = 0.066$; Figure 2.4C).

Fourth-corner analysis revealed significant patterns in the correlations between habitat characteristics, bee life history traits, and bee species abundances (model deviance = 3.377, $P < 0.001$). Bee body size (ITD) was positively associated with floral richness, indicating that captured bees tended to be larger as floral richness increased. Bee nest locations were correlated with habitat classification, and below-ground nesters were more abundant in cattle grazed and cheatgrass-colonized, whereas above-ground nesters were less abundant in these areas. Diet breadth was also correlated with environmental conditions and oligolectic bees were less abundant when floral cover was high but more abundant with high grass cover, whereas the opposite was true for polylectic species; kleptoparasitic bee abundances were unrelated to cover or habitat classification. Solitary bees were less abundant in areas where floral cover and richness were high but increased in abundance in areas with high grass cover and bare ground,

whereas social species were more abundant with increasing floral richness but were negatively associated with grass and bare ground cover. Variation in abundances of kleptoparasitic species and species with flexible social behaviors were not related to cover or habitat classification. Only bee species exhibiting early-season phenologies were affected by cover, and early season species were more abundant in areas colonized by cheatgrass. Abundances of bee species also varied due to interactions between pollen collection-related traits and environmental conditions. Bees with scopa pollen collection structures were positively associated with high grass and soil cover but negatively associated with high floral richness and rock cover, whereas bees with corbicula were positively associated with high floral richness and rock cover but negatively associated with cheatgrass and bare ground cover. Variation in tibial hair densities had complex relationships with environmental conditions; bees with high tibial hair densities were more abundant in areas with high grass and soil cover, whereas bees with low tibial hair densities were more abundant in areas with high floral richness and rock cover, and bees with intermediate tibial hair densities were most abundant in areas with high floral and cheatgrass cover (Figure 2.5).

4. Discussion

Cattle-grazing and cheatgrass colonization of shortgrass steppe sites were not associated with large differences in bee foraging habitats (floral cover and species richness) but did reflect a difference in wild bee nesting habitats in terms of the proportion of non-*Bromus* grass cover and woody material on the ground surface (Table 2.1). Despite the modest differences in cover composition across habitat classifications our data provide evidence that cattle grazing is associated with significant reductions in early- and mid-season bee diversity and FDis, but this was not the case in non-grazed sites with dense cheatgrass cover (Figure 2.2). There were distinct differences in community composition between cattle-grazed and non-grazed sites with

high cheatgrass cover that was reflected by turnover in the ratio of *Bombus* spp: *Melissodes* spp.; however, bee assemblages in non-grazed sites with low cheatgrass cover were similar to both grazed sites and those with high cheatgrass cover and were the most variable overall (Figure 2.3). Collectively, these results indicate that FDis in bee communities is more strongly predicted by broad-scale habitat classification (i.e., cattle-grazed vs. high- or low-cheatgrass cover) than cover composition within specific sites, with potential consequences for pollination services in rangelands.

Landscapes in the study region share a long evolutionary history with bison, elk, and other wild grazing and browsing species (Milchunas et al. 1988) and forbs may therefore be well-adapted to tolerate grazing, which could partially explain why no differences in floral cover were observed across site classifications. Nonetheless, floral cover predicted bee abundances with more bees captured from sites with abundant flowering forbs (Figure 2.4). In other recent studies, locations with high floral density have been associated with fewer bee captures in passive traps (e.g., (Rhoades et al., 2017) due to reduced attractiveness of traps when abundant floral resources are available.

Analysis of bee functional traits relative to floral cover and richness revealed that the preponderance of bees at sites with high floral cover were those with life history traits that included sociality, polylecty, and large body size. In our collections, this combination of traits is mostly represented by bumblebees (*Bombus* spp.). Accordingly, management efforts aimed at increasing or restoring local floral densities may be more likely to benefit *Bombus* spp. than other taxa. Interestingly, both cattle-grazed sites and those with high cheatgrass cover had similar relationships with bee functional traits and were positively associated with higher abundances of bees with below-ground nesting habits (Figure 2.5). In some landscapes cattle may trample

sensitive arthropod species resulting in reduced abundances (Bonte and Maes, 2008), but this does not appear to be the case for below-ground nesting bees in our system. Although bee abundances did not differ between grazed and non-grazed sites, cattle grazing was associated with significant reductions in bee FDis, indicating that cattle presence may result in a loss of bee functional diversity. The mechanisms underlying this pattern merit further study, as pollination services are generally improved with increasing bee functional diversity (Martins et al., 2015). Since floral abundance and richness were not negatively affected at grazed sites, I hypothesize that impacts of cattle on bee assemblage functional diversity are mediated via nesting habitats, rather than through indirect consumption-mediated effects on foraging habitat. In other systems cattle grazing has been documented to have positive effects on bee abundances even at very high grazing intensities (Vulliamy et al., 2006), so it may be difficult to generalize cattle-driven effects on bee assemblages.

To our knowledge, this is among the first studies to evaluate the effects of a non-native grass on pollinator assemblages. Our findings suggest that sites with high cover of cheatgrass were not associated with significant reductions in bee abundance, diversity, or FDis; instead, cheatgrass-dominated sites tended to have higher bee abundance and diversity early in the growing season (Figure 2.2). This contrasts with findings from other recent studies; for instance, Bhandari et al., 2018 determined that pollinator abundances in semi-arid pastures were reduced under high densities of non-native forage species. Several non-mutually exclusive hypotheses could potentially explain this pattern. First, it is possible that at cheatgrass-colonized sites vane traps were more visually apparent due to the relatively homogenous structure of the vegetation and thus more attractive to foraging bees. For example, some authors have suggested that passive traps tend to become increasingly attractive when floral displays are not abundant (Rhoades et

al., 2017; Prendergast et al., 2020; Portman et al., 2020). Similarly, bees captured in sites with high cheatgrass cover may be responding from nearby patches of foraging habitat or recruited from other distal locations. Alternatively, sites that are occupied by cheatgrass may simply be on highly productive or suitable soils; in other words, highly productive sites may be generally superior for invasive grasses, forbs, and pollinators alike. However, this seems unlikely as floral cover did not differ between site classifications (Table 2.1), and there was no evidence of a correlation between cheatgrass cover and floral cover (Pearson's $r = 0.08$). Another possibility is that sites with high cheatgrass cover provide some as-of-yet undetermined benefit to nesting habitat, such that bees are more likely to occupy sites with high cheatgrass cover even if there is little relationship between cheatgrass cover and foraging habitat (floral cover). In future studies it will be important to determine whether the effects of cheatgrass colonization are consistently associated with high early-season bee abundance and diversity and, if so, whether these effects are an artifact of sampling strategy or due to some ecological effect such as improved nesting habitat. Accordingly, our findings do not currently suggest a need to mitigate cheatgrass occurrence for pollinator conservation efforts in the shortgrass steppe of Colorado's Front Range.

Seasonal variation in wild bee assemblage richness and functional diversity were considerable, and our sample underscores the importance of making collections across the growing season to generate reliable estimates of bee richness and diversity. There was evident turnover in taxa with certain species of *Eucera*, *Melecta*, and *Osmia* prevalent early in the growing season, but by June and July *Bombus*, *Halictus*, *Lasioglossum*, and *Melissodes* were predominant in study sites (Table 2). Altogether, bee taxa richness and diversity were lowest in the early growing season, which is consistent with other reports (Rhoades et al., 2018) and was

mostly due to the relative inactivity of many social and semi-social species in the spring. Our collection had a lower rate of species detection than other regional studies focusing on bees in Colorado grasslands. For example, Kearns and Oliveras (2009) detected 108 species in grasslands of Boulder County, Colorado and an earlier study by Cockerell (1907) detected 116 species. This could be due in part to differing collection methods used among the different studies; for instance, blue vane traps, hand netting, and bowl traps are known to differ slightly in terms of the community they sample (Rhoades et al., 2017). In general, blue vane traps are biased towards capturing larger bees such as bumblebees, though blue vane traps also tend to capture the greatest overall number of taxa. In addition, these earlier studies found that floral resources were generally positively associated with intermediate levels of cattle grazing. In both earlier studies, collections were continued for several years (up to 5) and using hand netting methods—which is often associated with a higher rate of species detection than passive sampling methods (Rhoades et al., 2017), though rates of species detection in netting-based collections are presumably influenced by observer bias and skill (Westphal et al. 2008). However, bee abundances in the present study were similar to those found in both earlier works. The largest effects on bee diversity and FDis occurred early in the growing season (Figure 2.2), potentially indicating that species active primarily in spring have behavioral or life history traits that predispose them to site disturbance by livestock.

Our study has several limitations that should be considered when interpreting our results. First, this study does not address the potential effects of grazing intensities on pollinator assemblages due to poor availability of stocking rate data across the sites sampled for bee collection, limiting inferential scope to sites that were either grazed or non-grazed. Second, cover data recorded on transects grouped non-flowering forbs into a litter/wood category, which does

not allow us to interpret the effects of non-flowering forbs as a separate category; instead, we considered only flowering forbs (floral cover) as bee foraging resources, but this may not reflect the overall potential of a site to serve as bee habitat. Thirdly, this study did not consider other invasive plants beside *Bromus* spp.; however, other invasive grasses, forbs, or woody species may alter foraging and nesting habitats with consequences for pollinator assemblages. For instance, sites with a high proportion of ruderal or invasive species may fundamentally alter nutritional availability or pollen quality by outcompeting high-value native species. Nonetheless, our study design is useful for making conclusions about whether cattle grazing, invasive grass (*Bromus* spp.) abundance, and seasonality interact to impact regional bee assemblages.

Collectively, our results have several implications for managers concerned with maintaining site occupancy by wild bee assemblages in rangelands where livestock production is a common land use. First, our results do not suggest that floral resources are enhanced in sites managed for cattle grazing as some earlier studies do. Neither did I find any evidence that grazed sites exhibited any reduction in floral resources, likely indicating that grazing practices in the region do not strongly affect bee foraging habitats. Other recent studies indicate that increasing grazing intensities or higher stocking rates are generally associated with a reduction in available floral resources (Lazaro et al., 2016). Here, floral resource availability was an important predictor of bee abundances. Second, bee assemblage composition did vary between grazed and non-grazed sites, and this was reflected by shifts in the ratios of *Bombus* spp: *Melissodes* spp. Further experimental work could help to elucidate whether this turnover in bee taxa is associated with variation in pollination services. Third, both cattle grazing and high cheatgrass cover were associated with reduced site occupancy by above-ground nesting bees but increased site occupancy by below-ground nesting bees. Fourth, cattle grazing was associated with reduced

FD is in early season bee assemblages, and these effects may be mediated by cattle-driven impacts on nesting habitats rather than floral cover. Lastly, our study does not indicate that high cheatgrass cover negatively impacts bee abundance or diversity and suggests it may provide good nesting habitat. The mechanisms underlying this relationship are beyond the scope of the current study, but could have consequences for bee conservation, especially under widespread policies aimed at restoring cheatgrass-invaded habitats. For example, cheatgrass-dominated rangeland and forest sites are often treated with chemical (Baker, Garner & Lyon, 2009), cultural (Cox & Anderson, 2004), and physical (Yong & Clements, 2000) control methods with the general objective of reducing cheatgrass cover. Given that our study found an increased abundance of wild bees in cheatgrass sites, it will be important to determine whether cheatgrass control methods have deleterious, beneficial, or null impacts on bee assemblages to make appropriate management decisions about whether management of invasive grasses is likely to impact native bee conservation.

Table 2.1. A comparison of ground cover (%) and floral richness across grazed and non-grazed rangeland sites.

Variable	Cattle-grazed	High cheatgrass cover	Low cheatgrass cover	<i>F</i> (2,27)	<i>P</i>
Non-<i>Bromus</i> grass	41.4^a± 2.8	13.9^b±1.2	30.7^a±2.0	23.5	<0.001
Cheatgrass (<i>Bromus</i> spp.)	5.5^a±1.4	26.3^b±3.5	2.1^a±0.6	18.6	0.003
Floral cover	8.2 ^a ±3.7	10.2 ^a ±3.1	10.39 ^a ±4.1	0.2	0.890
Litter/wood cover	12.5 ^a ±2.2	10.7 ^a ±1.4	18.9 ^a ±2.2	2.6	0.090
Bare ground cover	12.3 ^a ±1.9	5.8 ^a ±1.1	8.84 ^a ±1.4	2.4	0.110
Rock	1.1 ^a ±0.5	1.9 ^a ±0.6	0.4 ^a ±0.2	1.3	0.291
Floral richness	16.0 ^a ±0.7	19.0 ^a ±0.5	19.0 ^a ±0.5	0.7	0.475

Table 2.2. Table 2. Summary of all bee taxa captured during the study (γ -diversity) and their abundances (total number of trapped individuals).

Family	Genus	species	Habitat category		
			Cattle-grazed	High cheatgrass cover	Low cheatgrass cover
Apidae	<i>Anthophora</i>	<i>affabilis</i>	37	40	31
		<i>bomboides</i>	0	5	2
		<i>montana</i>	43	50	29
		<i>occidentalis</i>	56	26	26
		<i>mellifera</i>	18	33	40
	<i>Apis</i>	<i>mellifera</i>	18	33	40
		<i>appositus</i>	36	60	59
	<i>Bombus</i>	<i>bifarius</i>	1	0	1
		<i>californicus</i>	0	0	2
		<i>centralis</i>	0	2	2
		<i>fervidus</i>	36	113	104
		<i>griseocollis</i>	9	18	19
		<i>huntii</i>	4	28	19
		<i>insularis</i>	0	1	0
		<i>nevadensis</i>	46	157	85
		<i>pensylvanicus</i>	114	197	170
		<i>rufocinctus</i>	2	7	4
		<i>sylvicola</i>	2	6	3
		<i>enavata</i>	23	2	4
		<i>hamata</i>	14	60	30
		<i>lepida</i>	0	4	0
		<i>pacifica</i>	3	15	10
		<i>agilis</i>	120	49	90
		<i>communis</i>	148	131	117
		<i>coreopsis</i>	28	16	37
		sp.1	6	3	6
		<i>tristis</i>	143	64	65
	<i>Svastra</i>	<i>obliqua</i>	45	104	94
		<i>petulca</i>	3	5	10
	<i>Xeromelecta</i>	<i>interrupta</i>	5	16	5
Colletidae	<i>Colletes</i>	Sp.1	2	0	0
Halictidae	<i>Agapostemon</i>	<i>angelicus</i>	24	18	11
		<i>coloradinus</i>	7	1	3
		<i>texanus</i>	14	29	25
		<i>virescens</i>	27	37	14
		<i>aurata</i>	8	14	3
	<i>Halictus</i>	<i>halictus.spp</i>	20	24	33
		<i>ligatus</i>	8	9	3
		<i>tripartitus</i>	139	114	96

Megachilidae	<i>Lasioglossum</i>	<i>dialictus</i>	51	117	64
		Sp.1	8	12	13
	<i>Anthidium</i>	<i>anthidium.spp</i>	0	7	6
	<i>Lithurgopsis</i>	<i>apicalis</i>	6	10	10
	<i>Megachile</i>	<i>dentitarsus</i>	1	1	2
		sp.1	2	13	4
		sp.2	23	38	25
		sp.3	2	0	2
	<i>Osmia</i>	sp.1	6	18	2
		sp.2	4	15	1
		sp.3	1	1	2

Table 2.3: Summary of post-hoc tests comparing bee community metrics across collection period and habitat type.

Response variable	Factors	Factor levels	Mean \pm SE	Grouping (Tukey's HSD)
(a) Bee abundance	Habitat	Cattle-grazed	29.76 \pm 3.06	B
		Low cheatgrass cover	34.85 \pm 3.17	AB
		High cheatgrass cover	42.25 \pm 3.12	AB
	Month	May	15.67 \pm 3.03	C
		June	40.32 \pm 3.65	AB
		Jul	49.83 \pm 5.00	AB
		Aug	36.58 \pm 2.25	B
	Habitat * month	n.s.	-	-
(b) Bee richness	Habitat	Cattle-grazed	9.14 \pm 0.73	B
		Low cheatgrass cover	10.61 \pm 0.71	B
		High cheatgrass cover	12.50 \pm 0.69	AB
	Month	May	6.21 \pm 0.77	C
		June	11.96 \pm 0.74	AB
		Jul	13.38 \pm 0.84	A
		Aug	10.90 \pm 0.46	B
	Habitat * month	n.s.	-	-
(c) Shannon diversity	Habitat	Cattle-grazed	1.58 \pm 0.10	B
		Low cheatgrass cover	1.85 \pm 0.09	A
		High cheatgrass cover	2.05 \pm 0.06	A
	Month	May	1.27 \pm 0.13	B
		June	2.02 \pm 0.07	A
		Jul	2.09 \pm 0.08	A
		Aug	1.93 \pm 0.05	A
	Habitat * month	Cattle-grazed, May	0.85 \pm 0.24	C
		Cattle-grazed, Jun	1.87 \pm 0.15	A
		Cattle-grazed, Jul	1.78 \pm 0.15	AB
		Cattle-grazed, Aug	1.87 \pm 0.09	A
		Low cheatgrass cover, May	1.14 \pm 0.18	BC
		Low cheatgrass cover, Jun	2.05 \pm 0.12	A
		Low cheatgrass cover, Jul	2.29 \pm 0.08	A
		Low cheatgrass cover, Aug	1.93 \pm 0.08	A

		High cheatgrass cover, May	1.85 ± 0.15	A
		High cheatgrass cover, Jun	2.15 ± 0.11	A
		High cheatgrass cover, Jul	2.22 ± 0.12	A
		High cheatgrass cover, Aug	1.99 ± 0.12	A
(d) Functional disp.	Habitat	Cattle-grazed	0.23 ± 0.01	B
		Low cheatgrass cover	0.32 ± 0.00	A
		High cheatgrass cover	0.26 ± 0.00	A
	Month	May	0.26 ± 0.02	A
		June	0.30 ± 0.01	A
		Jul	0.30 ± 0.01	A
		Aug	0.30 ± 0.01	A
	Habitat * month	-	-	-

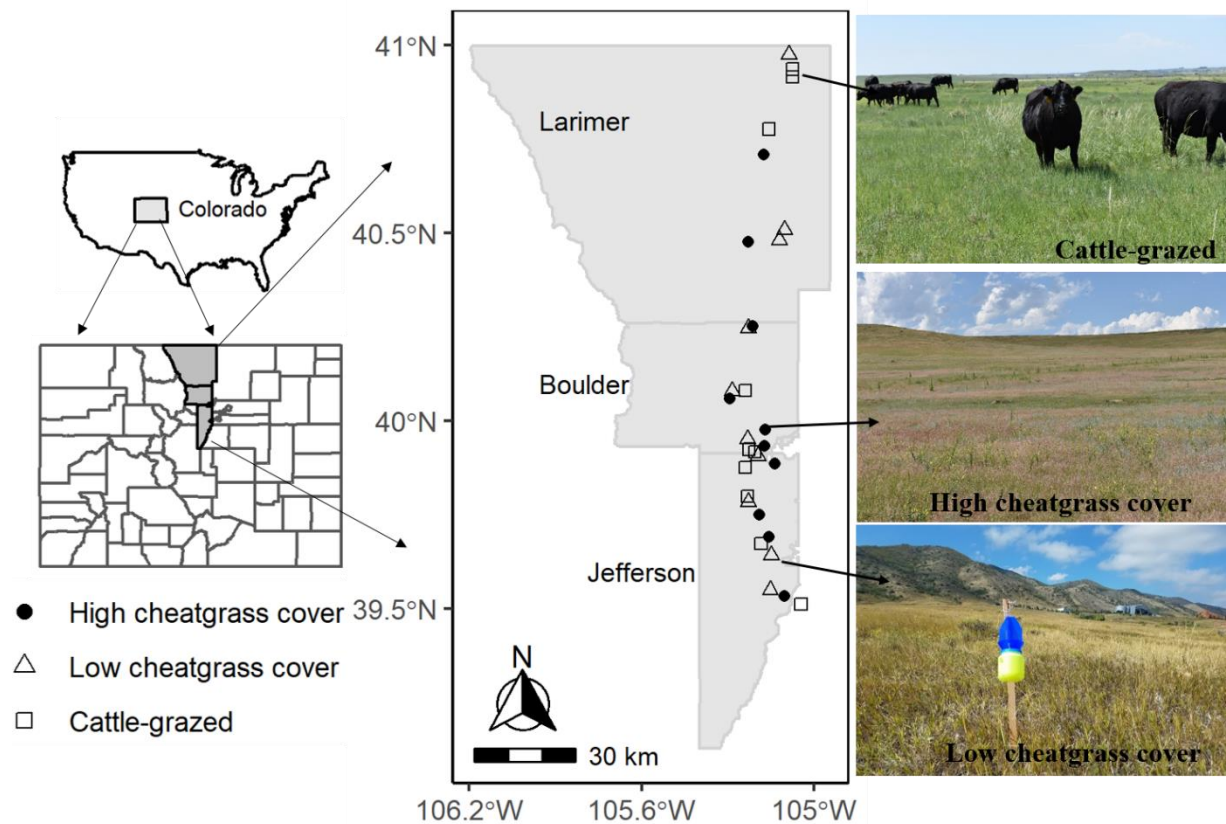


Figure 2.1. Approximate location of 30 shortgrass steppe study sites distributed across the Colorado Front Range and representative photographs of sites. Study locations were comprised of cattle-grazed sites, sites heavily colonized by cheatgrass (*Bromus* spp.), and sites that were non-grazed and with minimal cheatgrass cover.

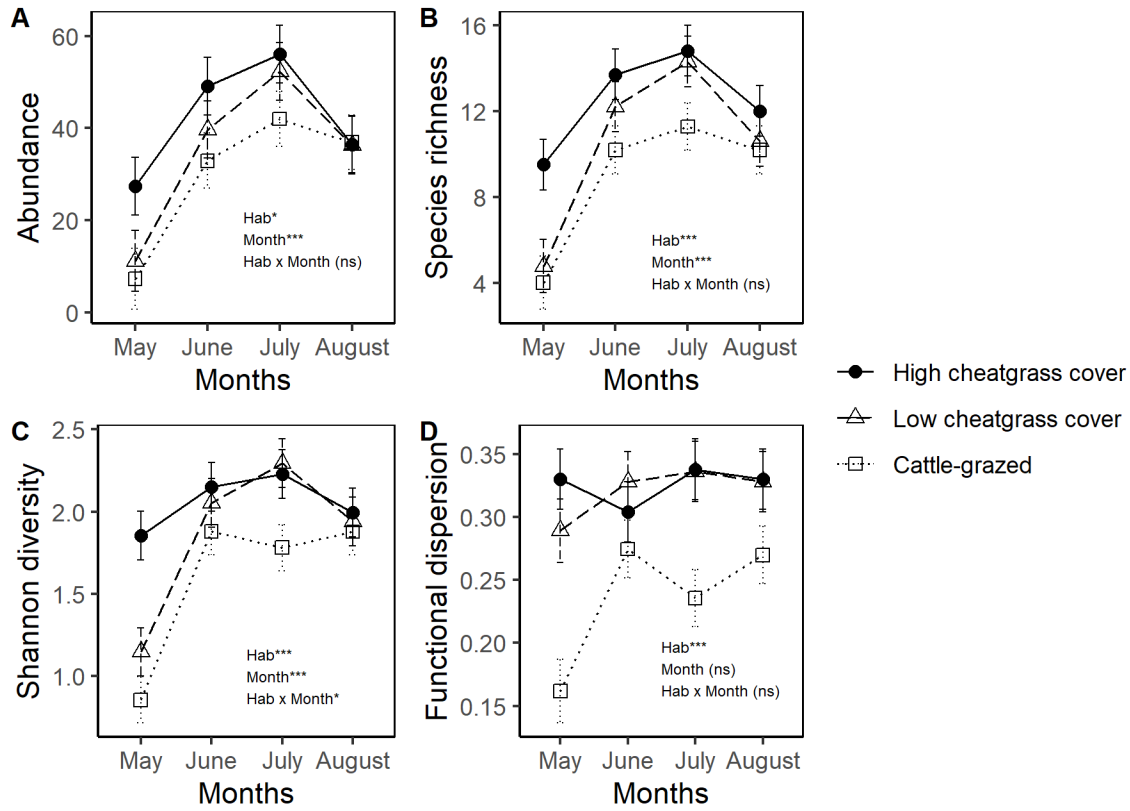


Figure 2.2. Bee community metrics vary across grazing treatments and seasonality. Variation in mean (A) bee abundance, (B) species richness (C) diversity, and (D) FDis is represented as a habitat classification \times collection period interaction. Asterisks denote significance of main effects (habitat, month of collection) and their interaction, and error bars show ± 1 SE of the mean. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

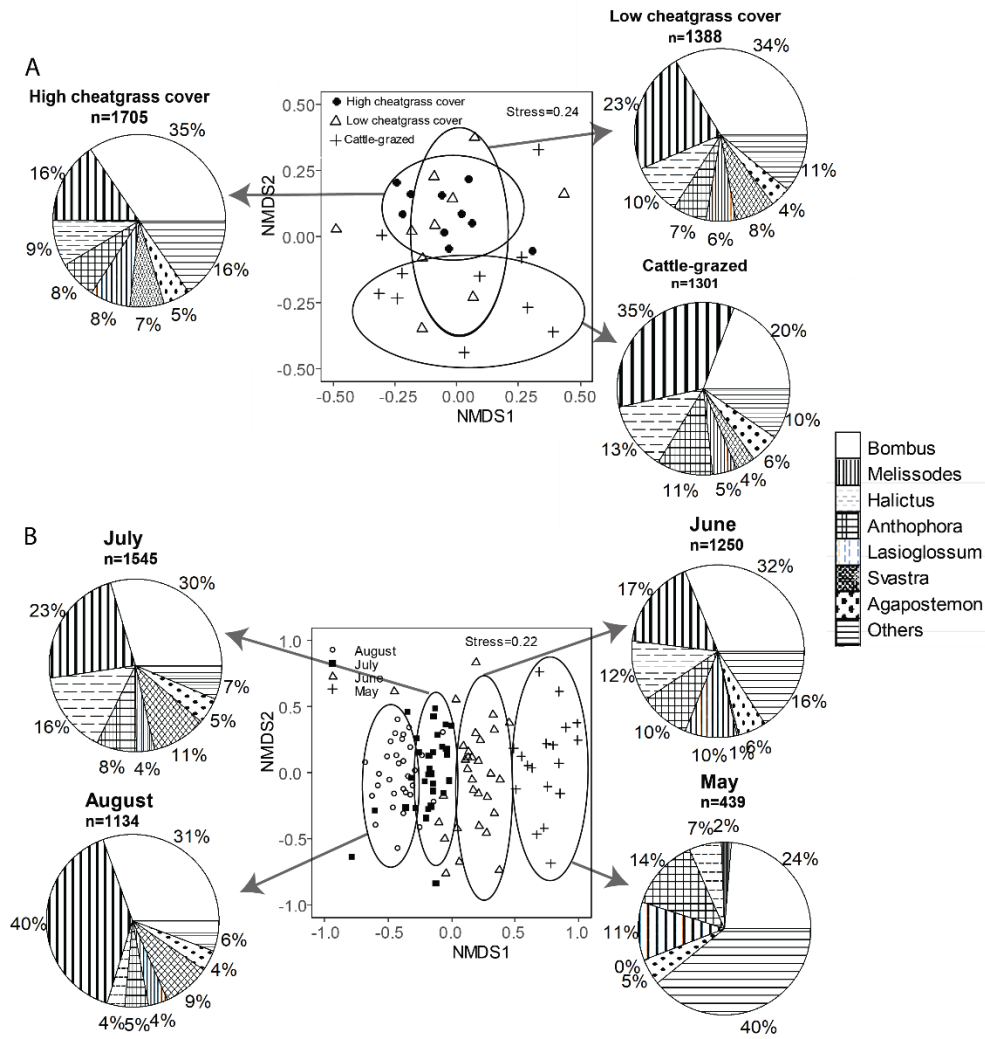


Figure 2.3. Variation in bee assemblages in cattle-grazed, cheatgrass-colonized, and non-grazed sites. Non-metric multidimensional scaling (NMDS) plots of bee assemblages (grouped by genera) pooled across (A) habitat classifications and (B) collection periods.

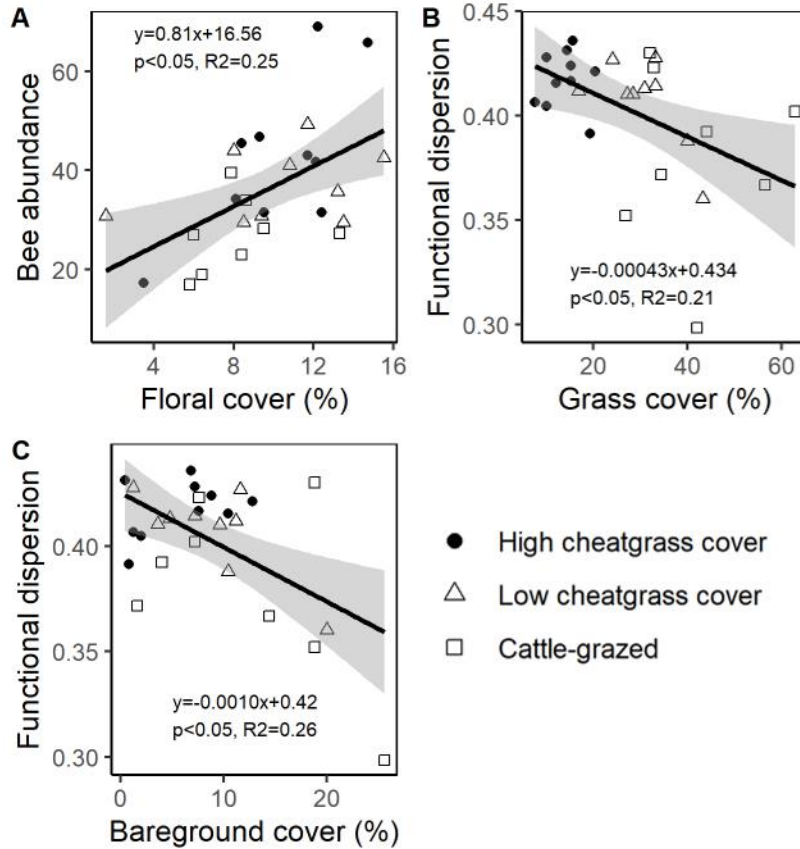


Figure 2.4. Ground cover impacts bee abundance and functional dispersion. (A) Floral cover is associated with increases in bee abundances, but both (B) non-*Bromus* grass cover and (C) bare ground cover are associated with reduced functional dispersion in bee assemblages. Gray shading shows 95% confidence intervals and regression equations are provided in each panel.

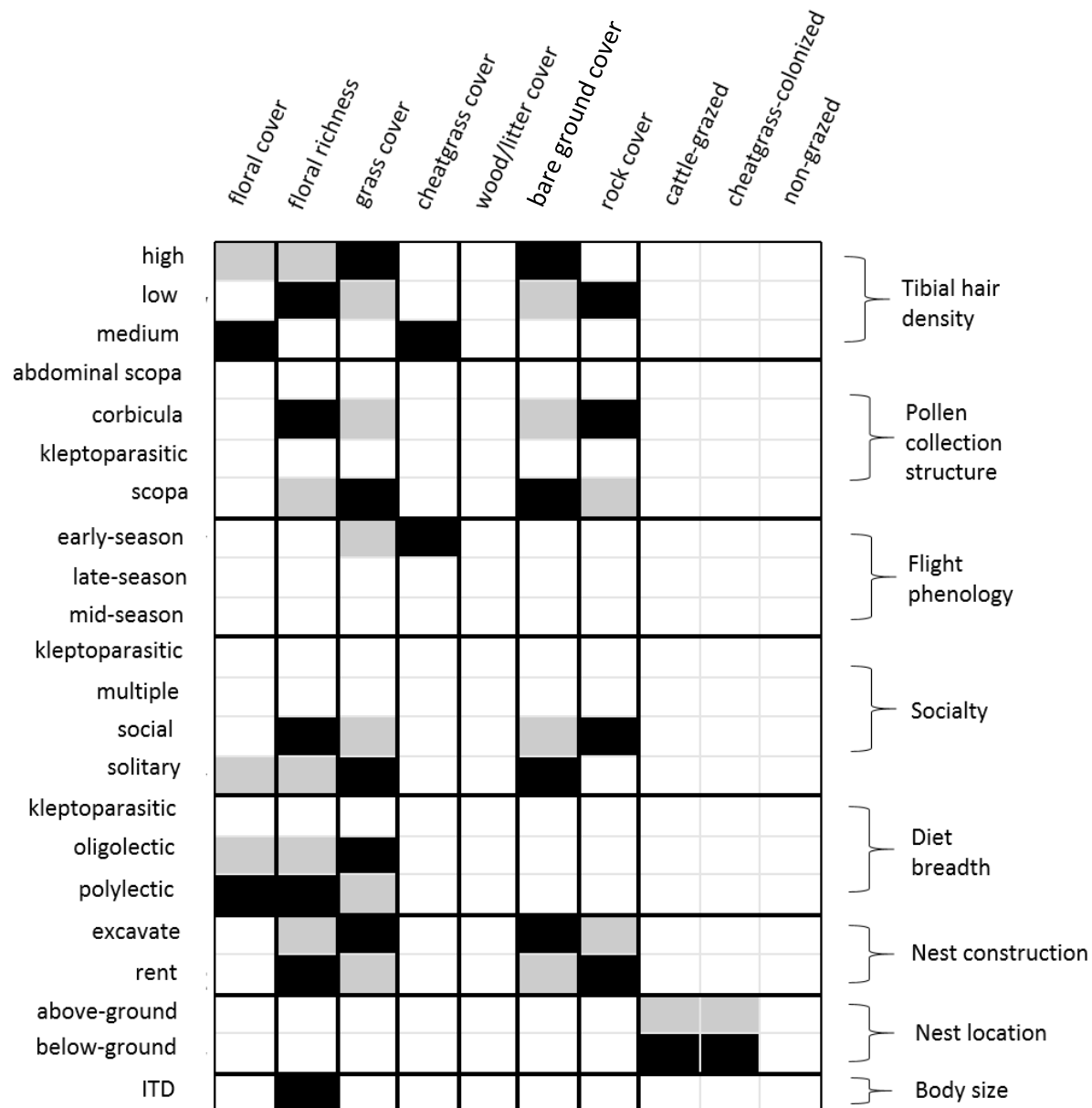


Figure 2.5. Relationship between habitat factors and frequency of bee functional traits. Summary of fourth-corner analysis to model bee species abundances as a function of life history trait \times environment interactions. Black cells indicate positive regression coefficients, gray cells indicate negative coefficients. Blank cells indicate no relationship. Identified correlations are significant at $P < 0.10$.

REFERENCES

- Alkemade R., Reid R. S., van den Berg M., de Leeuw J., & Jeuken M. (2013). Assessing the impacts of livestock production on biodiversity in rangeland ecosystems. *Proceedings of the National Academy of Sciences of the USA* 110:20900–20905.
- Baker W.L., Garner J., & Lyon P. (2009). Effects of imazapic on cheatgrass and native plants in Wyoming big sagebrush restoration for Gunnison sage-grouse. *Natural Areas Journal* 29:204–209.
- Balch J.K, Bradley B.A., D’Antonio C.M., & Go ’mez-Dans J. (2013). Introduced annual grass increases regional fire activity across the arid western USA (1980–2009). *Global Change Biology* 19:173–183.
- Banks R. E., & Baker W. L. (2011). Scale and pattern of cheatgrass (*Bromus tectorum*) invasion in Rocky Mountain National Park. *Natural Areas Journal* 31:377–390.
- Bauer A. A., Clayton M. K., & Brunet J. (2017). Floral traits influencing plant attractiveness to three bee species: Consequences for plant reproductive success. *American Journal of Botany* 104:772–781.
- Bhandari K. B., West C. P., Longing S. D., Brown C. P., Green P. E., & Barkowsky E. (2018). Pollinator abundance in semiarid pastures as affected by forage species. *Crop Science* 58:2665–2671.
- Bonte D., & Maes D. (2008). Trampling affects the distribution of specialised coastal dune arthropods. *Basic and Applied Ecology* 9:726–734.
- Brady, W.W., Mitchell, J.E., Bonham, C.D., & Cook, J.W. 1995. Assessing the power of the point-line transect to monitor changes in basal plant cover. *Journal of Range Management* 48: 187-190.
- Goodall, D.W. 1952. Some considerations in the use of point quadrats for the analysis of vegetation. *Australian Journal of Scientific Research, Series B* 5: 1-41.
- Brown A. M., Warton D. I., Andrew N. R., Binns M., Cassis G., & Gibb H. (2014). The fourth-corner solution—using predictive models to understand how species traits interact with the environment. *Methods in Ecology and Evolution* 5:344–352.
- Burke I. C., Lauenroth W. K., Antolin M. F., Derner J. D., Milchunas D. G., Morgan J. A., et al. (2008). The future of the shortgrass steppe. In: Lauenroth W. K. & Burke I. C., eds. *Ecology of the Shortgrass Steppe: A Long-Term Perspective*. Oxford University Press, New York. P 484–510.
- Cariveau D. P., Nayak G. K., Bartomeus I., Zientek J., Ascher J. S., Gibbs J., et al. (2016). The allometry of bee proboscis length and its uses in ecology. *PLOS ONE* 11:e0151482.
- Carvell C. (2002). Habitat use and conservation of bumblebees (*Bombus* spp.) under different grassland management regimes. *Biological Conservation* 103:33–49.
- Cockerell, T. D. A. (1907) The bees of Boulder County, Colorado. *Univ Colo Stud* IV (4), Boulder. Ca. Paper 373.
- Cook W., & Redente E. (1993). Development of the ranching industry in Colorado. *Rangelands* 15:204–207.
- Cox R.D., & Anderson V.J. (2004). Increasing native diversity of cheatgrass-dominated rangeland through assisted succession. *Rangeland Ecology and Management* 57:203–210.

- Danforth B. N., Minckley R. L., Neff J. L., & Fawcett F. (2019). *The Solitary Bees: Biology, Evolution, Conservation*. Princeton University Press, New Jersey. 488 p.
- Goergen E. M., Leger E. A., & Espeland E. K. (2011). Native perennial grasses show evolutionary response to *Bromus tectorum* (cheatgrass) invasion. *PLOS ONE* 6: e18145.
- Greenleaf S. S., & Kremen C. (2006). Wild bees enhance honey bees' pollination of hybrid sunflower. *Proceedings of the National Academy of Sciences of the USA* 103:3890–3895.
- Hall M. A. (2018). Blue and yellow vane traps differ in their sampling effectiveness for wild bees in both open and wooded habitats. *Agricultural and Forest Entomology* 20:487–495.
- Hall M. A., Nimmo D. G., Cunningham S. A., Walker K., & Bennett A. F. (2019). The response of wild bees to tree cover and rural land use is mediated by species' traits. *Biological Conservation*, 231, 1–12.
- Hsieh T.C., Ma K.H., & Chao A. (2016). iNEXT: an R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution* 7:1451–1456.
- Jones P. L., & Agrawal A. A. (2017). Learning in insect pollinators and herbivores. *Annual Review of Entomology* 62:53–71.
- Kearns C. A., & Oliveras D. M. (2009). Environmental factors affecting bee diversity in urban and remote grassland plots in Boulder, Colorado. *Journal of Insect Conservation* 13:655–665.
- Kearns C. A., Inouye D. W., & Waser N. M. (1998). Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics* 29:83–112.
- Kimoto C., DeBano S. J., Thorp R. W., Taylor R. V., Schmalz H., DelCurto T., et al. (2012). Short-term responses of native bees to livestock and implications for managing ecosystem services in grasslands. *Ecosphere* 3:1–19.
- Kremen C., Williams N. M., Aizen M. A., Gemmill-Herren B., LeBuhn G., Minckley R., et al. (2007). Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecology Letters* 10:299–314.
- Kruess A., & Tscharnkte T. (2002a). Contrasting responses of plant and insect diversity to variation in grazing intensity. *Biological Conservation* 106:293–302.
18. Kruess A., & Tscharnkte T. (2002b). Grazing intensity and the diversity of grasshoppers, butterflies, and trap-nesting bees and wasps. *Conservation Biology* 11:570–1580.
- Laliberte ´ E., & Legendre P. (2010). A distance-based framework for measuring functional diversity from multiple traits. *Ecology* 91:299–305.
- Laliberte ´ , E., Legendre, P. & Shipley, B. (2015). Package 'FD': Measuring functional diversity (FD) from multiple traits, and other tools for functional ecology. V.1.0–12.
- Lazaro A., Tscheulin T., Devalez J., Nakas G., & Petanidou T. (2016). Effects of grazing intensity on pollinator abundance and diversity, and on pollination services. *Ecological Entomology* 41:400–412.
- Legendre P., Galzin R., & Harmelin-Vivien M. L. (1997). Relating behavior to habitat: solutions to the fourth-corner problem. *Ecology* 78:547.
- Levine, J. M., Vilà, M., Antonio, C. M. D., Dukes, J. S., Grigulis, K., & Lavorel, S. (2003). Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270:775–781.
- MacInnis G., & Forrest J. R. K. (2019). Pollination by wild bees yields larger strawberries than pollination by honey bees. *Journal of Applied Ecology* 56:824–832.

- Martins K. T., Gonzalez A., & Lechowicz M. J. (2015). Pollination services are mediated by bee functional diversity and landscape context. *Agriculture, Ecosystems & Environment* 200:12–20.
- Michener C. D. (2007). *The bees of the world*, 2nd edition. The Johns Hopkins University Press, Baltimore. 953 p.
- Milchunas D. G., Sala O. E., & Lauenroth W. K. (1988). A generalized model of the effects of grazing by large herbivores on grassland community structure. *American Naturalist* 13:87–106.
- Moreira X., Castagneyrol B., Abdala-Roberts L., & Traveset A. (2019). A meta-analysis of herbivore effects on plant attractiveness to pollinators. *Ecology* 100:e02707.
- Murray T. E., Fitzpatrick U., Byrne A., Fealy R. Brown M. J., & Paxton R. J. (2012). Local-scale factors structure wild bee communities in protected areas. *Journal of Applied Ecology* 49:998–1008.
- Ollerton J., Winfree R., & Tarrant S. (2011). How many flowering plants are pollinated by animals? *Oikos* 120:321–326.
- Parkinson H., Zabinski C., & Shaw N. (2013). Impact of native grasses and cheatgrass (*Bromus tectorum*) on Great Basin forb seedling growth. *Rangeland Ecology & Management* 66:174–180.
- Portman Z.M., Bruninga-Socolor B., & Cariveau D.P. (2020). The state of bee monitoring in the United States: a call to refocus away from bowl traps and towards more effective methods. *Annals of the Entomological Society of America* 113:337–342.
- Potts S. G., Biesmeijer J. C., Kremen C., Neumann P., Schweiger O., & Kunin W. E. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution* 25:345–353.
- Potts S. G., Vulliamy B., Roberts S., O'Toole C., Dafni A., Ne'eman G., et al. (2005). Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecological Entomology* 30:78–85.
- Prendergast K.S., Menz M.H., Dixon K.W., & Bateman P.W. (2020). The relative performance of sampling methods for native bees: an empirical test and review of the literature. *Ecosphere* 11: e03076.
- Rafferty N. E. (2017). Effects of global change on insect pollinators: multiple drivers lead to novel communities. *Current Opinion in Insect Science* 23:22–27.
- Rhoades P. R., Davis T. S., Tinkham W. T., & Hoffman C. M. (2018). Effects of seasonality, forest structure, and understory plant richness on bee community assemblage in a southern Rocky Mountain mixed conifer forest. *Annals of the Entomological Society of America* 111:278–284.
- Rhoades P., Griswold T., Waits L., Bosque-Perez N. A., Kennedy C. M., & Eigenbrode S. D. (2017). Sampling technique affects detection of habitat factors influencing wild bee communities. *Journal of Insect Conservation* 21:703–714.
- Roulston T. H., & Goodell K. (2011). The role of resources and risks in regulating wild bee populations. *Annual Review of Entomology* 56:293–312.
- Schneider C. A., Rasband W. S., & Eliceiri K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9:671–675. <https://doi.org/10.1038/nmeth.2089> PMID: 22930834
37. Michener C. D. (1999). The corbiculae of bees. *Apidologie* 30:67–74.

- Scott V. L., Ascher J. S., Griswold T., & Nufio C. R. (2011). The bees of Colorado (Hymenoptera: Apoidea: Anthophila). Natural History Inventory of Colorado, University of Colorado Natural History Museum, Boulder. 101 p.
- Sjodin N. E. (2007). Pollinator behavioural responses to grazing intensity. *Biodiversity and Conservation* 16:2103–2121.
- Sjodin N. E., Bengtsson J., & Ekbom B. (2007). The influence of grazing intensity and landscape composition on the diversity and abundance of flower-visiting insects. *Journal of Applied Ecology* 45:763– 772.
- Sugden E.A. (1985). Pollinators of *Astragalus monoensis* Barneby (Fabaceae): new host records: potential impact of sheep grazing. *The Great Basin Naturalist* 45:299–312.
- Vanbergen A. J., & The Insect Pollinators Initiative. (2013). Threats to an ecosystem service: pressures on pollinators. *Frontiers in Ecology and the Environment* 11:251–259.
- Vulliamy B. G., Potts S. G., & Willmer P. (2006). The effects of cattle grazing on plant-pollinator communities in a fragmented Mediterranean landscape. *Oikos* 114:529–543.
- Wang, Y., Naumann, U., Eddelbuettel, D., Wilshire, J., Warton, D., Byrnes, J., et al. (2020). Package ‘mvabund’: Statistical methods for analyzing multivariate abundance data. V. 4.1.3.
- Westphal C., Bommarco R., Carre ´ G., Lamborn E., Morison N., Petanidou T., et al. (2008). Measuring bee diversity in different European habitats and biogeographical regions. *Ecological Monographs* 78:653–671.
- Young J.A., & Clements C.D. (2000). Cheatgrass control and seeding. *Rangelands* 22:3–7.

CHAPTER 3: BUMBLEBEE (HYMENOPTERA: APIDAE) VISITATION FREQUENCY DRIVES SEED YIELDS AND INTERACTS WITH SITE-LEVEL SPECIES RICHNESS TO DRIVE POLLINATION SERVICES IN SUNFLOWER

1. Introduction

Wild bees provide pollination services to wild plants and economically valuable crops in both natural and agriculture ecosystems and are important for maintaining biodiversity and ecosystem function (Kearns et al. 1998, Kremen et al. 2007). Roughly 90% of the world's angiosperm species are animal pollinated, and in temperate ecosystems native bee communities are the primary flower visitors and are responsible for most plant-pollinator interactions (Ollerton et al. 2011). Multiple studies demonstrate that in addition to cultured bees (i.e, *Apis mellifera* L.), wild bees are also of vital importance to numerous agricultural crops (Greenleaf and Kremen 2006, Albrecht et al. 2012). For example, in strawberry crops visitation by wild bees is associated with heavier fruit yield than when flowers are visited by honeybees alone (Horth and Campbell 2018; MacInnis and Forrest 2019). Similar patterns have been shown for wild bee visitations and yields of pumpkin (Petersen et al. 2013), blueberry (Isaacs and Kirk 2010), sunflower (Greenleaf and Kremen 2006), and a variety of other crop species (Kremen 2008). Consequently, it is now widely understood that native bee communities play a vital role in supporting agricultural economies and are critical for the maintenance of food systems important to human wellbeing.

Many researchers, agriculturalists, and policy makers have expressed significant concern over recent reports of decline in native pollinator population abundance, richness, and diversity, and the corresponding potential consequences for agronomic yields in food systems (Gallai et al.

2009). However, it is difficult to generalize about relationships between native bee assemblages and yields; diversity-yield relationships are not uniform and may vary due to effects of environmental conditions, crop species, and species interactions (Albrecht et al. 2012). For example, bee behaviors may be better predictors of pollination services than diversity metrics alone, as bee taxa differ in their floral visitation times, foraging distances, and pollen collection structures (Michener et al. 1994). Taxa also vary in their dietary breadth—some genera forage on a wide variety of floral resources (e.g., *Bombus* spp.) whereas others are restricted in terms of the flowers they visit or overlap with phenologically (e.g., *Svastra* spp.). Accordingly, the evenness or richness of bee assemblages may be less important than the presence of specific taxa or bee functional traits for pollination services in some environments.

Here, our objective is to quantify variation in the visitation behaviors of predominant bee taxa found in shortgrass steppe sites distributed along the Front Range of Colorado and test effects of bee community composition and richness on pollination services. I used sunflower (*Helianthus annuum* L.) as a focal floral species for studies of pollination services; sunflower is naturalized in the Front Range region and grows in wild populations, but also requires outcrossing for optimal seed development. In addition, sunflower has the advantage of being easily grown in the greenhouse for controlled experiments and produces large individual inflorescences that can be experimentally excluded from visitation by pollinators. Specifically, I asked the following questions: (1) what are the primary bee taxa visiting sunflowers in the field and how do their visitation behaviors (alightment frequency and average time of visit per bee) differ?; and (2) does localized bee richness drive pollination services in experimental or natural sunflower populations?

2. Materials and methods

2.1. Study area

The experiments were conducted in shortgrass steppe habitats distributed across three counties (Larimer, Boulder, and Jefferson County) in the Front Range region of Colorado. All study locations were selected to maintain a minimum distance of one kilometer between sites to reduce the likelihood of bee movement among sites and to control for potential autocorrelation effects. Studies performed with potted sunflower spanned 18 sites that were selected *a priori* to reflect a range of bee α -diversities (from low to high); sites were selected based on data from recent regional studies of bee diversity (Thapa-Magar et al. 2020). Studies performed on wild sunflower were conducted at 15 sites selected to represent locations with high abundances of naturally occurring sunflower (Figure 1).

2.2. Experimental design

I conducted three experiments to test effects of bee community assemblages on sunflower (*H. annuum*) seed yields. The first experiment tested how differences in bee visitation behaviors (alightment frequency and total visitation time) impact sunflower seed yields, and the second and third experiments tested effects of bee assemblage richness on yields in potted and wild sunflowers, respectively.

2.2.1. Greenhouse conditions for potted sunflower.

Potted sunflowers were grown from organic seed (var ‘Big Smile’; Johnny’s Selected Seeds, Portland, ME); ‘Big Smile’ is an open-pollinated dwarf variety (20-30 cm height) that was selected to maintain plants at a manageable size for movement between the greenhouse and field. This variety typically matures to inflorescence within 50-60 days and produces a gold-to-yellow-colored inflorescences similar to wild sunflower. I sowed seed of sunflowers into 4 L pots containing a sphagnum peat moss-based growing medium (PRO MIX BX-General Purpose,

PRO MIX Inc., Quakertown, PA); the greenhouse photoperiod was 16L:8D and mean day and night temperatures were 23°C and 18°C, respectively. The first sowing was in April and several staggered plantings were made to ensure a ready supply of test flowers. Pots were watered *ad libitum* while in the greenhouse. Mesh bags constructed of fine screening (No See Um' Mosquito Netting, Seattle Fabrics, Seattle, WA) were placed over inflorescences during development and secured using binder clips to prevent insect damage in the greenhouse and to serve as a treatment for inducing pollination deficit in the field. At 50 days post-sowing (late May and early June), inflorescences began to open, and plants were considered ready for transfer to the field.

2.2.2. Experiment 1: effects of visitation frequency and foraging time of bee genera on seed yields.

Bee behaviors were intensively monitored at a single site to quantify variability in bee alightment frequencies (number of visits by different taxa) and visitation times (recorded as min:sec per visitation event) to potted sunflowers, and to link visits by different taxa to pollination services. Between June 1-3 (2019) a total of 39 potted sunflowers with bagged inflorescences were deployed in the field for testing. Inflorescences were unbagged for a period of 12 minutes and were monitored simultaneously by two observers trained in bee taxonomy to record bee visitors to the inflorescence. Observers recorded alightment of bees on inflorescences, and a stopwatch was used to record the length of time that bees were in contact with inflorescences. Bees visiting inflorescences were recorded at a genus level to ensure accurate classification and were restricted to *Agapostemon*, *Bombus*, *Halictus*, *Lasioglossum*, *Megachile*, *Melissodes*, and *Svastra*; these genera comprised 83% of all captured bees in a recent regional study (Thapa-Magar et al. 2020). Other visitors were excluded from visitation by observers to prevent simultaneous visitations by multiple bees that would have complicated the ability to

accurately time contact duration. This process of unbagging, recording visitation frequency and time, and classifying bee visitor genus was performed for only one inflorescence at a time (n=13 per day). Observations took place under sunny/fair weather conditions and during morning and early afternoon hours; observations were summarized to yield (a) total number of visits per taxa per inflorescence and (b) total (summed) visitation time per taxa per inflorescence.

After allowing bees to visit and forage on flowers, inflorescences were re-bagged and potted plants were returned to the greenhouse for maturation where *ad libitum* watering was resumed. Sunflower plants were monitored until dry-drown, at which point plants were cut at the base. Inflorescences were removed from stalks, and seeds were collected. Stalks and empty inflorescences were subsequently dried at 70°C in an oven for 24 h, and plant dry biomass was weighed. Collected seed was sorted based on grain fill; those with unfilled or incomplete grains were scored as ‘inviable’ and those with filled grains were scored as ‘viable’. Viable and inviable seeds were counted, and total mass of viable seed was recorded as a metric of agronomic yield (g viable seed/plant).

This study design allowed us to quantify bee visitation times to sunflower by genus and determine how both visitation frequency and total visitation time of different genera affected pollination services, as measured by seed yield. A one-way ANOVA was used to compare differences in mean visitation times among genera, and all pairwise comparisons of means were made using Tukey’s HSD test. A forward-stepwise model selection procedure was used to analyze the effects of visitation frequency (# visits) and visitation time (min: sec) of each recorded bee genus on the response variable of viable seed yield (g), where each individual inflorescence was treated as an experimental replicate. Criteria for selection of the final model was minimization of Akaike’s information criteria (AIC; Akaike 1974). Significance was

assigned to modeled effects using *F*-tests; a Type I error rate of $\alpha=0.05$ was used to interpret statistical significance. Prior to model interpretation, homoscedasticity was verified by visual inspection of residuals.

2.2.3. Experiments 2 and 3: effects of bee richness on pollination services in sunflower.

To evaluate how local bee richness and the proportion of bees that are ‘long visitors’ interact to impact pollination services, two additional experiments were performed to allow for short-term (4-d) and long-term (one month) access of bee assemblages to inflorescences of potted and wild growing sunflowers.

2.2.3.1. Potted sunflower test.

During June 2019, eighteen sites (Figure 3.1) were each provisioned with ten 50-d old potted sunflower plants (N=180 experimental plants), pots were arranged in an approximately equidistant array with pots spaced >1 m apart. To control effects of landscape variability on our tests, cover type (shortgrass steppe) was consistent within a 400 m buffer of sampling locations. Inflorescences from seven potted sunflowers at each site were randomly selected and unbagged so bee assemblages could freely access inflorescences for a period of 4-d; inflorescences on three plants remained bagged as a means of excluding bees (and other visitors) and experimentally inducing pollination deficit. While potted plants were in the field, they were placed in filled watering trays to provide access to water. After 4-d, unbagged inflorescences were re-bagged and all pots were returned to the greenhouse for sunflower maturation and seed collection.

Immediately following collection of plants, bee assemblages were sampled at study sites using passive trapping methods. I chose not to sample bee assemblages during the period where bees could access inflorescences so that sampling did not unintentionally induce pollination deficit or otherwise impact bee-flower interactions. Blue vane traps (Springstar, Inc.,

Woodinville, WA) were hung from construction stakes at a height of 1 m for a period of 48-h; after this period trap contents were collected into plastic bags, placed on dry ice, and returned to the laboratory for curation and specimen identification (Figure 3.2). All taxa were identified to genus, and many to species. Bee collections were subsequently used to compute species-level richness and diversity.

2.2.3.2. Wild sunflower test.

The same experiment was repeated later in the field season during August 2019 across 16 sites (Figure 3.1), except that the second experiment investigated effects of pollination deficit and bee richness on stands of wild sunflowers growing in the field. Individual plants (stalks) and flowers were selected randomly for the experiment, and two immature (i.e., not yet fully emerged) inflorescences of similar size and height were selected on each experimental plant. One of the inflorescences was selected to receive a pollination deficit treatment (bagged) in early July, and the other inflorescence was left open; the number of plants treated at sites ranged from 3-12 (6 to 24 experimental flowers per site). Test sunflowers were harvested for seed collection 25 days after pollination deficit treatments were implemented and it was evident that petals had senesced. As a covariate of bee richness, abundance of sunflowers actively in bloom in the immediate area (1 ha) was counted and ranged from 9-900 flowers per hectare. As described above in the experiment testing effects of bee richness on potted sunflower yields, bee assemblages were sampled immediately post-harvest of sunflower using blue vane traps.

In both experiments, the list of taxa captured at each site immediately following harvest of flowers was used to generate metrics of bee assemblage diversity (species-level richness and Shannon-Weiner diversity) and compute the proportion of the sample that was comprised of ‘long visitors’ at study sites (i.e., the relative abundance [%] *Bombus* in the sample). These

metrics were used as factors to analyze effects of species-level bee richness and relative abundance of a specific genus (*Bombus* spp.) on pollination services. I elected to exclude Shannon-Weiner diversity as a model effect due to high collinearity with bee richness.

‘Pollination service’ was defined as the quotient of mean seed yields between open-pollinated plants (non-bagged) and those with experimentally induced pollination deficit (bagged), where a larger value indicates greater difference between seed yields in plants with access to native bees as compared to those with native bees excluded. Accordingly, a single pollination quotient was computed for each study site, treating individual bagged and non-bagged plants as subsamples rather than replicates. Both experiments were analyzed using generalized linear models with an identity link function to test the effects of bee richness, relative abundance of *Bombus* spp. (% *Bombus*), and their interaction on mean site-level pollination quotient. In the analysis of pollination quotients in the experiment testing wild sunflower, the abundance of sunflowers at the site was also incorporated as an effect potentially influencing pollination quotient. Pollination quotient was log-transformed prior to analysis, and the interaction between bee richness and relative abundance of *Bombus* spp. was visualized using the ‘effects’ package (Fox 2020). Statistical significance was assigned to modeled effects using a Student’s *t* statistic and a Type I error rate of $\alpha=0.05$.

3. Results

3.1. Experiment 1: effects of visitation frequency and foraging time of bee genera on seed yields.

A total of 223 observations of visitation times were recorded across the seven focal bee genera (per-genus visitation frequency and summed contact time with study plants is provided in Appendix 3, Table A.3.1), and mean visitation times differed significantly by bee genus (F_6 ,

$t_{16}=5.468$, $P<0.001$). On average, *Bombus* spp. visited flowers for the longest time; *Svastra* spp. and *Halictus* spp. were intermediate in visitation times, whereas *Melissodes* spp., *Agapostemon* spp., *Lasioglossum* spp., and *Megachile* spp. spent significantly less time foraging on flowers (Table 3.1). Overall, very few visits were recorded for *Lasioglossum* spp.

Selection of a model of seed yield based on visitation time and visitation frequency of the above bee genera converged on a best model that indicated mean seed yields (g) were positively associated with the number of *Bombus* visits to inflorescences and slightly negatively associated with total *Halictus* visitation time to inflorescences (Table 3.2). Although both effects were important for constructing a general model of seed yield, interpretation of coefficients and statistical probability values (i.e., P -values) indicated that visitation by *Bombus* spp. was the only statistically significant effect for predicting seed yield (Figure 3.3).

3.2. Experiments 2 and 3: effects of bee richness and pollination deficit on pollination services.

As expected, in both experiments bagging of inflorescences with fine mesh was sufficient to induce pollination deficit. In the experiment testing potted sunflower, mean seed yields in open pollinated inflorescences was 3.616 ± 0.195 SE grams per inflorescence and mean seed yields in bagged inflorescences was 2.566 ± 0.210 SE grams per inflorescence, and this difference (~30%) was statistically significant ($t_{19}=5.381$, $P<0.001$). Effects of bagging on inducing pollination deficit were stronger in wild growing stands of sunflower, and mean seed yields in open pollinated plants was 0.900 ± 0.120 SE grams per inflorescence and mean seed yields in bagged inflorescences was 0.168 ± 0.042 SE grams per inflorescence, and this difference (~81%) was also statistically significant ($t_{14}=6.061$, $P<0.001$; Figure 3.4). When comparing between the two experiments, seed yields in the potted sunflower cultivar (var 'Big

Smile') was on average 82% higher than seed yields from wild growing sunflower ($t_{68}=12.370$, $P<0.001$), indicating substantially greater mean seed yields from inflorescences of the cultivar as compared to wild-growing plants.

3.2.1. Potted sunflower test.

In potted sunflowers, there was no evidence that bee richness, the proportion of *Bombus* (%) in the sample, nor the interaction between richness and % *Bombus* affected variation in mean pollination quotients (Table 3.4).

3.2.2. Wild sunflower test.

A different pattern was detected for wild growing sunflowers: although bee richness was modestly positively associated with pollination quotients, the proportion (%) of *Bombus* in the sample was strongly positively related to pollination quotient. However, there was also evidence that a bee richness \times % *Bombus* interaction strongly impacted pollination quotients. Local sunflower density had no detectable impact on pollination quotient (Table 3.4). Although there was not a statistically significant correlation between bee richness and relative abundance of *Bombus* spp. in the sample (Pearson's $r=-0.210$, $P=0.451$), shifts in site-level species richness modified the effects of *Bombus* spp. relative abundances on pollination quotients. At low levels of bee richness, *Bombus* spp. abundance strongly positively predicted pollination quotients, but as bee richness increased this relationship switched and *Bombus* spp. abundances were no longer positively associated with seed yields (Figure 3.5).

4. Discussion

Our data show that seven bee genera are common visitors to sunflowers in shortgrass rangeland sites of northern Colorado and the studied taxa vary considerably in their mean visitation times to sunflower: *Bombus* spp. and *Halictus* spp. foraged on inflorescences the

longest while *Agapostemon* spp., *Lasioglossum* spp., and *Megachile* spp. exhibited much shorter visitation times. This variation was related to differences in pollination efficacy, and inflorescences that had more interactions with certain taxa (*Bombus* spp., the longest floral visitors) exhibited higher mean seed yields. In experiments that compared pollination services across a range of bee species richness, pollination quotient (the difference in mean seed yield between inflorescences treated with experimental pollination deficit vs. open-pollinated inflorescences) was not associated with bee richness or the proportion of *Bombus* spp. in the sample for sunflower cultivars with short-term exposure to pollinators. However, when inflorescences in wild sunflower were allowed long-term exposure to pollinators, pollination quotients increased congruent with the proportion of *Bombus* spp. captured at the site, but this effect waned as bee richness increased, suggesting that site α -diversity interacts with the effects of specific bee functional groups to drive pollination services. I interpret our results to indicate that bee taxonomic groups (here, bumblebees) that exhibited specific functional behaviors (long foraging times) were an important predictor of pollination services when bee richness is low, but the importance of these taxa may be reduced when bee species richness is high.

This interpretation is consistent with evidence from recent studies of pollination services in different regions. For instance, in an analysis of four experimental datasets, Winfree et al. (2015) established that fluctuations in abundance of several common bee taxa was a more important predictor of pollination service in agricultural systems than bee richness. In addition, increased bee richness was not necessarily associated with increased deposition of conspecific pollen grains. In our study, *Bombus* was the only specific taxa whose visitation had detectable impacts on pollination services, and significant seed yields were still achieved even with low visitation frequencies (one or two visitations, Parker 1981a, b). This pattern can potentially be

explained by pollen deposition rates. Often, *Bombus* spp. deposit more pollen grains per visit than other bee taxa (Parker 1980, Winfree et al. 2015). In addition, *Bombus* spp. are large in mass and body size relative to other taxa and there is general evidence for a positive correlation between bee body size and pollen deposition during foraging (Földesi et al. 2020). Here, both *Bombus* spp. and *Halictus* spp. exhibited mean visitation times to sunflower inflorescences that were significantly longer than the average visitation time pooled across all bee taxa. However, visitation time of *Halictus* spp. was associated with slight negative effects on seed yields in sunflower (Table 3.2), potentially indicating that *Halictus* spp. did not transfer pollen grains among sunflower inflorescences. Recently published data on functional variation (intertegular distance, ITD, a metric of bee body size, Cane 1987) in prairie bee assemblages of the southern Rocky Mountains indicates that regional *Bombus* spp. are approximately 4-fold larger than regional *Halictus* spp. (*Bombus* spp. ITD: 6.082 ± 0.411 mm; *Halictus* spp. ITD: 1.423 ± 0.187 mm; data from Thapa-Magar et al. 2020). Accordingly, I hypothesize that superior pollination services of regional sunflower crops are likely provided by bees that are both large and that visit inflorescences for long periods. Further work could test this prediction by evaluating pollen grain transfer rates among these two taxa.

There is a considerable body of research investigating effects of variation in pollinator assemblages on sunflower yield and seed quality; this is in part due to the ease with which sunflower can be grown and manipulated as a model system, but also due to the agronomic importance of the species as a crop. The overall patterns of diversity-yield relationships are variable in this literature: some authors reporting strong positive effects of bee diversity or diversity on seed yield and quality (e.g., Greenleaf and Kremen 2006, Carvalheiro et al. 2011, Hevia et al. 2016), but others report null or even negative (e.g., Pisanty et al. 2014) associations

between α -diversity and yields. In addition, there appear to be regional differences associated with which bee taxa are good pollinators of sunflower (Mallinger and Prasifka 2017). For example, Mallinger et al. (2019) found that *Andrena helianthi* (Hymenoptera: Andrenidae) and *Melissodes* spp. were the most common and effective pollinators of confection sunflower grown in the northern Great Plains region, and that *Bombus* and *Halictus* spp. were infrequent visitors and less effective at depositing pollen per visit. This differs from our results from the southern Rocky Mountain region, where *Bombus* spp. were the only flower visitors in our study with detectable effects on seed yields, and *Melissodes* spp. visitations were not clearly associated with yields. Pollination services are also further mediated by landscape context, where adjacency of natural land cover to agricultural systems can have strong effects on regional bee abundance and diversity (e.g. Chaplin-Kramer et al. 2011, Hevia et al. 2016, Zou et al. 2017, among others). Here I do not consider landscape or genetic factors, which could help to further explain variation in patterns of sunflower yields.

Our study has several limitations that should be considered when interpreting our results. First, our experiments use different sunflower genotypes in different experiments, and the highly-selected cultivar (var ‘Big Smile’) had much larger inflorescences, shorter plant heights, and was less limited by pollination deficit than wild-type sunflowers—accordingly, this cultivar might be generally less responsive to variation in bee assemblages than wild-type sunflower. In addition, there were differences in overall length of exposure between potted (short exposure times) and wild sunflower (long exposure times), and the substrate in which sunflower plants grew (as well as water availability) was carefully controlled for potted flowers but not for wild stands of sunflower, which likely impacts variability in seed yields. Second, our two field experiments were performed at different times during the growing season, and potted sunflowers

were exposed primarily to early-season bee species whereas wild sunflower was exposed to late-season bee species. This difference in timing of experiments was associated with a turnover in bee taxa; for example, during the early-season *Agapostemon*, *Eucera*, and *Lasioglossum* spp. were abundant and comprised a majority of sampled bee assemblages, but by late in the season *Melissodes* spp. were the predominant taxa. However, overall bee abundances and species richness was comparable between the two periods (Table 3). Variability in bee β -diversity is known to be associated with changes in pollination services (e.g., Winfree et al. 2018). Lastly, variability in pollination quotients in field experiments with stands of wild-type sunflower was high, and several values weighted regression models in analyses testing effects of site-level richness and relative *Bombus* abundances.

In summary, I report that seven genera are common visitors to sunflower in shortgrass-steppe ecosystems of northern Colorado, and that genera can be separated based on relative differences in mean floral visitation times. Although two genera (*Bombus* and *Halictus*) were ‘long visitors’ (Table 1), *Bombus* spp. were the longest visitors on average and even one or two visits by *Bombus* spp. to inflorescences was sufficient to overcome pollination deficit in potted sunflower. Wild-type sunflower growing in the field was more strongly limited by pollination deficit than a potted sunflower cultivar, suggesting that genetic variation (in addition to differences in pollinator assemblages and soil or water conditions) play a strong role in predicting variability in sunflower seed production. Only sunflower with long-term exposure to pollinators was responsive to variability in bee assemblages, and pollination services increased along with the proportion of *Bombus* spp. captured at a site but tended to decline as bee species richness increased (Figure 5). Accordingly, I conclude that presence of a single common taxa (i.e., *Bombus* spp.) is an important factor for predicting pollination services of an important crop

species in the Front Range of Colorado when site-level species richness is low. This is promising for maintenance of ecosystem services and function, as *Bombus* populations in grassland ecosystems can be managed indirectly by manipulation of vegetation characteristics (height and structure), floral species composition (Carvell 2002), and landscape factors (Diaz-Forero et al. 2013). To best manage regional pollination services, further research in this system is merited to characterize plant-pollinator interaction networks of local *Bombus* populations, especially in relation to transfer of pollen in agricultural crops, and evaluate factors directly impacting *Bombus* colony fitness.

Table 3.1. A summary of recorded visitations and estimated mean visitation times from seven bee genera alighting on potted sunflower (*H. annuum*, var ‘Big Smile’). Lettering denotes the groupings identified by Tukey’s HSD test; genera not connected by the same letter differ significantly ($P<0.05$) in mean visitation time.

Bee genus	Number of recorded visitations	Mean (SE) visitation time	Tukey’s HSD grouping
<i>Agapostemon</i>	39	1.42 (0.33)	D
<i>Bombus</i>	24	3.73 (0.42)	A
<i>Halictus</i>	29	2.44 (0.38)	BC
<i>Lasioglossum</i>	3	0.45 (1.20)	BCD
<i>Megachile</i>	23	1.15 (0.43)	D
<i>Melissodes</i>	73	1.60 (2.44)	CD
<i>Svastra</i>	32	2.72 (0.39)	AB

Table 3.2. Summary of a model selection procedure to test effects of visitation frequency and visitation time of seven bee genera on seed yields in potted sunflower (*H. annuum*, var ‘Big Smile’). The model shown below is the best model identified using a forward-stepwise approach with minimization of AIC as the criteria. Significant ($P < 0.05$) model effects are highlighted in bold text.

Parameter	Estimate (β)	df	SS	<i>F</i> - score	<i>P</i>
Intercept	0.232	1	0.000	0.000	1.000
<i>Bombus</i> visit frequency	0.120	1	0.460	7.810	0.008
<i>Halictus</i> visit time	-0.025	1	0.147	2.501	0.122
Residual error	0.242	36	2.120	-	-

Table 3.3. Summary of bee taxa captured using blue vane traps in two experiments testing the effects of bee richness on pollination quotient in sunflower (*H. annuum*). One experiment was carried out using potted sunflower (var ‘Big Smile’) and the other in naturally occurring stands of wild sunflower.

Family	Genus	species	Experiment	
			Potted plants	Wild plants
Andrenidae	<i>Andrena</i>	sp 1	1	0
Apidae	<i>Anthophora</i>	<i>affabilis</i>	16	2
		<i>montana</i>	1	1
		<i>occidentalis</i>	5	21
		sp 1	1	0
	<i>Bombus</i>	<i>appositus</i>	0	5
		<i>fervidus</i>	1	11
		<i>griseocollis</i>	2	1
		<i>huntii</i>	0	1
		<i>insularis</i>	2	0
		<i>nevadensis</i>	14	5
		<i>pennsylvanicus</i>	7	34
	<i>Ceratina</i>	sp 1	1	0
	<i>Eucera</i>	<i>hamata</i>	30	2
	<i>Melecta</i>	<i>pacifica</i>	9	1
	<i>Melissodes</i>	<i>communis</i>	15	19
		<i>coreopsis</i>	12	72
		<i>tristis</i>	0	89
	<i>Svastra</i>	<i>obliqua</i>	0	16
		sp 1	0	1
Halictidae	<i>Agapostemon</i>	<i>angelicus</i>	13	9
		<i>virescence</i>	32	6
	<i>Augochlorella</i>	<i>aurata</i>	5	26
		sp 1	0	3
	<i>Halictus</i>	sp 1	33	5
		sp 2	0	2
		sp 3	1	1
		sp 4	1	25
	<i>Lasioglossum</i>	<i>dialictus</i>	133	21
		sp 1	7	4
		sp 2	10	1
Megachilidae	<i>Megachile</i>	sp 1	17	0
		sp 2	14	2
		sp 3	8	7
	<i>Osmia</i>	sp 1	16	0
		SUM	407	393

Table 3.4. Summary of two generalized linear models analyzing the effects of bee richness and relative abundances of *Bombus* spp. on pollination services in sunflower. Pollinations services were defined as the quotient of mean seed yields for ‘open pollinated’ and ‘bagged’ inflorescences.

Experiment	Parameter	Estimate (β)	SE	<i>t</i> -score	<i>P</i>
Potted sunflower	Intercept	0.459	0.193	2.375	0.030
	Bee richness	-0.023	0.026	-0.876	0.394
	% <i>Bombus</i> in sample	-0.030	0.032	-0.948	0.357
	Bee richness \times % <i>Bombus</i>	0.004	0.003	1.217	0.241
Wild sunflower	Intercept	-35.476	30.331	-1.170	0.269
	Bee richness	6.941	3.528	1.196	0.077
	% <i>Bombus</i> in sample	6.585	0.993	6.631	<0.001
	Bee richness \times % <i>Bombus</i>	-0.827	0.149	0.712	<0.001
	Sunflower relative abundance	0.015	0.021	0.712	0.492

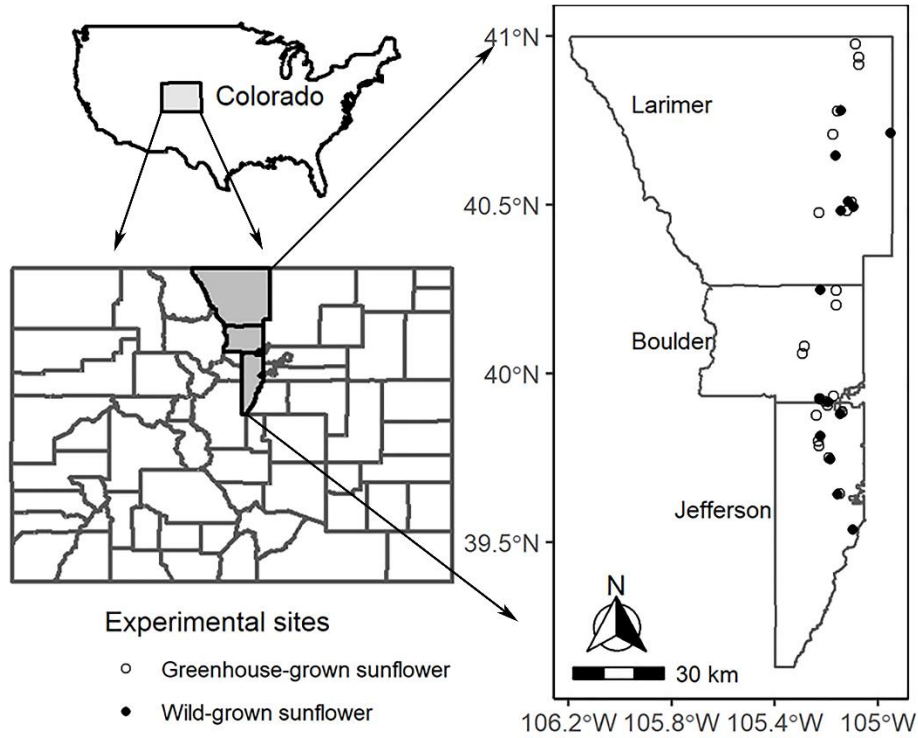


Figure 3.1. Shortgrass steppe study sites in the Front Range of Colorado where experiments were conducted with potted sunflowers (*Helianthus annuum* var ‘Big Smile’) (open symbols) and wild growing common sunflower (closed symbols) to test effects of wild bee visitation on pollination services.



Figure 3.2. Photo series showing (a) greenhouse-grown potted sunflower (*Helianthus annuus* L. var 'Big Smile') and (b) a stand of common wild sunflower. In (c) and (d) potted sunflower cultivars have been placed in the field and experimentally bagged to induce pollination deficit, whereas (e) in wild sunflower individual inflorescences were bagged. (f) Collection of bees using blue vane traps immediately following harvest of experimental sunflower and (g) dried-down flowers ready for seed collection.

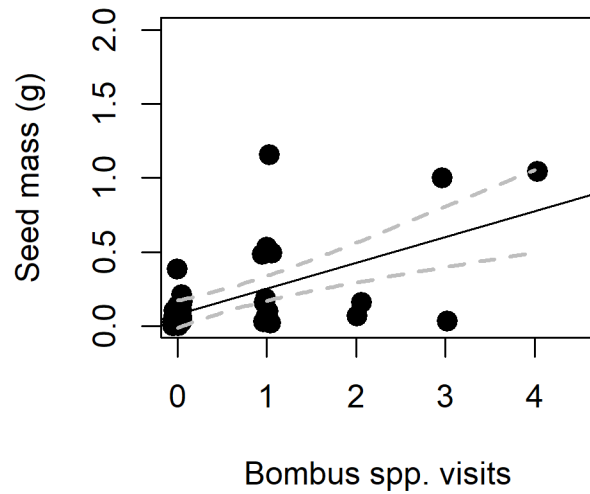


Figure 3.3. The relationship between the frequency of *Bombus* spp. visitations (i.e., number of visits by bumblebees) and seed yields in potted sunflower (*H. annuum*, var 'Big Smile'). The solid line is the regression model ($y=0.080+0.174x$) and dashed gray lines are 95% confidence intervals.

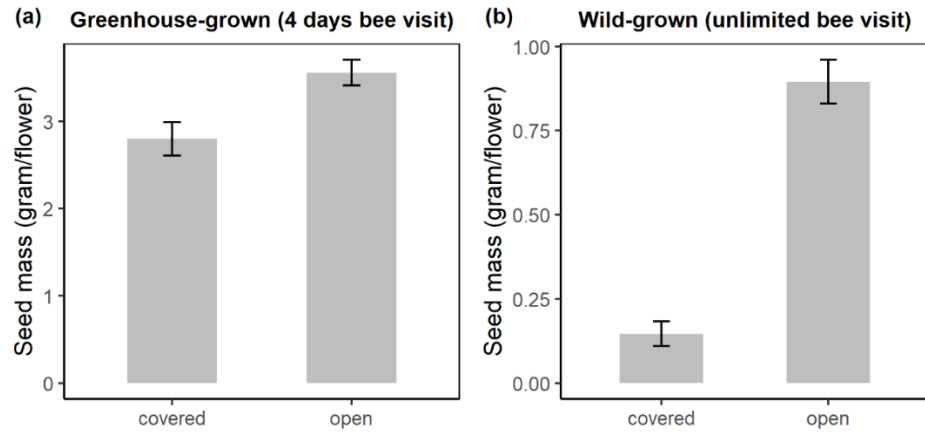


Figure 3.4. Differences in mean seed yield (g seed/inflorescence) compared between open pollinated sunflowers and those with visitation by native bees excluded for (a) potted (var 'Big Smile') and (b) common wild sunflower (*H. annuum*). Bars show plus or minus one standard error.

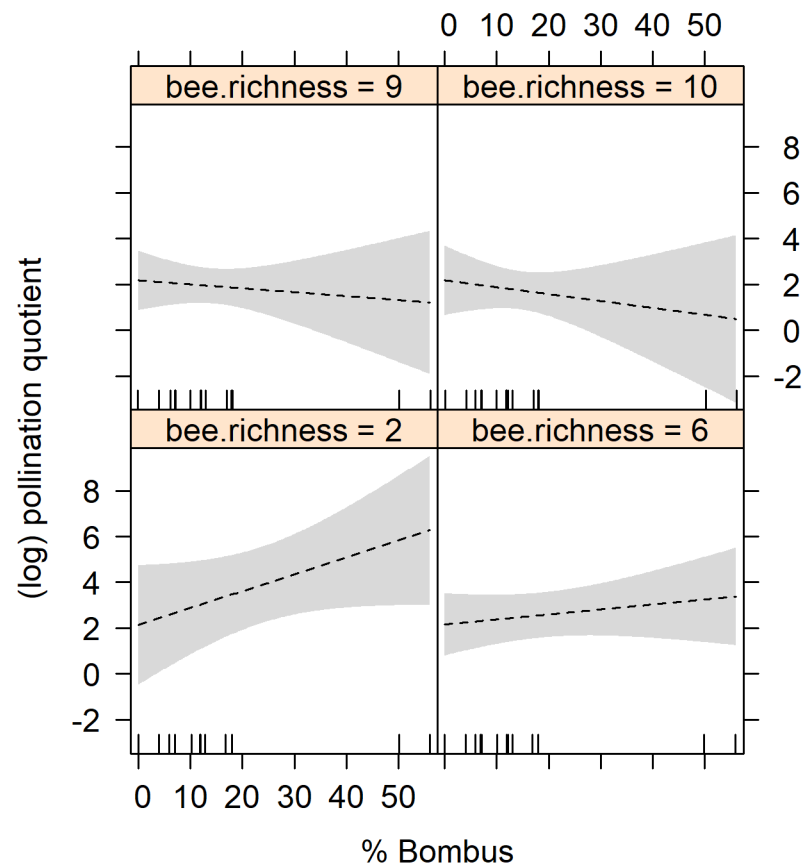


Figure 3.5. The effects of *Bombus* spp. relative abundance in community samples on pollination quotient in stands of wild sunflower (*H. annuum*), as modified by bee richness. Dashed lines show modeled effects and shaded regions show 95% confidence intervals.

REFERENCES

- Akaike, H. 1974. A new look at the statistical model identification. *IEEE T. Automat. Contr.* 19:716-723.
- Albrecht, M., B. Schmid, Y. Hautier, and C. B. Müller. 2012. Diverse pollinator communities enhance plant reproductive success. *Proc. Royal Soc. B* 279:4845-4852.
- Cane, J. H. 1987. Estimation of bee body size using intergular span (Apoidea). *J. Kansas Entomol. Soc.* 60:145-147.
- Carvalho, L. G., Veldtman, R., Shenkute, A. G., Tesfay, G. B., Pirk, C. W. W., and J. S. Donaldson, et al. 2011. Natural and within-farmland biodiversity enhances crop productivity. *Ecol. Lett.* 14:251-259.
- Carvell, C. 2002. Habitat use and conservation of bumblebees (*Bombus* spp.) under different grassland management regimes. *Biol. Conserv.* 103:33-49.
- Chaplin-Kramer, R., Tuxen-Bettman, K., and C. Kremen, C. 2011. Value of wildland habitat for supplying pollination services to Californian agriculture. *Rangelands* 33:33-41.
- Diaz-Forero, I., Kuusemets, V., Mänd, M., Liivamägi, A. Kaart, T., and J. Luig. 2012. Influence of local and landscape factors on bumblebees in semi-natural meadows: a multiple-scale study in a forested landscape. *J. Insect Conserv.* 17:113-125.
- Földesi, R., Howlett, B. G., Grass, I., and P. Batáry. 2020. Larger pollinators deposit more pollen on stigmas across multiple plant species—a meta-analysis. *J. Appl. Ecol.* 58:699-707.
- Fox, J. 2020. Package ‘effects’: effect displays for linear, generalized linear, and other models. V.4.2-0.
- Gallai, N., J.-M. Salles, J. Settele, and B. E. Vassiere. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecol. Econ.* 68:810-821.
- Greenleaf, S. S., and C. Kremen. 2006. Wild bees enhance honey bees’ pollination of hybrid sunflower. *Proc. National Acad. Sci. USA* 103:13890-13895.
- Hevia, V., Bosch, J., Azcárate, F. M., Fernández, E., Rodrigo, A., Barril-Graells, H., et al. 2016. Bee diversity and abundance in a livestock drove road and its impact on pollination and seed set in adjacent sunflower fields. *Agr. Ecosyst. Environ.* 232:336-344.
- Horth, L., and L. A. Campbell. 2018. Supplementing small farms with native mason bees increases strawberry size and growth rate. *J. Appl. Ecol.* 55:591-599.
- Isaacs, R., and A. K. Kirk. 2010. Pollination services provided to small and large highbush blueberry fields by wild and managed bees. *J. Appl. Ecol.* 47:841-849.
- Kearns, C. A., D. W. Inouye, and N. M. Waser. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. *Ann. Rev. Ecol. Syst.* 29:83-112.
- Kremen, C. 2008. Crop pollination services from wild bees. In: James, R., Pitts-Singer, T. L., eds. *Bee Pollination in Agricultural Ecosystems*. Oxford University Press, New York, USA.
- Kremen, C., N. M. Williams, M. A. Aizen, B. Gemmill-Herren, G. LeBuhn, and R. Minckley, et al. 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecol. Lett.* 10:299-314.
- MacInnis, G., and J. R. Forrest. 2019. Pollination by wild bees yields larger strawberries than pollination by honeybees. *J. Appl. Ecol.* 56:824-832.

- Mallinger, R. E., Bradshaw, J., Varenhorst, A. J., and J. R. Prasifka. 2019. Native solitary bees provide economically significant pollination services to confection sunflowers (*Helianthus annuus* L.) (Asterales: Asteraceae) grown across the northern Great Plains. *J. Econ. Entomol.* 112:40-48.
- Mallinger, R., and J. Prasifka. 2017. Benefits of insect pollination to confection sunflowers differ across plant genotypes. *Crop Sci.* 57:3264-3272.
- Michener, C. D., McGinley, R. J., and B. N. Danforth. 1994. *The Bee Genera of North and Central America: Hymenoptera, Apoidea*. Smithsonian Institution Press, Washington, DC, USA. 209 p.
- Ollerton, J., R. Winfree, and S. Tarrant. 2011. How many flowering plants are pollinated by animals? *Oikos* 120:321-326.
- Parker, F. D. 1980. Sunflower pollination: abundance, diversity and seasonality of bees and their effect on seed yields. *J. Apicult. Res.* 20:49-61.
- Parker, F. D. 1981a. How efficient are bees in pollinating sunflowers? *J. Kansas Entomol. Soc.* 54:61-67.
- Parker, F. D. 1981b. Sunflower pollination: abundance, diversity, and seasonality of bees on male-sterile and female-fertile cultivars. *Environ. Entomol.* 10:1012-1017.
- Petersen, J. D., S. Reiners, and B. A. 2013. Pollination services provided by bees in pumpkin fields supplemented with either *Apis mellifera* or *Bombus impatiens* or not supplemented. *PLOS ONE*, 8: e69819.
- Pisanty, G., Klein, A.-M., and Y. Mandelik. 2014. Do wild bees complement honeybee pollination of confection sunflowers in Israel? *Apidologie* 45:235-247.
- Thapa-Magar, K., T. S. Davis, and B. Kondratieff. 2020. Livestock grazing is associated with seasonal reduction in pollinator biodiversity and functional dispersion but cheatgrass invasion is not: variation in bee assemblages in a multi-use shortgrass prairie. *PLOS ONE* 15:e0237484.
- Winfree, R., Fox, J. W., Williams, N. M., Reilly, J. R., and D. P. Cariveau. 2015. Abundance of common species, not species richness, drives delivery of a real-world ecosystem service. *Ecol. Lett.* 18:626-635.
- Winfree, R., Reilly, J. R., Bartomeus, I., Cariveau, D. P., Williams, N. P., and J. Gibbs. 2018. Species turnover promotes the importance of bee diversity for crop pollination at regional scales. *Science* 359:791-793.
- Zhou, Y., Bianchi, F. J., Jauker, F., Xiao, H., Chen, J., and S. Luo, et al. 2017. Landscape effects on pollinator communities and pollination services in small-holder agroecosystems. *Agr. Ecosyst. Environ.* 246:109-116.

APPENDIX 1: A META-ANALYSIS OF THE EFFECTS OF HABITAT ARIDITY, EVOLUTIONARY HISTORY OF GRAZING, AND GRAZING INTENSITY ON POLLINATOR COMMUNITIES WORLDWIDE

List A.1.1. List of studies included in the quantitative synthesis.

- (1) Báldi, A., Batáry, P., Kleijn, D., 2013. Effects of grazing and biogeographic regions on grassland biodiversity in Hungary – analysing assemblages of 1200 species. *Agric. Ecosyst. Environ.* 166, 28–34.
- (2) Batáry, P., Báldi, A., Sárospataki, M., Kohler, F., Verhulst, J., Knop, E., Herzog, F., Kleijn, D., 2010. Effect of conservation management on bees and insect-pollinated grassland plant communities in three European countries. *Agric. Ecosyst. Environ.* 136, 35–39.
- (3) Berg, Å., Cronvall, E., Eriksson, Å., Glimskär, A., Hiron, M., Knape, J., Pärt, T., Wissman, J., Żmihorski, M., Öckinger, E., 2019. Assessing agri-environmental schemes for semi-natural grasslands during a 5-year period: can I see positive effects for vascular plants and pollinators? *Biodivers. Conserv.* 28, 3989–4005.
- (4) Buckles, B.J., Harmon-Threatt, A.N., 2019. Bee diversity in tallgrass prairies affected by management and its effects on above- and below-ground resources. *J. Appl. Ecol.* 56, 2443–2453.
- (5) Cagnolo, L., Molina, S.I., Valladares, G.R., 2002. Diversity and guild structure of insect assemblages under grazing and exclusion regimes in a montane grassland from Central Argentina. *Biodivers. Conserv.* 11, 407–420.
- (6) Carvell, C., 2002. Habitat use and conservation of bumblebees (*Bombus* spp.) under different grassland management regimes. *Biol. Conserv.* 103, 33–49.
- (7) Conforti, M. 2019. "The effects of native and domestic grazers on the health of bumble bee (*Bombus* spp.) populations in a historical tallgrass prairie ecosystem". Dissertation.
- (8) Davidson, K.E., Fowler, M.S., Skov, M.W., Forman, D., Alison, J., Botham, M., Beaumont, N., Griffin, J.N., 2020. Grazing reduces bee abundance and diversity in saltmarshes by suppressing flowering of key plant species. *Agric. Ecosyst. Environ.* 291, 106760.
- (9) Debano, S.J., 2006. Effects of livestock grazing on aboveground insect communities in semi-arid grasslands of southeastern Arizona. *Biodivers. Conserv.* 15, 2547–2564.
- (10) Debinski, D.M., Moranz, R.A., Delaney, J.T., Miller, J.R., Engle, D.M., Winkler, L.B., McGranahan, D.A., Barney, R.J., Trager, J.C., Stephenson, A.L., Gillespie, M.K., 2011. A cross-taxonomic comparison of insect responses to grassland management and land-use legacies. *Ecosphere* 12, art131.
- (11) Elwell, S.L., Griswold, T., Elle, E., 2016. Habitat type plays a greater role than livestock grazing in structuring shrubsteppe plant–pollinator communities. *J. Insect Conserv.* 20, 515–525.
- (12) Garrido, P., Mårell, A., Öckinger, E., Skarin, A., Jansson, A., Thulin, C., 2019. Experimental rewilding enhances grassland functional composition and pollinator habitat use. *J. Appl. Ecol.* 56, 946–955.

- (13) Hall, M.A., Nimmo, D.G., Cunningham, S.A., Walker, K., Bennett, A.F., 2019. The response of wild bees to tree cover and rural land use is mediated by species' traits. *Biol. Conserv.* 231, 1–12.
- (14) Hatfield, R.G., LeBuhn, G., 2007. Patch and landscape factors shape community assemblage of bumble bees, *Bombus* spp. (Hymenoptera: Apidae), in montane meadows. *Biol. Conserv.* 139, 150–158.
- (15) Hopfenmüller, S., Holzschuh, A., Steffan-Dewenter, I., 2020. Effects of grazing intensity, habitat area and connectivity on snail-shell nesting bees. *Biol. Conserv.* 242, 108406.
- (16) Jerrentrup, J.S., Wrage-Mönnig, N., Röver, K.-U., Isselstein, J., 2014. Grazing intensity affects insect diversity via sward structure and heterogeneity in a long-term experiment. *J. Appl. Ecol.* 51, 968–977.
- (17) Johansson, V., Knape, J., Franzén, M., 2017. Population dynamics and future persistence of the clouded Apollo butterfly in southern Scandinavia: The importance of low intensity grazing and creation of habitat patches. *Biol. Conserv.* 206, 120–131.
- (18) Kämper, W., Blüthgen, N., Eltz, T., 2017. Bumblebee footprints on bird's-foot trefoil uncover increasing flower visitation with land-use intensity. *Agric. Ecosyst. Environ.* 240, 77–83.
- (19) Kimoto, C., DeBano, S.J., Thorp, R.W., Taylor, R. V., Schmalz, H., DelCurto, T., Johnson, T., Kennedy, P.L., Rao, S., 2012. Short-term responses of native bees to livestock and implications for managing ecosystem services in grasslands. *Ecosphere* 3, art88.
- (20) Kohler, F., Verhulst, J., Knop, E., Herzog, F., Kleijn, D., 2007. Indirect effects of grassland extensification schemes on pollinators in two contrasting European countries. *Biol. Conserv.* 135, 302–307.
- (21) Kormann, U., Rösch, V., Batáry, P., Tschardtke, T., Orci, K.M., Samu, F., Scherber, C., 2015. Local and landscape management drive trait-mediated biodiversity of nine taxa on small grassland fragments. *Divers. Distrib.* 21, 1204–1217.
- (22) Kovács-Hostyánszki, A., Elek, Z., Balázs, K., Centeri, C., Falusi, E., Jeanneret, P., Penksza, K., Podmaniczky, L., Szalkovszki, O., Báldi, A., 2013. Earthworms, spiders and bees as indicators of habitat quality and management in a low-input farming region—A whole farm approach. *Ecol. Indic.* 33, 111–120.
- (23) Kruess, A., Tschardtke, T., 2002. Grazing Intensity and the Diversity of Grasshoppers, Butterflies, and Trap-Nesting Bees and Wasps. *Conserv. Biol.* 16, 1570–1580.
- (24) Lazaro, A., Tscheulin, T., Devalez, J., Nakas, G., Petanidou, T., 2016. Effects of grazing intensity on pollinator abundance and diversity, and on pollination services. *Ecol. Entomol.* 41, 400–412.
- (25) MacDonald, M.A., Cobbold, G., Mathews, F., Denny, M.J.H., Walker, L.K., Grice, P. V., Anderson, G.Q.A., 2012. Effects of agri-environment management for circl buntings on other biodiversity. *Biodivers. Conserv.* 21, 1477–1492.
- (26) Mazalova, M., Sipos, J., Rada, S., Kasak, J., Srapatka, B., Kuras, T., 2015. Responses of grassland arthropods to various biodiversity-friendly management practices: Is there a compromise? *Eur. J. Entomol.* 112, 734–746.
- (27) Minckley, R.L., 2014. Maintenance of richness despite reduced abundance of desert bees (Hymenoptera: Apiformes) to persistent grazing. *Insect Conserv. Divers.* 7, 263–273.
- (28) Moranz, R.A., Debinski, D.M., McGranahan, D.A., Engle, D.M., Miller, J.R., 2012. Untangling the effects of fire, grazing, and land-use legacies on grassland butterfly communities. *Biodivers. Conserv.* 21, 2719–2746.

- (29) Moranz, R.A., Fuhlendorf, S.D., Engle, D.M., 2014. Making sense of a prairie butterfly paradox: The effects of grazing, time since fire, and sampling period on regal fritillary abundance. *Biol. Conserv.* 173, 32–41.
- (30) Mwangi, D., Kasina, M., Nderitu, J., Hagen, M., Gikungu, M., Kraemer, M., 2012. Diversity and abundance of native bees foraging on hedgerow plants in the Kakamega farmlands, western Kenya. *J. Apic. Res.* 51, 298–305.
- (31) Noreika, N., Bartomeus, I., Winsa, M., Bommarco, R., Öckinger, E., 2019. Pollinator foraging flexibility mediates rapid plant-pollinator network restoration in semi-natural grasslands. *Sci. Rep.* 9, 15473.
- (32) Öckinger, E., Eriksson, A.K., Smith, H.G., 2006. Effects of grassland abandonment, restoration and management on butterflies and vascular plants. *Biol. Conserv.* 133, 291–300.
- (33) Odanaka, K.A., Rehan, S.M., 2019. Impact indicators: Effects of land use management on functional trait and phylogenetic diversity of wild bees. *Agric. Ecosyst. Environ.* 286, 106663.
- (34) Osgathorpe, L.M., Park, K., Goulson, D., 2012. The use of off-farm habitats by foraging bumblebees in agricultural landscapes: implications for conservation management. *Apidologie* 43, 113–127.
- (35) Pöyry, J., Lindgren, S., Salminen, J., Kuussaari, M., 2004. Restoration of butterfly and moth communities in semi-natural grasslands by cattle grazing. *Ecol. Appl.* 14, 1656–1670.
- (36) Redpath, N., Osgathorpe, L.M., Park, K., Goulson, D., 2010. Crofting and bumblebee conservation: The impact of land management practices on bumblebee populations in northwest Scotland. *Biol. Conserv.* 143, 492–500.
- (37) Robson, D.B., 2019. Impact of Grazing History on Pollinator Communities in Fescue Prairie. *Blue Jay* 77, 10–15.
- (38) Rosenberger, D.W., Conforti, M.L., 2020. Native and agricultural grassland use by stable and declining bumble bees in Midwestern North America. *Insect Conserv. Divers.* 13, 585–594.
- (39) Saarinen, K., Jantunen, J., 2005. Grassland Butterfly Fauna under Traditional Animal Husbandry: Contrasts in Diversity in Mown Meadows and Grazed Pastures. *Biodivers. Conserv.* 14, 3201–3213.
- (40) Sárospataki, M., Báldi, A., Batáry, P., Józán, Z., Erdős, S., Rédei, T., 2009. Factors affecting the structure of bee assemblages in extensively and intensively grazed grasslands in Hungary. *Community Ecol.* 10, 182–188.
- (41) Shapira, T., Henkin, Z., Dag, A., Mandelik, Y., 2020. Rangeland sharing by cattle and bees: moderate grazing does not impair bee communities and resource availability. *Ecol. Appl.* 30, e02066.
- (42) Simons, N.K., Lewinsohn, T., Blüthgen, N., Buscot, F., Boch, S., Daniel, R., Gossner, M.M., Jung, K., Kaiser, K., Müller, J., Prati, D., Renner, S.C., Socher, S.A., Sonnemann, I., Weiner, C.N., Werner, M., Wubet, T., Wurst, S., Weisser, W.W., 2017. Contrasting effects of grassland management modes on species-abundance distributions of multiple groups. *Agric. Ecosyst. Environ.* 237, 143–153.
- (43) Sjödin, N.E., 2007. Pollinator behavioural responses to grazing intensity. *Biodivers. Conserv.* 16, 2103–2121.
- (44) Sjödin, N.E., Bengtsson, J., Ekbom, B., 2007. The influence of grazing intensity and landscape composition on the diversity and abundance of flower-visiting insects. *J. Appl. Ecol.* 45, 763–772.

- (45) Smith, G.W., Debinski, D.M., Scavo, N.A., Lange, C.J., Delaney, J.T., Moranz, R.A., Miller, J.R., Engle, D.M., Toth, A.L., 2016. Bee Abundance and Nutritional Status in Relation to Grassland Management Practices in an Agricultural Landscape. *Environ. Entomol.* 45, 338–347.
- (46) Smith, L.M., Cherry, R., 2014. Effects of Management Techniques on Grassland Butterfly Species Composition and Community Structure. *Am. Midl. Nat.* 172, 227–235.
- (47) Söderström, B., Svensson, B., Vessby, K., Glimskär, A., 2001. Plants, insects and birds in semi-natural pastures in relation to local habitat and landscape factors. *Biodivers. Conserv.* 10, 1839–1863.
- (48) Steffan-Dewenter, I., Leschke, K., 2003. Effects of habitat management on vegetation and above-ground nesting bees and wasps of orchard meadows in Central Europe. *Biodivers. Conserv.* 12, 1953–1968.
- (49) Stein, D.S., Debinski, D.M., Pleasants, J.M., Toth, A.L., 2020. Evaluating Native Bee Communities and Nutrition in Managed Grasslands. *Environ. Entomol.* 49, 717–725.
- (50) Tadey, M., 2015. Indirect effects of grazing intensity on pollinators and floral visitation. *Ecol. Entomol.* 40, 451–460.
- (51) Thapa-Magar, K.B., Davis, T.S., Kondratieff, B., 2020. Livestock grazing is associated with seasonal reduction in pollinator biodiversity and functional dispersion but cheatgrass invasion is not: Variation in bee assemblages in a multi-use shortgrass prairie. *PLoS One* 15, e0237484.
- (52) van Klink, R., Nolte, S., Mandema, F.S., Lagendijk, D.D.G., WallisDeVries, M.F., Bakker, J.P., Esselink, P., Smit, C., 2016. Effects of grazing management on biodiversity across trophic levels—The importance of livestock species and stocking density in salt marshes. *Agric. Ecosyst. Environ.* 235, 329–339.
- (53) Vanbergen, A.J., Woodcock, B.A., Gray, A., Grant, F., Telford, A., Lambdon, P., Chapman, D.S., Pywell, R.F., Heard, M.S., Cavers, S., 2014. Grazing alters insect visitation networks and plant mating systems. *Funct. Ecol.* 28, 178–189.
- (54) Vogel, J.A., Debinski, D.M., Koford, R.R., Miller, J.R., 2007. Butterfly responses to prairie restoration through fire and grazing. *Biol. Conserv.* 140, 78–90.
- (55) Vulliamy, B., G. Potts, S., G. Willmer, P., 2006. The effects of cattle grazing on plant-pollinator communities in a fragmented Mediterranean landscape. *Oikos* 114, 529–543.
- (56) Wallis De Vries, M.F., Parkinson, A.E., Dulphy, J.P., Sayer, M., Diana, E., 2007. Effects of livestock breed and grazing intensity on biodiversity and production in grazing systems. 4. Effects on animal diversity. *Grass Forage Sci.* 62, 185–197.
- (57) Welti, E.A.R., Joern, A., 2018. Fire and grazing modulate the structure and resistance of plant–floral visitor networks in a tallgrass prairie. *Oecologia* 186, 517–528.
- (58) Xie, Z., Williams, P.H., Tang, Y., 2008. The effect of grazing on bumblebees in the high rangelands of the eastern Tibetan Plateau of Sichuan. *J. Insect Conserv.* 12, 695–703.
- (59) Yoshihara, Y., Chimeddorj, B., Buuveibaatar, B., Lhagvasuren, B., Takatsuki, S., 2008. Effects of livestock grazing on pollination on a steppe in eastern Mongolia. *Biol. Conserv.* 141, 2376–2386.
- (60) Zhao, Z., Wei, J., Zhang, K., Li, H., Wei, S., Pan, X., Huang, W., Zhu, M., Zhang, R., 2018. Asymmetric response of different functional insect groups to low-grazing pressure in Eurasian steppe in Ningxia. *Ecol. Evol.* 8, 11609–11618.

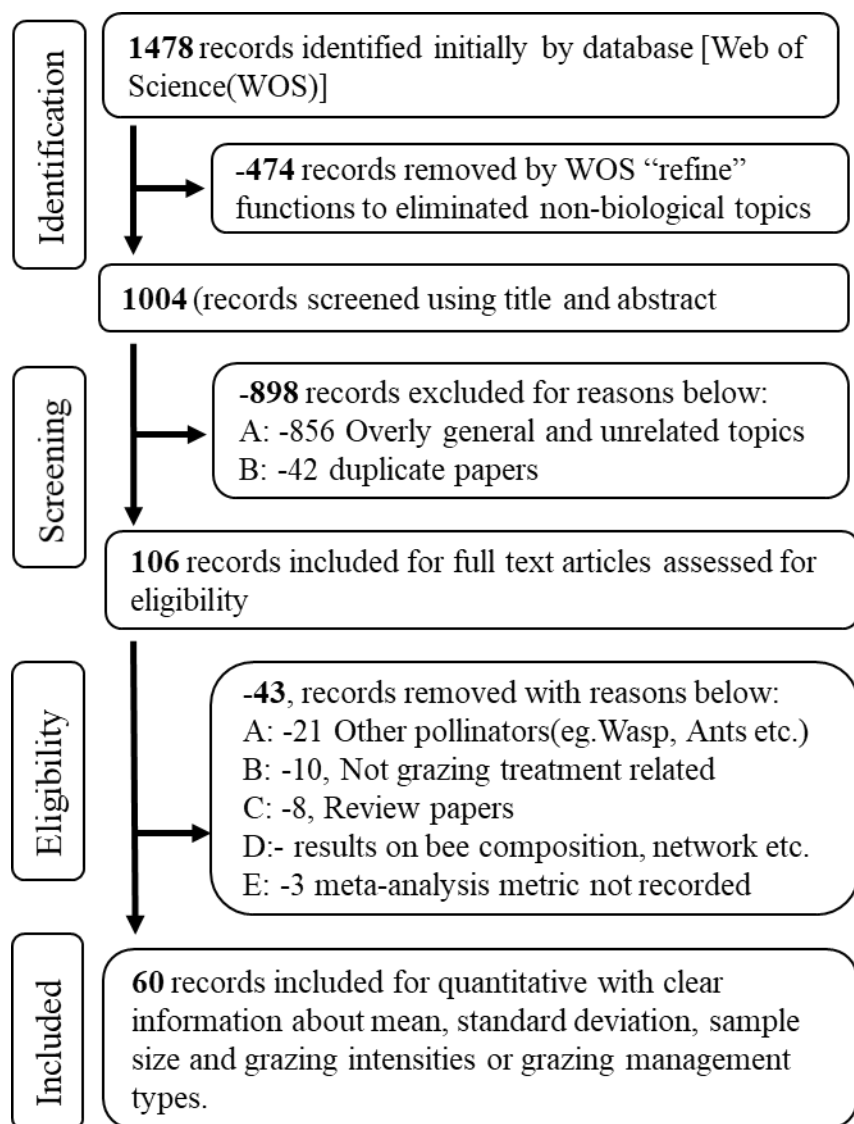


Figure A.1.1. Flow chart (PRISMA diagram) documenting literature search process and screening criteria.

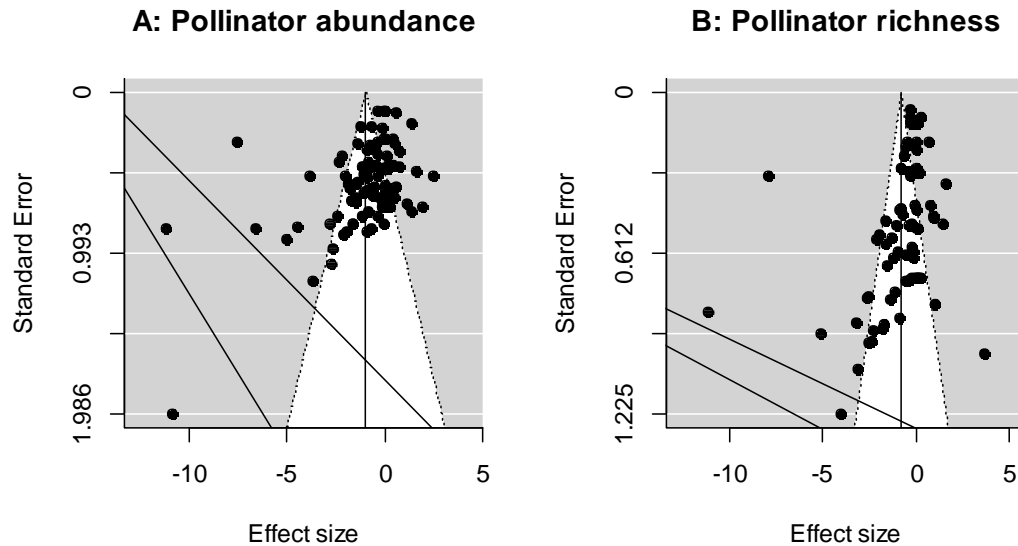


Figure A.1.2: Funnel plots of effect size on standard errors for publish bias. I analyzed potential publication bias in our meta-analysis results using three different approaches. First, I did a visual assessment of the relationship between effect sizes and standard errors (funnel plots) to evaluate plot symmetry (shown below). Funnel plots were symmetrical indicating that it was unlikely published studies preferentially reported extreme positive or extreme negative effect size. Further, a trim-and-fill analysis estimated zero missing studies for abundance and richness (Duval and Tweedie 2000). Second, I used Spearman rank correlation to examine the relationship between the effect size and sample size across the studies. A significant correlation would indicate a publication bias whereby larger effect size are more likely to be published than smaller effect size. However, our statistical results demonstrated no correlation between effect size and sample size in either pollinator abundance (Spearman's $\rho=0.026$, $P=0.788$) or species richness (Spearman's $\rho=0.044$, $P=0.685$). Third, I calculated Rosenberg's fail-safe number, which indicated that 1,684 studies with null results for effects of grazing on bee richness would be needed to make our reported aridity effect sizes non-significant (i.e., $P>0.05$) and 2,906 such studies would be needed to make the effect of grazing on bee abundances non-significant. This result does not necessarily prove a lack of publication bias but indicates any potential publication biases have a negligible impact on effect sizes (Rosenberg 2005).

References

- Duval, S., & Tweedie, R. 2000. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 56:455-463.
<http://doi.org/10.1111/j.0006-341x.2000.00455.x>.
- Rosenberg, M. S. 2005. The file-drawer problem revisited: a general weighted method for calculating fail-safe numbers in meta-analysis. *Evolution* 59:464-468.
<https://doi.org/10.1111/j.0014-3820.2005.tb01004>.

APPENDIX 2: LIVESTOCK GRAZING IS ASSOCIATED WITH SEASONAL REDUCTION
IN POLLINATOR BIODIVERSITY AND FUNCTIONAL DISPERSION BUT CHEATGRASS
INVASION IS NOT: VARIATION IN BEE ASSEMBLAGES IN A MULTI-USE
SHORTGRASS STEPPE

Table A.2.1. Bee functional traits considered in this study and their descriptions.

Trait	Categories or values	Description and sources
Lecty (dietary specialization)	1: Oligolectic 2: Polylectic 3: Kleptoparasite	Oligolectic are bee species forage on one to four forb genera. Polylectic are bees forage on five or more genera (Scott et al. 2011) (Murray et al. 2009). Species with no evident lecty status are kleptoparasites; these species may be oligolectic or polylectic depending on the diet breadth of species they parasitize.
Nesting construction	1: Excavators 2: Rent 3: Kleptoparasite	For excavator species female bees excavate their own nests. Renters inhabit pre-existing cavities or nesting spaces (Potts et al. 2005). Kleptoparasites rob the nests of other bees and nest construction related to the nesting habits of species they parasitize.
Nesting location	1: Above ground 2: Below ground 3: Kleptoparasite	Bees living above ground are above-ground nesters, while those bees which nest below ground are below-ground nesters. Kleptoparasites may inhabit either type of nest as they exploit nests already constructed by other bees.
Tibia hair density	1: High 2: Medium 3: Low	High tibial hair density was defined by setae density that totally obscures viewing the tibia. Medium tibial hair density was defined as dense setae but able to view the tibia. Low tibia hair density was defined as sparse or intermittent setae with a corbicula.
Tibia pollen collecting structure	1: Corbicula 2: Scopa 3: Abdomen scopa 4: Kleptoparasite	A corbicula is a specialized concave structure (pollen basket) that carries pollen (Michener 1999), whereas a scopa is a setal structure that carries pollen and may be located on the abdomen or tibia. Kleptoparasitic bees do not have pollen collection structures.

Flight phenology	1: Early 2: Middle 3: Late	Early phenology was defined as peak abundance in May and June. Mid-season phenology was defined as peak abundance in late June through July. Late-season phenology was defined as peak abundance in August.
Sociality	1: Solitary 2: Social 3: Multiple 4: Kleptoparasite	In solitary bees, females do everything required to raise offspring including excavating/renting nests, laying eggs and provisioning larvae (Linsely 1958, Danforth et al. 2019). Social bees divide labor of among queens, drones, and workers. Social bees typically have a higher foraging and reproductive capacity and have a faster response to resource provision than solitary bees (Michener 2007). ‘Multiple’ refers to bee species which exhibit both solitary and primitive communal behaviors, and kleptoparasites are typically solitary bees that rob nests of other bees.
Body size (mm)	Intertegular distance (ITD)	ITD is the distance between the inner nodes of the wing base. ITD is a proxy of dry weight and foraging distance (Greenleaf and Kremen 2006) and tongue length (Cariveau et al. 2016).

Table A.2.2. Functional trait values used to inform computation of bee assemblage functional dispersion.

Family	Genus	species	ITD	Nest location	Nest construction	Sociality	Lecty	Pollen collection structure	Tibial hair density	Flight phenology
Apidae	<i>Anthophora</i>	<i>affabilis</i>	4.42	below	excavate	solitary	poly	scopa	high	middle
		<i>bomboides</i>	3.69	below	excavate	solitary	poly	scopa	high	early
		<i>montana</i>	3.69	below	excavate	solitary	poly	scopa	high	middle
		<i>occidentalis</i>	4.20	below	excavate	solitary	poly	scopa	high	middle
	<i>Apis</i>	<i>mellifera</i>	5.01	above	excavate	social	poly	corbicula	low	middle
	<i>Bombus</i>	<i>appositus</i>	7.47	below	rent	social	poly	corbicula	low	middle
		<i>bifarius</i>	5.18	below	rent	social	poly	corbicula	low	late
		<i>californicus</i>	5.96	below	rent	social	poly	corbicula	low	middle
		<i>centralis</i>	3.37	below	rent	social	poly	corbicula	low	middle
		<i>fervidus</i>	7.82	below	rent	social	poly	corbicula	low	middle
		<i>griseocollis</i>	7.78	below	rent	social	poly	corbicula	low	late
		<i>huntii</i>	7.14	below	rent	social	poly	corbicula	low	late
		<i>insularis</i>	5.45	klepto	klepto	klepto	klepto	klepto	low	early
		<i>nevadensis</i>	4.94	below	rent	social	poly	corbicula	low	early
		<i>pennsylvanicus</i>	7.42	below	rent	social	poly	corbicula	low	late
		<i>rufocinctus</i>	4.87	below	rent	social	poly	corbicula	low	middle
		<i>sylvicola</i>	5.55	below	rent	social	poly	corbicula	low	late
	<i>Diadasia</i>	<i>enavata</i>	4.38	below	excavate	solitary	oligo	scopa	high	middle
	<i>Eucera</i>	<i>hamata</i>	2.65	below	excavate	solitary	poly	scopa	high	early
		<i>lepida</i>	3.86	below	excavate	solitary	poly	scopa	high	early
	<i>Melecta</i>	<i>pacifica</i>	3.42	klepto	klepto	klepto	klepto	klepto	low	early
	<i>Melissodes</i>	<i>agilis</i>	3.81	below	excavate	solitary	oligo	scopa	high	late
		<i>communis</i>	3.98	below	excavate	solitary	oligo	scopa	high	middle
		<i>coreopsis</i>	3.44	below	excavate	solitary	oligo	scopa	high	middle
		<i>sp 1</i>	3.56	below	excavate	solitary	oligo	scopa	high	middle
		<i>tristis</i>	3.19	below	excavate	solitary	oligo	scopa	high	late
	<i>Svastra</i>	<i>obliqua</i>	3.88	below	excavate	solitary	oligo	scopa	high	late
		<i>petulca</i>	3.60	below	excavate	solitary	oligo	scopa	high	middle

	<i>Xeromelecta</i>	<i>interrupta</i>	2.98	klepto	klepto	klepto	klepto	klepto	low	middle	
Colletidae	<i>Colletes</i>	<i>sp 1</i>	3.04	below	excavate	solitary	poly	scopa	medium	middle	
Halictidae		<i>angelicus</i>	2.56	below	excavate	multiple	poly	scopa	medium	late	
		<i>coloradinus</i>	2.56	below	excavate	multiple	poly	scopa	medium	middle	
		<i>texanus</i>	2.91	below	excavate	multiple	poly	scopa	medium	late	
		<i>Agapostemon</i>	<i>virescens</i>	3.48	below	excavate	multiple	poly	scopa	medium	middle
		<i>Augochlorella</i>	<i>aurata</i>	2.03	below	excavate	social	poly	scopa	medium	late
		<i>Lasioglossum</i>	<i>dialictus</i>	1.13	below	excavate	multiple	poly	scopa	medium	middle
			<i>sp 1</i>	1.84	below	excavate	multiple	poly	scopa	medium	early
		<i>Halictus</i>	<i>sp 1</i>	1.42	below	excavate	multiple	poly	scopa	medium	middle
			<i>ligatus</i>	1.75	below	excavate	multiple	poly	scopa	medium	middle
			<i>tripartitus</i>	1.10	below	excavate	multiple	poly	scopa	medium	middle
Megachilidae	<i>Anthidium</i>	<i>sp 1</i>	3.26	klepto	klepto	solitary	klepto	klepto	low	middle	
	<i>Lithurgopsis</i>	<i>apicalis</i>	3.64	above	rent	multiple	oligo	abdomen	low	late	
	<i>Megachile</i>	<i>dentitarsus</i>	4.24	above	rent	solitary	poly	abdomen	low	middle	
		<i>sp1</i>	2.77	above	rent	solitary	poly	abdomen	low	middle	
		<i>sp2</i>	3.75	above	rent	solitary	poly	abdomen	low	middle	
		<i>sp 3</i>	4.28	above	rent	solitary	poly	abdomen	low	middle	
	<i>Osmia</i>	<i>sp1</i>	2.73	above	rent	solitary	poly	scopa	medium	early	
		<i>sp2</i>	2.10	above	rent	solitary	poly	scopa	medium	early	
		<i>sp 3</i>	2.40	above	rent	solitary	poly	scopa	medium	late	

Table A.2.3. Summary of bee trap captures by month of collection.

Family	Genus	species	Month			
			May	June	July	August
Apidae	<i>Anthophora</i>	<i>affabilis</i>	43	54	10	1
		<i>bomboides</i>	2	3	2	0
		<i>montana</i>	14	49	17	42
		<i>occidentalis</i>	0	13	88	7
	<i>Apis</i>	<i>mellifera</i>	6	54	19	12
	<i>Bombus</i>	<i>appositus</i>	6	40	75	34
		<i>bifarius</i>	0	0	1	1
		<i>californicus</i>	0	2	0	0
		<i>centralis</i>	0	2	2	0
		<i>fervidus</i>	3	87	112	51
		<i>griseocollis</i>	2	6	20	18
		<i>huntii</i>	11	3	15	22
		<i>insularis</i>	1	0	0	0
		<i>nevadensis</i>	71	177	38	2
		<i>pensylvanicus</i>	10	68	191	212
		<i>rufocinctus</i>	1	7	2	3
		<i>sylvicola</i>	0	2	5	4
	<i>Diadasia</i>	<i>enavata</i>	0	26	2	1
	<i>Eucera</i>	<i>hamata</i>	82	22	0	0
		<i>lepida</i>	4	0	0	0
	<i>Melecta</i>	<i>pacifica</i>	27	1	0	0
	<i>Melissodes</i>	<i>agilis</i>	0	1	64	194
		<i>communis</i>	7	170	153	66
		<i>coreopsis</i>	0	19	38	24
		sp. 1	0	8	4	3
		<i>tristis</i>	1	15	94	162
	<i>Svastra</i>	<i>obliqua</i>	0	4	146	93
		<i>petulca</i>	0	0	11	7
	<i>Xeromelecta</i>	<i>interrupta</i>	1	22	2	1
Colletidae	<i>Colletes</i>	sp. 1	0	1	1	0
Halictidae	<i>Agapostemon</i>	<i>angelicus</i>	5	11	23	14
		<i>coloradinus</i>	0	6	3	2
		<i>texanus</i>	2	2	41	23
		<i>virescens</i>	15	48	10	5
	<i>Augochlorella</i>	<i>aurata</i>	3	4	4	14
	<i>Halictus</i>	sp. 1	17	36	21	3
		<i>ligatus</i>	2	2	14	2
		<i>tripartitus</i>	8	105	203	33

Megachilidae	<i>Lasioglossum</i>	<i>dialictus</i>	37	108	48	39
		sp. 1	11	15	5	2
	<i>Anthidium</i>	sp. 1	0	3	7	3
	<i>Lithurgopsis</i>	<i>apicalis</i>	0	6	16	4
	<i>Megachile</i>	<i>dentitarsus</i>	0	1	2	1
		sp. 1	3	7	3	6
		sp. 2	6	32	28	20
		sp. 3	0	2	1	1
	<i>Osmia</i>	sp. 1	23	2	1	0
		sp. 2	14	4	2	0
		sp. 3	1	0	1	2

Table A.2.4. Summary of generalized linear model results to predict the effects of cover composition and floral richness on bee community assemblages.

Response	Parameter	β	SE	<i>t</i> -score	<i>P</i>
Bee abundance	(Intercept)	-0.007	0.166	-0.040	0.968
	floral cover	0.549	0.243	2.255	0.037
	floral richness	-0.513	0.322	-1.596	0.128
	bareground cover	0.221	0.251	0.881	0.390
	litter/wood cover	-0.219	0.304	-0.719	0.481
	cheatgrass cover	-0.487	0.520	-0.935	0.362
	grass cover	-0.811	0.491	-1.650	0.116
	rock cover	-0.096	0.186	-0.518	0.611
Bee richness	(Intercept)	0.011	0.175	0.062	0.951
	floral cover	0.298	0.257	1.162	0.261
	floral richness	-0.209	0.340	-0.617	0.545
	bareground cover	0.232	0.265	0.874	0.394
	litter/wood cover	0.056	0.321	0.175	0.863
	cheatgrass cover	-0.066	0.549	-0.119	0.906
	grass cover	-0.712	0.518	-1.373	0.187
	rock cover	-0.292	0.196	-1.488	0.154
α -diversity	(Intercept)	0.002	0.185	0.013	0.990
	floral cover	0.123	0.271	0.454	0.655
	floral richness	0.002	0.358	0.005	0.996
	bareground cover	0.150	0.280	0.536	0.598
	litter/wood cover	0.299	0.339	0.883	0.389
	cheatgrass cover	0.221	0.580	0.381	0.708
	grass cover	-0.428	0.547	-0.782	0.444
	rock cover	-0.240	0.207	-1.162	0.260
Functional dispersion	(Intercept)	0.030	0.146	0.206	0.839
	floral cover	-0.293	0.215	-1.367	0.188
	floral richness	-0.170	0.284	-0.599	0.556
	bareground cover	-0.673	0.221	-3.041	0.007
	litter/wood cover	0.020	0.268	0.076	0.940
	cheatgrass cover	-0.704	0.459	-1.535	0.142
	grass cover	-0.848	0.433	-1.958	0.066
	rock cover	0.166	0.164	1.016	0.323

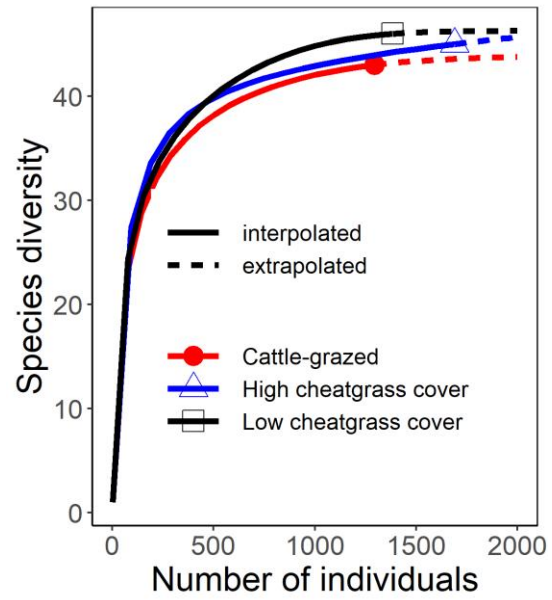


Figure A.2.1. Species detection curves for wild bees in shortgrass steppe in three habitat types.

APPENDIX 3: BUMBLEBEE (HYMENOPTERA: APIDAE) VISITATION FREQUENCY
DRIVES SEED YIELDS AND INTERACTS WITH SITE-LEVEL SPECIES RICHNESS TO
DRIVE POLLINATION SERVICES IN SUNFLOWER

Table A.3.1. A summary of recorded visitation frequency for seven bee genera visiting inflorescences of 39 potted sunflower plants (*Helianthus annuum* L., var ‘Big Smile’) in shortgrass steppe sites over 12-min observation windows in an experiment to test how variation in bee genus visitation frequency and time impact sunflower yields.

Plant ID	Number of visitations per inflorescence by bee genus						
	<i>Agapostemon</i>	<i>Bombus</i>	<i>Halictus</i>	<i>Lasioglossum</i>	<i>Megachile</i>	<i>Melissodes</i>	<i>Svastra</i>
1	0	1	1	0	0	1	0
2	1	0	1	0	1	3	3
3	0	0	0	0	0	11	0
4	1	1	1	0	0	2	0
5	2	0	1	0	2	1	0
6	1	0	7	0	1	1	1
7	0	0	0	0	0	6	0
8	1	4	0	0	0	0	0
9	1	1	0	0	0	2	0
10	0	0	0	0	1	0	2
11	1	1	2	0	3	0	1
12	0	0	0	0	0	3	1
13	0	0	0	0	0	6	0
14	0	0	0	0	0	1	1
15	0	0	0	0	0	0	3
16	1	0	1	0	0	1	0
17	2	0	0	0	2	0	2
18	0	1	0	0	0	0	0
19	2	3	0	0	0	1	2
20	3	0	1	0	0	3	0
21	0	1	0	0	0	3	1
22	0	0	1	0	1	3	0
23	0	0	0	0	0	1	2
24	2	0	1	0	0	2	1
25	1	1	0	0	0	0	0
26	2	1	0	2	0	1	0
27	8	0	0	0	2	0	1
28	1	0	0	0	1	1	1
29	2	1	3	0	1	2	0

30	1	2	0	0	1	3	1
31	1	0	4	0	1	3	1
32	0	2	0	0	0	0	0
33	0	0	1	0	3	1	1
34	0	3	0	1	0	0	0
35	1	0	2	0	0	5	3
36	0	0	0	0	0	0	2
37	2	0	1	0	1	2	1
38	1	1	0	0	0	2	0
39	1	0	1	0	2	2	1
SUM	39	24	29	3	23	73	32

Table A.3.2. A summary of pooled visitation time for seven bee genera visiting inflorescences of 39 potted sunflower plants (*Helianthus annuum* L., var ‘Big Smile’) in shortgrass steppe sites over 12-min observation windows in an experiment to test how variation in bee genus visitation frequency and time impact sunflower yields.

Plant ID	Summed visitation time (min) per inflorescence by bee genus						
	<i>Agapostemon</i>	<i>Bombus</i>	<i>Halictus</i>	<i>Lasioglossum</i>	<i>Megachile</i>	<i>Melissodes</i>	<i>Svastra</i>
1	0.00	0.13	0.25	0.00	0.00	0.20	0.00
2	1.17	0.00	4.00	0.00	1.83	5.17	4.50
3	0.00	0.00	0.00	0.00	0.00	11.80	0.00
4	6.08	1.33	1.03	0.00	0.00	4.33	0.00
5	7.33	0.00	5.25	0.00	5.17	2.00	0.00
6	0.77	0.00	6.37	0.00	1.25	0.40	0.90
7	0.00	0.00	0.00	0.00	0.00	10.95	0.00
8	0.12	11.48	0.00	0.00	0.00	0.00	0.00
9	1.13	0.13	0.00	0.00	0.00	4.17	0.00
10	0.00	0.00	0.00	0.00	0.33	0.00	5.33
11	1.83	3.33	3.58	0.00	5.17	0.00	4.00
12	0.00	0.00	0.00	0.00	0.00	6.17	5.00
13	0.00	0.00	0.00	0.00	0.00	10.53	0.00
14	0.00	0.00	0.00	0.00	0.00	10.00	0.92
15	0.00	0.00	0.00	0.00	0.00	0.00	10.08
16	0.33	0.00	6.00	0.00	0.00	3.00	0.00
17	2.00	0.00	0.00	0.00	3.67	0.00	6.67
18	0.00	10.00	0.00	0.00	0.00	0.00	0.00
19	2.17	6.42	0.00	0.00	0.00	0.50	1.25
20	3.68	0.00	5.83	0.00	0.00	3.80	0.00
21	0.00	6.00	0.00	0.00	0.00	1.17	3.07
22	0.00	0.00	3.00	0.00	0.83	5.52	0.00
23	0.00	0.00	0.00	0.00	0.00	0.45	10.17
24	1.62	0.00	7.58	0.00	0.00	1.92	1.00
25	0.50	7.15	0.00	0.00	0.00	0.00	0.00
26	5.57	8.25	0.00	0.85	0.00	0.67	0.00
27	7.17	0.00	0.00	0.00	0.80	0.00	1.83
28	0.25	0.00	0.00	0.00	0.50	5.17	5.00
29	2.33	1.33	1.33	0.00	1.00	4.33	0.00
30	0.50	6.08	0.00	0.00	0.67	5.50	0.33
31	2.33	0.00	6.50	0.00	1.17	1.67	4.67
32	0.00	4.67	0.00	0.00	0.00	0.00	0.00
33	0.00	0.00	5.33	0.00	1.92	0.58	3.50
34	0.00	12.22	0.00	0.50	0.00	0.00	0.00
35	0.50	0.00	6.67	0.00	0.00	3.57	5.42
36	0.00	0.00	0.00	0.00	0.00	0.00	10.55
37	3.98	0.00	5.20	0.00	0.33	3.95	1.55
38	0.35	11.00	0.00	0.00	0.00	2.33	0.00

39	3.67	0.00	3.00	0.00	1.83	7.00	1.50
SUM	55.38	89.53	70.93	1.35	26.47	116.83	87.23