RESTORING WILD BEES ACROSS FRAGMENTED LANDSCAPES

By

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ABSTRACT

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This dissertation focuses on the effects of ecological restoration on wild bees across human-impacted landscapes, with the goal of informing restoration methods to best encourage rapid bee establishment in these restored habitats. Bees play an important functional role as plant pollinators but are declining globally due in large part to habitat loss and fragmentation. This has created widespread interest in restoring wild bees, especially across agricultural lands. Because bees depend on flowers for all food and nutrition, restoration efforts for bees generally focus on reintroducing flowers into otherwise resource-poor landscapes. However, restoration efforts rarely take other factors into account, including the effects of standard management practices, spatial aspects of restored habitats, distance to existing habitats, and landscape context. An understanding of how these factors structure bee communities and populations would allow the development of conservation methods that better target this important group.

I conducted three related studies to better understand how the design of restored habitats affects the establishment and persistence of bees across fragmented landscapes. My first chapter examines the effects of restoration management on bee abundance and richness, and disentangles the effects of management and landscape context from those of the local floral community. To do this, I monitored bee and flower communities for three years across an actively managed restored tallgrass prairie and used structural equation modeling to examine the relationships between management, landscape context, flowers, and bees. Contrary to expectations, I found that bee abundance and richness were driven by the presence of bison and the landscape context of the restoration, rather than local floral resources. I conclude that restoration management that only focuses on improving local floral resources may not adequately protect bee communities.

My second chapter tests the effects of a common conservation strategy, landscape corridors, on dispersal and colonization by a solitary bee *Megachile rotundata*. I released bees within experimentally fragmented landscapes that separate two spatial aspects of fragments altered by corridors, connectivity and edge-to-area ratio, allowing study of the relative importance of these factors on the colonization process. I found that the initial occupation of habitat patches was increased by the additive effects of both connectivity and higher edge-to-area ratio, but that fragment colonization was ultimately determined by higher edge-to-area ratio. I conclude that corridors and high-edge habitats can successfully increase bee colonization.

My third chapter tests the interacting effects of land-use and patch size on the process of bee dispersal and colonization across agricultural landscapes. I conducted another managed release of *M. rotundata* bees across two experimental landscapes that were both composed of patches of high- and low-flowering habitats, but that differed in patch size. I found that bee colonization was not affected by habitat type when patches were small, but bees showed a strong preference and higher reproductive success in high-flowering habitats when patches were large. I conclude that restoration efforts for bees across agricultural landscapes should emphasize increased landscape heterogeneity, smaller crop field sizes, and higher incorporation of high-quality flowering habitats within the agricultural matrix.

These three chapters show that a consideration of habitat configuration and landscape context in restoration is vital to the establishment and success of wild bees. Though floral resources are one necessary component for bees, a more holistic approach to restoration that also incorporates other factors can better protect this important group.

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INTRODUCTION

The world is currently in a biodiversity crisis, marked by widespread extinctions and dramatic declines in local species abundances (Dirzo et al. 2014). The most important drivers of these biodiversity declines are the loss and resulting fragmentation of natural habitats due to human activities, most often through the conversion of natural land to large-scale agriculture (Haddad et al. 2015; Newbold et al. 2015). Human-impacted landscapes can be homogenous and lack important resources for most organisms, leaving only small habitat fragments for native biodiversity (Benton et al. 2003). In highly degraded areas where protection or preservation of existing natural habitats is no longer possible, ecological restoration may be the best option to rescue threatened ecosystems (Hobbs and Norton 1996; Palmer et al. 2016).

Restoration is the “process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed” (SER 2004), in which degraded lands are converted back into high-diversity natural habitat like prairie or forest (Palmer et al. 2016). The goals of habitat restoration differ depending on the project, but generally involve increasing biodiversity and creating self-sustaining ecosystems that persist over time (Hobbs and Norton 1996; Ehrenfeld 2000; Palmer et al. 2016). Restoration has gained popularity over the last several decades with new government initiatives, non-profits starting large projects across the country, and even citizens starting restoration projects on their own land (Hobbs 2007; Suding 2011). Such widespread use of restoration necessitates an understanding of how well current restoration efforts are serving native biodiversity, as well as the development of restoration methods to better reinstate ecological systems.

Because ecosystems are highly complex, many interacting components must be restored for a habitat restoration project to be successful and self-sustaining over time (Hobbs 2007; Palmer et al. 2016). First, the source of degradation must be identified and stopped, thus allowing the repair of the physical environment. At this stage, restoration actions can include reinstating natural hydrology, topology, and soil composition, and often involve massive alterations to the landscape (Palmer et al. 2016). Second, there is a need for the development or reinstatement of natural vegetation structure. In many restoration projects, this requires actively replanting native plants and conducting ongoing, long-term restoration management to maintain target plant composition and community structure (Palmer et al. 2016; Suding et al. 2016). Third, there must be a return of natural communities, including non-plant organisms such as arthropods, fungi, mammals, and reptiles (Metzger and Brancalion 2016; Palmer et al. 2016). Fourth, there needs to be the reinstatement of essential ecological functions such as seed dispersal, decomposition, pollination, and soil cycling (Palmer et al. 2016; Suding et al. 2016). All of these factors exist within the context of habitat configuration, including the spatial aspects of the habitat itself such as habitat size and shape, and the landscape context of the restoration, including composition of the surrounding landscape and the restoration's isolation from other natural habitats (Metzger and Brancalion 2016; Suding et al. 2016) (see Figure 0.1 for a diagram of this conceptual framework).

Despite the importance of restoring many interacting components in order to recreate self-sustaining ecosystems, many restoration efforts focus primarily on restoring the physical environment and natural vegetative structure rather than taking a more holistic approach to restoration (Young 2000). This may be due to limitations in funding, time, and effort, as well as a lack of knowledge of ways to actively address the other components of a functioning ecosystem (Miller and Hobbs 2007). Approaches that focus solely on restoration of the physical environment and vegetative structure assume that non-target organisms such as arthropods and birds will colonize newly restored habitats unassisted, and subsequently establish diverse communities (Young 2000; Williams 2011). This approach also assumes that important ecological functions will follow the natural, unassisted establishment of organisms that contribute those functions. However, it remains largely unknown whether non-plant organisms in restorations do indeed follow this trajectory and how current restoration actions affect their establishment and persistence (Hobbs and Norton 1996).

In addition to incomplete assessments of the return of non-plant organisms, restoration projects often lack consideration of how landscape context and spatial aspects of habitats factor into the restoration process (Holl et al. 2003; Leite et al. 2013; Tambosi et al. 2014). Restoration practitioners often initiate restoration projects on patches of land that are convenient or easily available, rather than basing decisions on landscape context such as connectivity or proximity to other natural habitats (Tambosi et al. 2014; Metzger and Brancalion 2016). Further, restoration projects focused on restoring local patches of habitat rarely consider specific spatial aspects of the restoration such as habitat shape and the amount of edge. This could be a major oversight for some restoration projects, because spatial aspects of habitats may have a strong role in processes such as habitat colonization and movement of organisms between habitats (Gutzwiller and Anderson 1992; Hanski 1998; Ewers and Didham 2007; Leite et al. 2013; Metzger and Brancalion 2016). In turn, this could affect the ability of restored habitats to meet restoration goals such as the establishment of diverse communities of organisms, higher metapopulation persistence across fragmented landscapes, and the reinstatement of ecological functions (Hobbs and Norton 1996; Metzger and Brancalion 2016).

A better understanding of how non-plant organisms respond to restoration efforts and spatial aspects of restored habitats can help us design habitats that better address restoration goals. Fortunately, two aspects of restored habitats make them an excellent system for studying these processes. The first is that large-scale restoration is increasingly popular and broadly implemented (Hobbs 2007; Suding 2011), creating opportunities for observational studies to assess how well the restoration process is serving natural communities. Second, because restoration already involves manipulating aspects of the landscape and biotic environment, there are many opportunities for large-scale experiments to study ways to improve existing restoration methods (Choi et al. 2008; Palmer et al. 2016).

The focus of my dissertation are the wild bees, an extremely diverse group of functionally-important organisms often found in restored habitats (Handel 1997). Bees are the primary pollinators of both natural (Ollerton et al. 2011) and crop systems (Klein et al. 2007; Garibaldi et al. 2013), and thus may be essential for ecosystem recovery due to their role in the pollination of restored plant communities (Handel 1997; Dixon 2009). However, bees are declining worldwide, largely due to habitat loss and other human impacts (Potts et al. 2010; Goulson et al. 2015). Habitat restoration is being used as a primary conservation strategy to counter and reverse declines in wild bees because restoration re-introduces important food and nesting resources back into degraded landscapes (Winfree 2010). Thus, it is necessary to understand bee responses to restoration to better design restoration methods to protect this important group.

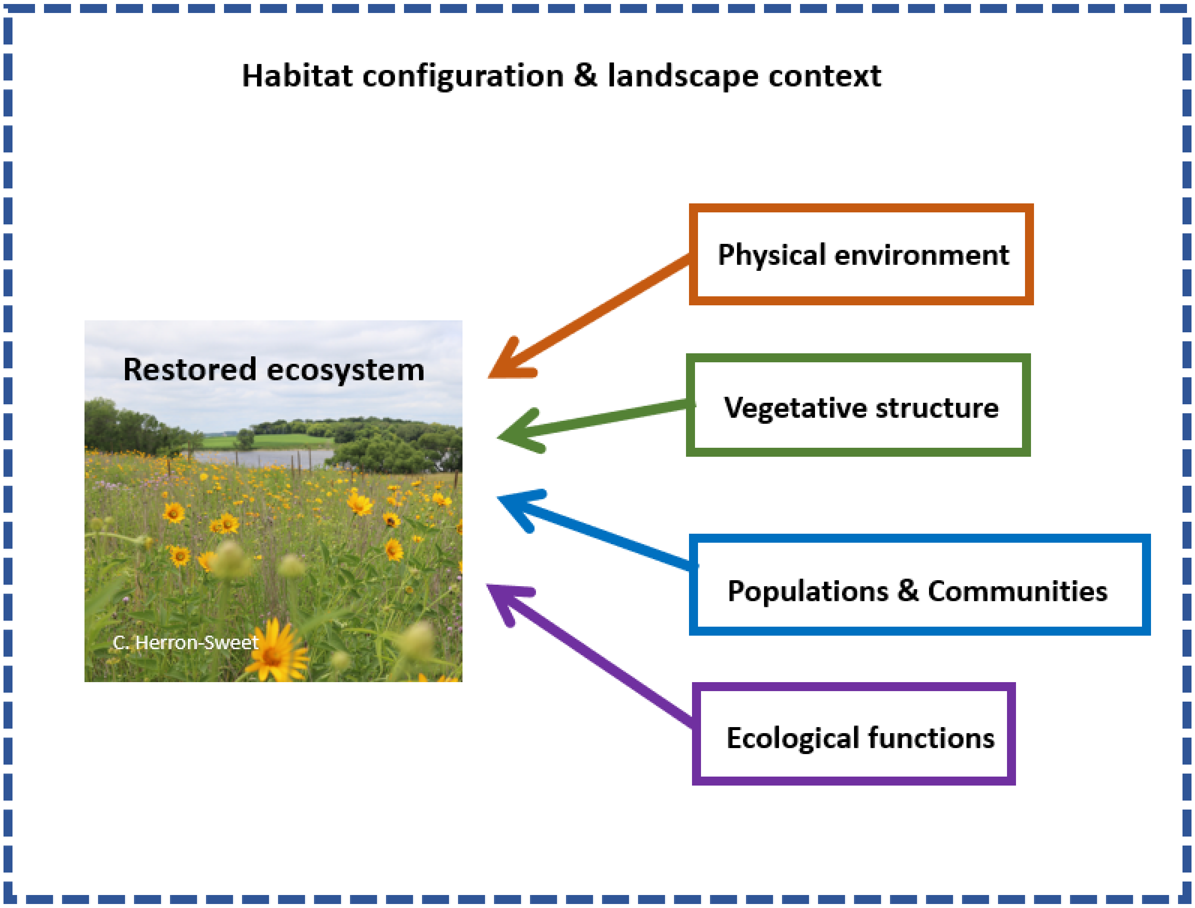
Because bees rely on flowers as their primary source of food and nutrition (Roulston and Goodell 2011), restoration efforts for bees generally focus on increasing the abundance of local floral resources (Winfree 2010; Scheper et al. 2015; Vaudo et al. 2015; Williams et al. 2015).

These habitat restoration efforts have been shown to have strong, positive effects on bee abundance and richness compared to degraded habitats (Tonietto and Larkin 2018). In addition, bee colonization of restored habitats can happen rapidly, with some studies showing bee communities that resemble those of remnant habitats within only a few years following initial restoration (Exeler et al. 2009; Griffin et al. 2017). However, much about the effects of restoration on wild bees remains unknown. Few studies have examined the effects of plant-focused management on bees and whether management can be tailored specifically for bee communities. Further, despite the importance of rapid bee population establishment for both restoration goals and ecosystem functioning, the process of bee colonization in restored habitats has been largely unstudied.

In my dissertation, I present three chapters focused on ways to improve restoration methods for wild bees. The first chapter uses an observational approach to examine the effects of ongoing, plant-focused restoration management on bee communities, with the goal of determining whether bees are sufficiently protected by plant-focused methods. My second and third chapters use landscape-scale experiments to examine the effects of spatial aspects of habitats on bee colonization, with the goal of identifying ways to alter habitat configuration to increase bee establishment and population viability in restored habitats. I conclude this work with a discussion of the overall implications of my findings, as well as some specific recommendations for restoration practitioners. My hope is that by increasing our understanding of bee responses to restoration methods, this work will help to improve restoration methods for wild bees and other functionally important organisms.

APPENDIX

Figure 0.1 Conceptual framework for restored habitats. To encourage high biodiversity and be self-sustaining over time, reconstructed ecosystems require restoration of the physical environment, vegetative structure, natural populations and communities, and ecological functions. All of these factors exist within the context of landscape and habitat configuration.



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CHAPTER ONE

Wild bee communities are structured by landscape and management, not local floral resources, in a restored tallgrass prairie[1]

Abstract

Restored habitats require long-term management to maintain biodiversity and ensure ecosystem functions, but management strategies are often developed for plant communities with little knowledge of the effects on non-plant organisms. In our study system, restored tallgrass prairie, managers frequently employ prescribed fire and grazing by bison to structure plant communities and promote habitat heterogeneity. Wild bees are often expected to respond to such management actions via their effects on local floral resource availability, but management may also affect bees by altering survival and nesting independently from plant community responses. Understanding how wild bees respond to specific management methods is important because bees are essential pollinators likely critical to the success of restored habitats. Working in restoration plantings within a large, actively-managed tallgrass prairie preserve, we separated the effects of management and landscape context on bee community abundance and richness from the effects of these covariates on bees mediated through the abundance and richness of the local flowering plant community. We found that bee communities responded primarily to landscape and bison rather than aspects of the local floral community. In contrast, flowers responded to restoration age, prescribed burning, bison and the amount of prairie habitat in the greater

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[1] Manuscript will be submitted for publication with coauthors Bethanne Bruninga-Socolar and Jason Gibbs

landscape. Because bees respond to different factors and at a different landscape scale than local plant communities, we conclude that management designed for plants may not be sufficient for pollinators. We thus suggest the development of restoration plans that take both plants and other functionally important groups into account.

Introduction

Habitat restoration often relies on active, long-term management to maintain target conditions (Hobbs and Norton 1996; Suding et al. 2016). Restoration projects generally focus on the seeding and reestablishment of plants with the assumption that non-plant organisms will colonize and persist on their own (Young 2000; Williams 2011). Thus, long-term management strategies are primarily designed to aid the recovery of plant communities (Hobbs and Norton 1996; Suding 2011). Plant-focused restoration management regularly employs methods that disrupt aspects of both the abiotic and biotic environment, including burning, introduction of large grazers, supplemental seeding, and herbicide (e.g. Schramm 1992; Harmon-Threatt and Chin 2016). Such methods may be useful to remove exotic species (D'antonio and Meyerson 2002), maintain nutrient cycling (Wan et al. 2001), prevent dominance by certain plants like C4 grasses (Pywell et al. 2007), and ensure higher plant diversity (Martin and Wilsey 2006; Veen et al. 2008). However, these plant-focused strategies rarely take animals into account, despite the fact that many animals are also of conservation interest and provide ecological functions important to the long-term success of restoration projects (Handel 1997; Palmer et al. 1997; Young 2000; Suding et al. 2016). Animals may respond differently than plants to restoration practices (Hobbs and Norton 1996). Thus, to maintain ecological integrity, restoration management must protect biodiversity more broadly.

One group that can benefit from habitat restoration are the wild bees, the focus of our study. Bees are ecologically important due to their role as the predominant pollinators of flowering plants (Winfree 2010), but bees are suffering worldwide declines caused by loss of natural habitats and other global changes (Potts et al. 2010; Cameron et al. 2011; Goulson et al. 2015; Powney et al. 2019). It is well-established that restoration benefits wild bees by reintroducing floral resources and nesting habitats into otherwise inhospitable landscapes (Tonietto and Larkin 2018). Restored habitats are rapidly colonized by bees (Exeler et al. 2009) and can foster high bee abundance and richness only a few years following initial restoration (e.g. Exeler et al. 2009; Williams 2011; Griffin et al. 2017; Tonietto et al. 2017). In turn, pollination by bees is likely essential to the recovery of plant communities within most restored habitats (Dixon 2009; Menz et al. 2011). However, by altering aspects of abiotic environments in addition to biotic environments, plant-focused management may have unintended effects on bees (Harmon-Threatt and Chin 2016; Tonietto and Larkin 2018; Buckles and Harmon-Threatt 2019). An understanding of how management affects bee communities would allow restoration practitioners to better design recovery plans that incorporate this important group.

Due to the reliance of bees on flowers as their main source of food and nutrients, bee communities are thought to be primarily driven by the availability of floral resources (Potts et al. 2003; Roulston and Goodell 2011; Vaudo et al. 2015) and thus expected to be affected by management actions indirectly through their effects on the local flower community. As a result, conservation efforts for bees focus on improving the abundance and richness of local floral communities (Winfree 2010; Scheper et al. 2015; Vaudo et al. 2015; Williams et al. 2015). However, in addition to altering food resources, management actions such as prescribed burning or grazing may have additional, potentially negative effects on bee communities through their effects on bee survival or nesting habitats (Harmon-Threatt and Chin 2016; Buckles and Harmon-Threatt 2019). For example, burning or grazing could kill stem-nesting bees or change soil conditions or availability for ground-nesting species (Buckles and Harmon-Threatt 2019). Few studies have disentangled the drivers of bee community dynamics in restored habitats (Tonietto and Larkin 2018), and the relative importance of local floral resources versus management factors operating independently on bee communities remains largely unknown. This distinction may have widespread implications for the value of current management practices; if bees respond primarily to floral communities, plant-focused management may be sufficient to restore both groups, but if bees respond to management independently from the flowers, bees may require targeted management for restoration success.

In addition to responding to management and local conditions within restoration areas themselves, bee communities are likely to be affected by the larger landscape context surrounding the restored habitat (Scheper et al. 2015). Bees are known to be sensitive to agricultural land-use, and often show lower abundance and richness with increasing distance from natural habitats (Ricketts et al. 2008; Garibaldi et al. 2011; Kennedy et al. 2013). In a restoration context, this could mean that habitat patches surrounded by higher proportions of agriculture and lower natural habitat may have fewer bees and bee species than restorations embedded within high-quality natural habitats. Diversity of natural habitats may further affect bee communities; increased landscape heterogeneity may increase the diversity of bee communities by adding to the range of floral and nesting resources available to bees (Steffan-Dewenter 2003).

The aim of our study was to identify restoration practices and landscape factors in restored tallgrass prairie which affect the abundance and diversity of bee communities, and to separate out the effects of these factors from those mediated through the flowering plant communities. Tallgrass prairie is a critically endangered ecosystem with less than 1% of the original remnant prairie remaining (Samson and Knopf 1994), making restoration efforts essential for the preservation of prairie biodiversity. To study the effects of restoration management and landscape context, we conducted a three-year observational study of wild bees and flowering plants across 14 restoration plantings in an intensively managed, large-scale tallgrass prairie preserve in north-central Illinois, USA. Using a path diagram of all hypothesized causal relationships between our variables of interest (Figure 1.1), we conducted piecewise structural equation modeling (piecewise SEM; Shipley 2009, 2013; Lefcheck 2015) to identify the complex connections between restoration management factors, landscape context, floral communities, and bee communities. We hypothesized that floral communities would be strongly affected by current methods of restoration management. Due to the strong association between bee communities and their floral resources (Potts et al. 2003; Roulston and Goodell 2011; Crone 2013; Vaudo et al. 2015), we hypothesized that bee abundance and richness would be primarily determined by that of flowering plant communities rather than other effects of management. Further, based on the known sensitivity of bee communities to land-use context (Kennedy et al. 2013), we hypothesized that both bee abundance and richness would be increased by the amount of natural habitat available in the larger landscape.

Methods

Study sites

We conducted our research within restored prairies in The Nachusa Grasslands (41°89′ N, 89°34′ W), one of the largest prairie restoration projects in North America (Rowe 2010; Gerla et al.

2012). Managed by The Nature Conservancy (TNC) in north-central Illinois, Nachusa is composed of over 1,500 ha of tallgrass prairie and other natural habitats including oak savanna and wetlands (Hansen and Gibson 2014). Since restoration efforts began in 1986, TNC has converted over 130 patches of agricultural land into high-diversity tallgrass prairies ranging in size from 3 to 60 ha (Hansen and Gibson 2014). The goal of these restoration efforts is to establish a large, interconnected tract of tallgrass habitat that supports high levels of prairie biodiversity.

The Nachusa Grasslands is actively managed by a team of TNC restoration ecologists and volunteers to ensure maximum diversity of native plants. In the initial phase of restoration, cleared areas are tilled and seeded with mixes of native prairie plants obtained from remnant prairie patches, existing restoration plantings, and local nurseries. Following initial restoration, land managers assess restoration plantings yearly and plan targeted management for each site. All plantings receive prescribed burning every 1–3 years, a rate which was chosen to encourage plant growth and diversity. As needed, grasslands also receive supplemental seeding of underrepresented native plants and targeted removal of exotic species through herbicide treatments. In addition, to increase grazing intensity and thus limit the growth of grasses, a herd of North American Bison (*Bison bison* (L.)) was introduced in 2014 to a subset of the prairie plantings (see map of Nachusa Grasslands, Figure 1.2). Initially composed of 52 individuals, the herd was increased to 95 individuals by 2017. The landscape surrounding the Nachusa Grasslands is almost entirely agricultural and planted with corn and soybean row crops.

We sampled pollinator communities across 14 restoration plantings over the course of three summers: 2015, 2016, and 2017. We selected restoration plantings that were rated as high-quality by the Nachusa Grasslands land managers (B. Kleiman, Nachusa Grasslands Project

Director, pers. comm.), and differed in their management and site characteristics. Selected plantings ranged in size from 4.46 to 25.32 ha, were located between 0.42 and 5.02 km from each other, and were distributed across the landscape such that plantings of similar ages were not spatially clustered (Mantel r=-0.12, p value=0.86). Over the course of our three-year study, our plantings ranged in age from 2 to 29 years since initial planting. Plantings in our study were burned every 1-3 years, creating a mix of burned and unburned plantings every year of the study. Bison were confined to a subset of plantings (Figure 1.2). Landscape context is also known to affect bees (Kennedy et al. 2013) so we quantified the percentage of prairie, forest, and agricultural land within a 500 m radius of each site, a distance that covers the typical foraging range of most bee species (Greenleaf et al. 2007). We used pixel data from Landsat imagery in the Global Forest Change land cover data set (Hansen et al. 2013) to fit a RandomForests model, which applies RandomForests algorithms to classify pixels by land use type (‘randomForest’ package in R, Liaw and Wiener 2002). A detailed description of planting characteristics can be found in Table 1.1.

Bee sampling

We sampled bee communities at each restoration planting using bee bowl arrays and blue vane traps, two trapping methods which use bright coloration to attract and collect bees (see Griffin et al. 2017 for a full description of trapping methods). We placed three bee bowl arrays (Figure 1.3a) and one blue vane trap (Figure 1.3b) in each planting. These were raised to the height of dominant vegetation and spaced 10 m from each other along a 30 m transect positioned 60 m from a non-prairie edge (agricultural land or forest).

We sampled bees approximately every 2 weeks throughout the summer from May to September, including 8 sample rounds in 2015 and 2016, and 6 rounds in 2017. Each sample round consisted of placing traps at every planting on the morning of warm, sunny days and collecting all traps 24 hours later. Specimens were collected from traps directly into ethanol and pinned in the lab. All specimens were identified to species by Jason Gibbs, Laura Rericha-Anchor (Cook County Forest Preserve) and SRG based on taxonomic revisions (Mitchell 1960, 1962; LaBerge 1961, 1971, 1973, 1980, 1989; Ribble 1968; LaBerge and Bouseman 1970; Bouseman and LaBerge 1979; McGinley 1986; Gibbs 2010, 2011; Rehan and Sheffield 2011; Gibbs et al. 2013) and comparison to identified specimens collected at Nachusa and held at Rutgers and Michigan State Universities. All specimens are deposited in the lab of Nick Haddad at the W.K. Kellogg Biological Station, Michigan State University.

Floral sampling

Tallgrass prairie vegetation at the Nachusa Grasslands is dominated by a diversity of perennial forbs and C_4 grasses including big bluestem (*Andropogon gerardii*) and Indian grass (*Sorghastrum nutans*) (Hansen and Gibson 2014). Plants bloom between early April to October, with peak bloom in July and August.

To characterize the local floral community and measure floral resources across the growing season, we conducted floral counts at every restoration planting within 2–3 days after sampling bees. At each planting, we haphazardly selected eight 1x1 m areas by randomly throwing a quadrat square 2–4 meters from each of the 4 points along the bee collection transect, repeating throws twice at each of the 4 points. Within each of the 8 quadrats, we counted the total number of flowers of each forb species, only including open, functional flowers with unwilted petals and fresh anthers and/or stigmas. Methods of counting flowers were determined per flower species depending on aspects such as size and structure that could affect how bees interact with the flowers, in order to standardize floral "units" wherever possible. For example, we counted aster species by inflorescence rather than individual flower, and *Verbena* and *Liatris* species were counted by spike.

Analysis

To test the causal relationships between restoration management methods, landscape context, local floral communities, and bee communities, we used piecewise structural equation models (piecewise SEMs; Shipley 2009, 2013; Lefcheck 2015). Though piecewise SEMs are conceptually similar to classical path analysis in that they examine the multivariate relationships between variables, piecewise SEMs differ in that they do not use global estimation from a single variance-covariance matrix. Rather, they solve individual linear models and then piece the models together to form a full path model. Piecewise SEM therefore allows for different paths to have non-normal sampling distributions and can accommodate datasets with smaller sample sizes (Lefcheck 2015).

To create our piecewise SEMs, we first defined the response variables, management factors, and landscape variables of interest based on our knowledge of the tallgrass prairie study system. We used two response variables to characterize our flower and bee communities: abundance and richness. We calculated abundances by separately summing counts of flowers and bees across all sampling rounds within a given planting per year. Because richness estimates may be affected by the number of individuals and species collected (Chao and Jost 2012), we calculated species richness by rarefying richness estimates for each planting within a year to the lowest level of sampling coverage across the collection of plantings using the iNEXT package (Hsieh et al. 2016). We identified two management variables used by restoration managers to shape plant communities within tallgrass prairie restorations: presence of bison and burning regime. The presence of bison and burning were binary factors indicating whether each of the two treatments had been applied at a restoration planting in a given year. We also included another factor known to be important for plant communities within prairie restorations, restoration age. Restoration age was defined as the number of years since the planting was initially seeded. Because bees are known to be sensitive to the amount and type of natural habitats in a landscape, we chose two landscape variables: % prairie within 500 m of the sampling transect for each planting and % forest within 500 m. Percent prairie represents the amount of usable prairie habitat for bees in the surrounding landscape. Forest is the predominant natural habitat type in the landscape other than prairie, so % forest represents a measure of heterogeneity in natural habitats surrounding each planting. Both % prairie and % forest have a negative correlation with the amount of agricultural land in the surrounding landscape (r=-0.54 and r=-0.7, respectively).

We constructed a full causal path model based *a priori* on our hypothesized relationships between our identified management variables, landscape context, floral communities, and bee communities (Figure 1.1). We then fit component models to each of the four response variables (floral abundance, floral richness, bee abundance, and bee richness) according to their relationships with management factors, landscape context, and each other within our full causal path model. All management and landscape factors were included in each component model, with restoration planting and year included as random effects. The two abundance models were created with quasipoisson distributions (base R; R core team 2016), while the two rarefied richness models were created with normal distributions (nlme package; Pinheiro et al. 2019). Once we had created our full path model, we used a multi-step, informed model selection process to determine a best-fit global model. To do this, we ran the full model and used the p-values for the path coefficients to identify the least important paths within our full model set. We then removed the weakest path and reassessed the overall fit of the SEM using Shipley's test of d-separation (Shipley 2009, 2013; Lefcheck 2015), which identifies paths that are missing from the hypothesized relationships in the full model. If no paths were missing and the test of d-separation remained non-significant, we calculated the overall value of Akaike's information criterion corrected for small sample sizes (AICc) based on Fisher's C statistic generated from the tests of d-separation (Shipley 2013). Lower AICc scores indicate an improvement in model fit; if the model fit decreased by ΔAICc ≥ 2, then we permanently removed the non-significant path and proceeded with the model selection process. If the removal of any path in this way violated the conditions of removal, such as creating missing paths or increasing the AICc score, the path was added back into the full model. In this way, we removed all non-significant paths one by one until we determined a best-fit model. Tests of d-separation and extraction of coefficients were conducted using the R package 'piecewiseSEM' (Lefcheck 2015), and all statistical analyses were conducted in R version 3.2.4 (R Core Team 2016).

Results

We captured a total of 5,557 wild bee individuals representing 102 bee species (Table 1.2), ranging from 101 to 323 individuals per planting in 2015, 57 to 221 bees per planting in 2016, and 65 to 221 bees per planting in 2017. We counted a total of 33,282 individual flowers representing 84 flower species, ranging from 43 to 4593 flowers per planting in 2015, 16 to 2055 flowers per planting in 2016, and 42 to 893 flowers per planting in 2017.

Our best-fit model (Figure 1.4; Fisher's C= 19.29, p= 0.963, AICc= 165) had no missing paths and yielded a substantially better fit than our full model with all hypothesized links between variables (AICc= 3360). The best-fitting model retained no links between local floral variables and bee variables, and for the most part bee and flower communities responded to different aspects of local management and landscape context (Figure 1.4). Bee abundance was increased by % prairie within 500 m of the sample location (unstandardized coefficient=0.02, SE=0.004, p=0.0009; Figure 1.5a), and decreased by the presence of bison (unstandardized coefficient=-0.35, SE=0.11, p=0.003; Figure 1.5c). Bee richness was positively affected by the % forest within 500 m of the sample location (unstandardized coefficient=0.12, SE=0.04, p=0.02; Figure 1.5b). Local floral abundance was negatively affected by the age of the restoration planting (unstandardized coefficient=-0.09, SE=0.02, p=0.0001; Figure 1.6a) and the presence of bison (unstandardized coefficient=-0.62, SE=0.19, p=0.003; Figure 1.6c), but positively affected by burning (unstandardized coefficient=0.65, SE=0.2, p=0.004; Figure 1.6d) and the amount of prairie within 500 m from the sample location (unstandardized coefficient=0.03, SE=0.01, p=0.01; Figure 1.6e). Local floral richness was also negatively affected by the age of the restoration planting (unstandardized coefficient=-0.66, SE=0.1, p=0.0001; Figure 1.6b).

Discussion

By disentangling the effects of restoration management and landscape context on bees from those mediated by the local floral community, we found that the composition of the landscape and presence of bison, not local floral resources, were the primary determinants of wild bee abundance and richness across tallgrass prairie restorations. Thus, bees are not always affected by restoration management primarily through their interactions with the local flower community, as is often assumed. Our study shows that bees can instead respond to management and landscape context independently from the floral community and will therefore require targeted management strategies to maximize bee abundance and diversity.

We found that higher proportions of prairie habitat in the landscape increased bee abundance, while higher variety of natural habitats increased bee richness. This strong response of bees to landscape composition likely reflects the high sensitivity of bees to land-use often observed across agricultural landscapes (Kennedy et al. 2013). Most wild bees are unable to thrive in agricultural areas due to lack of food and nesting resources (Winfree 2010) and the negative effects of pesticides and other agricultural chemicals (Potts et al. 2010). Conversely, landscapes with more natural habitat can support more bee individuals and species due to increased foraging options (Williams and Kremen 2007; Kennedy et al. 2013), higher dispersal between nearby habitat patches (Ponisio et al. 2019), and more nesting habitat (Winfree 2010; Kennedy et al. 2013). At a population level, a higher proportion of natural habitat in a landscape has been shown to increase the number of bee offspring (Williams and Kremen 2007) and colony sizes for eusocial species like bumble bees (Crone and Williams 2016). Composition of the natural habitats themselves may further affect these processes; a more heterogeneous, diverse set of natural habitats can introduce a greater variety of food and nesting resources and thus allow occupation by more bee species (Steffan-Dewenter 2003), as seen in our restored prairies with higher proportions of forest in the landscape. Our results provide evidence for the importance of increasing habitat extent and variety when managing restorations for bees.

Bee communities responded to only a single management factor in our study, the presence of bison within restorations. Though bison were originally introduced to the restored prairies as a form of management for plants, their presence had a direct, negative relationship with bee abundance rather than affecting them through the floral community. One likely explanation for this pattern is that the presence of bison reduced bee nesting by changing soil conditions within restorations. Bison are known to impact the chemical properties of soil through the deposition of nutrient-rich urine and grazing, both of which can alter decomposition rates and amount of leaf litter (Knapp et al. 1999). Bison can also change the physical properties of the soil by directly disturbing the ground with their hooves, trampling vegetation, or compacting soil through the creation of wallows (Knapp et al. 1999; Truett et al. 2001; Veen et al. 2008). Such changes to the soil may prevent ground-nesting bees from establishing new nests or reduce the viability of existing nests. The effects of bison on soil conditions may be especially pronounced in habitat restorations like The Nachusa Grasslands, where bison are confined to a limited space and may therefore revisit specific parts of the landscape more commonly than if they had unlimited space to roam.

Contrary to our expectations, we found no links between local floral resources and bee communities in our restored prairies. This was surprising because bees are known to be highly dependent on flowers for all nutritional and energetic requirements (Roulston and Goodell 2011; Vaudo et al. 2015), and a number of previous studies have identified local floral resources as major drivers of bee community dynamics in restored habitats (Tonietto and Larkin 2018). However, many of these previous studies examined bee communities in relatively small-scale, isolated restorations (e.g. Pywell 2005; Hopwood 2008; Morandin and Kremen 2013; Blaauw and Isaacs 2014), while our study was conducted at a large, interconnected complex of restored and remnant prairies. Thus, differences in findings may be the result of differences in landscape context and the scale at which bees are responding to the flower community. Bees are mobile foragers, and even relatively small species can travel up to several hundred meters to locate flowers (Gathmann and Tscharntke 2002; Greenleaf et al. 2007). In a landscape of high-quality restored prairie habitat like at the Nachusa Grasslands, local floral resources may simply not be limiting to most bees due to overall high floral abundance across the landscape and the ability of bees to forage across long distances. Bees may instead respond to floral availability at larger spatial scales, as shown by bee response to overall habitat availability surrounding our restored prairies. In contrast, local floral resources may be more limiting in smaller, more isolated restorations in which bees are not able to access resources in the greater landscape (Scheper et al. 2015), causing bees to exhibit greater responses to local floral communities. Regardless, our results suggest that increasing the overall extent of habitat may be more effective for conserving wild bees than focusing efforts on improving the quality of floral resources at a local scale.

Though there were some similarities in the response of bees and flowers to management practices and landscape context, flower communities in restored prairies showed the greatest response to habitat age, a factor which did not affect bees. As prairie age increased, floral abundance and richness decreased, with the lowest levels of both experienced in the oldest restored plantings. Similar decreases in plant richness over time have been previously described at the Nachusa Grasslands (Hansen and Gibson 2014) and many other restored prairies (e.g. Sluis 2002; Camill et al. 2004; McLachlan and Knispel 2005), and are primarily the result of gradual dominance and replacement of forb species by C4 grasses (Camill et al. 2004). Preventing decreases in overall plant richness and forb abundance remains a central challenge in grassland restoration.

The two plant-focused management practices that we included in our study, prescribed burning and introduction of large grazers, both had discernible effects on the plant community. Prescribed burning increased floral abundance within restorations. These findings reflect the known short-term effects of prescribed burning; fire clears away dead vegetation and creates an influx of nutrients into the soil, thus stimulating new plant establishment, plant growth, and flower development (Veen et al. 2008). In the long-term, fire is also known to decrease forb abundance and plant richness by further encouraging dominance by fire-tolerant grasses (Collins and Smith 2006). However, the long-term effects of burning were not evident in our study due to our relatively short three-year observational period. Unlike burning, the presence of bison had a negative effect on floral abundance. Because bison primarily eat grasses, not flowering forbs, bison are introduced to restorations to suppress dominant grasses through grazing and subsequently encourage higher plant diversity and forb abundance over the long-term (Knapp et al. 1999; Veen et al. 2008). Again, the short-term nature of our study was unable to capture the expected long-term positive effects of bison, but instead tested the short term year-to-year effects of their presence. Over time, alterations in decomposition and soil nutrients can affect plant growth and flowering (Hobbs 1996; Knapp et al. 1999) and may provide a mechanism for long-term positive effect of bison on the plant community. In addition, though bison do not preferentially eat flowering plants, they may still impact them through trampling and the formation of wallows over time (Knapp et al. 1999; Truett et al. 2001; Veen et al. 2008).

Interestingly, the percentage of prairie habitat surrounding each restoration had a slightly positive effect on floral abundance. Though the mechanism of this pattern is not known, it could be due to biotic factors unmeasured in our study. For example, biotic interactions such as seed dispersal, herbivory, decomposition, and predation may be affected by the amount of habitat in the landscape due to increased movement between habitats or population-level effects of increased habitat area (Tscharntke et al. 2005) and could potentially alter the floral resources within a restored field (Kardol and Wardle 2010). Further study is needed to understand other potential drivers of floral resource distributions across restored landscapes.

Due to the large size of the prairie preserve used in our study (over 1,500 hectares), our findings may be especially relevant for other large-scale restoration projects. Most remaining patches of historic tallgrass prairie exist as small, low-quality fragments (Samson and Knopf 1994; Taft et al. 2006), necessitating large-scale restoration to reconnect habitats and create natural prairie landscapes which can support high levels of biodiversity (Rowe 2010; Gerla et al. 2012). The conservation community has increasingly recognized the importance of such restoration efforts at a landscape-scale, leading to an increase in large restoration projects across many ecosystems including grasslands (Holl et al. 2003; Rowe 2010; Gerla et al. 2012). In the face of worldwide bee declines (Potts et al. 2010; Goulson et al. 2015; Powney et al. 2019), these large-scale restored areas will undoubtably be important to wild bee recovery. Our study shows that an emphasis on increasing the extent and variety of natural habitats and developing bee-focused management will make these large-scale restorations even more effective at protecting bee communities.

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APPENDIX

Table 1.1. Attributes of 14 plantings used in our study. "Year" is the year of data collection and "Age" is the number of years since initial seeding. "Burned" is whether the planting was burned in the non-growing season before data collection and "Bison" is the presence of bison over the growing season. "% prairie" is the percentage of land devoted to prairie within a 500 m radius of the sample locations, and "% forest" is the percentage of land devoted to forest within a 500 m radius of the sample locations.

Planting	Year	Age	Burned	Bison	% prairie	% forest
108/MU	2015	2	no	yes	0.60	0.07
108/MU	2016	3	yes	no	0.60	0.07
108/MU	2017	4	yes	yes	0.60	0.07
2012/HPN	2015	3	yes	yes	0.51	0.05
2012/HPN	2016	4	yes	yes	0.51	0.05
2012/HPN	2017	5	yes	yes	0.51	0.05
103/L	2015	4	yes	yes	0.51	0.12
103/L	2016	5	yes	yes	0.51	0.12
103/L	2017	6	yes	yes	0.51	0.12
94/SB	2015	6	no	no	0.44	0.00
94/SB	2016	7	no	yes	0.44	0.00
94/SB	2017	8	no	no	0.44	0.00
85/CCKWest	2015	7	no	no	0.52	0.00
85/CCKWest	2016	8	no	no	0.52	0.00
85/CCKWest	2017	9	no	no	0.52	0.00
86/HPW	2015	7	yes	yes	0.47	0.32

Table 1.1 (cont'd)

86/HPW	2016	8	yes	no	0.47	0.32
86/HPW	2017	9	yes	no	0.47	0.32
66/CCKEast	2015	8	no	yes	0.48	0.00
66/CCKEast	2016	9	no	yes	0.48	0.00
66/CCKEast	2017	10	no	yes	0.48	0.00
73/FC	2015	8	no	no	0.26	0.16
73/FC	2016	9	no	yes	0.26	0.16
73/FC	2017	10	no	no	0.26	0.16
57/TC	2015	12	no	no	0.28	0.03
57/TC	2016	13	no	yes	0.28	0.03
57/TC	2017	14	no	no	0.28	0.03
49/SF	2015	14	no	yes	0.46	0.02
49/SF	2016	15	no	yes	0.46	0.02
49/SF	2017	16	no	yes	0.46	0.02
50/HPE	2015	14	yes	no	0.44	0.45
50/HPE	2016	15	yes	yes	0.44	0.45
50/HPE	2017	16	yes	no	0.44	0.45
37/WH	2015	23	no	no	0.34	0.46
37/WH	2016	24	yes	yes	0.34	0.46
37/WH	2017	25	yes	yes	0.34	0.46
5/8/MU	2015	25	no	no	0.75	0.02
5/8/MU	2016	26	yes	yes	0.75	0.02

Table 1.1 (cont'd)

5/8/MU	2017	27	yes	yes	0.75	0.02
24/25/MU	2015	27	no	no	0.66	0.26
24/25/MU	2016	28	yes	yes	0.66	0.26
24/25/MU	2017	29	yes	no	0.66	0.26

Table 1.2. List of bee species and total number of specimens collected across all 14 plantings.

Genus	Species	Taxonomic authority	Number collected
Agapostemon	*sericeus*	(Förster, 1771)	7
Agapostemon	*texanus*	(Cresson, 1872)	9
Agapostemon	*virescens*	(Fabricius, 1775)	531
Andrena	*asteris*	(Robertson, 1891)	1
Andrena	*commoda*	(Smith, 1879)	26
Andrena	*cressonii*	(Robertson, 1891)	2
Andrena	*erythronii*	(Robertson, 1891)	2
Andrena	*helianthiformis*	(Viereck & Cockerell, 1914)	7
Andrena	*imitatrix*	(Cresson, 1872)	1
Andrena	*nasonii*	(Robertson, 1895)	2
Andrena	*personata*	(Robertson, 1897)	1
Andrena	*rudbeckiae*	(Robertson, 1891)	5
Andrena	*simplex*	(Smith, 1853)	5
Andrena	*violae*	(Robertson, 1891)	2
Andrena	*wilkella*	(Kirby, 1802)	3
Andrena	*wilmattae*	(Cockerell, 1906	52
Anthidium	*oblongatum*	(Illiger, 1806)	1
Anthophora	*terminalis*	(Cresson, 1869)	7
Augochlora	*pura*	(Say, 1837)	8
Augochlorella	*aurata*	(Smith, 1853)	174
Augochlorella	*persimilis*	(Viereck, 1910)	127

Table 1.2 (cont'd)

Augochloropsis	*sumptuosa*	(Smith, 1853)	1
Bombus	*auricomus*	(Robertson, 1903)	63
Bombus	*bimaculatus*	(Cresson, 1863)	4
Bombus	*fervidus*	(Fabricius, 1798)	59
Bombus	*griseocollis*	(DeGeer, 1773)	74
Bombus	*impatiens*	(Cresson, 1863)	84
Bombus	*rufocinctus*	(Cresson, 1863)	74
Bombus	*vagans*	(Smith, 1854)	5
Calliopsis	*nebraskensis*	(Crawford, 1902)	2
Ceratina	*calcarata*	(Robertson, 1900)	4
Ceratina	*dupla*	(Say, 1837)	135
Ceratina	*mikmaqi*	(Rehan and Sheffield, 2011)	23
Ceratina	*strenua*	(Smith, 1879)	2
Colletes	*inaequalis*	(Say, 1837)	2
Dieunomia	*heteropoda*	(Say, 1824)	1
Dufourea	*monardae*	(Viereck, 1924)	4
Eucera	*hamata*	(Bradley, 1942)	542
Halictus	*confusus*	(Smith, 1853)	20
Halictus	*ligatus*	(Say, 1837)	129
Halictus	*parallelus*	(Say, 1837)	25
Halictus	*rubicundus*	(Christ, 1791)	1
Hoplitis	*pilosifrons*	(Cresson, 1864)	5

Table 1.2 (cont'd)

Hoplitis	*spoliata*	(Provancher, 1888)	1
Hylaeus	*affinis*	(Smith, 1853)	27
Hylaeus	*mesillae*	(Cockerell, 1896)	10
Hylaeus	*modestus*	(Say, 1837)	3
Lasioglossum	*admirandum*	(Sandhouse, 1924)	10
Lasioglossum	*albipenne*	(Robertson, 1890)	281
Lasioglossum	*anomalum*	(Robertson, 1892)	591
Lasioglossum	*bruneri*	(Crawford, 1902)	20
Lasioglossum	*callidum*	(Sandhouse, 1924)	1
Lasioglossum	*coreopsis*	(Robertson, 1902)	1
Lasioglossum	*coriaceum*	(Smith, 1853)	37
Lasioglossum	*cressonii*	(Robertson, 1890)	6
Lasioglossum	*ellisiae*	(Sandhouse, 1924)	26
Lasioglossum	*fedorense*	(Crawford, 1906)	1
Lasioglossum	*foveolatum*	(Robertson, 1902)	1
Lasioglossum	*fuscipenne*	(Smith, 1853)	2
Lasioglossum	*hitchensi*	(Gibbs, 2012)	32
Lasioglossum	*illinoense*	(Robertson, 1892)	7
Lasioglossum	*imitatum*	(Smith, 1853)	1
Lasioglossum	*leucocomum*	(Lovell, 1908)	193
Lasioglossum	*leucozonium*	(Schrank, 1781)	3
Lasioglossum	*lineatum*	(Walker, 1995)	2

Table 1.2 (cont'd)

Lasioglossum	*nelumbonis*	(Robertson, 1890)	1
Lasioglossum	*oceanicum*	(Cockerell, 1916)	226
Lasioglossum	*paradmirandum*	(Knerer and Atwood, 1966)	56
Lasioglossum	*pectorale*	(Smith, 1853)	67
Lasioglossum	*perpunctatum*	(Ellis, 1913)	100
Lasioglossum	*pilosum*	(Smith, 1853)	778
Lasioglossum	*pruinosum*	(Robertson, 1892)	423
Lasioglossum	*tegulare*	(Robertson, 1890)	2
Lasioglossum	*versatum*	(Robertson, 1902)	56
Lasioglossum	*viridatum*	(Lovell, 1905)	1
Lasioglossum	*weemsi*	(Mitchell, 1960)	2
Lasioglossum	*zephyrum*	(Smith, 1853)	7
Lasioglossum	*zonulum*	(Smith, 1848)	12
Megachile	*addenda*	(Cresson, 1878)	2
Megachile	*brevis*	(Say, 1837)	15
Megachile	*campanulae*	(Robertson, 1903)	1
Megachile	*inimica*	(Cresson, 1872)	1
Megachile	*latimanus*	(Say, 1823)	7
Megachile	*mendica*	(Cresson, 1878)	1
Megachile	*montivaga*	(Cresson, 1878)	6
Melissodes	*agilis*	(Cresson, 1878)	73
Melissodes	*bimaculatus*	(Lepeletier, 1825)	154

Table 1.2 (cont'd)

Melissodes	*communis*	(Cresson, 1878)	1
Melissodes	*coreopsis*	(Robertson, 1905)	3
Melissodes	*desponsus*	(Smith, 1854)	14
Melissodes	*druriellus*	(Kirby, 1802)	3
Melissodes	*trinodis*	(Robertson, 1901)	25
Nomada	*articulata*	(Smith, 1854)	1
Nomada	*superba*	(Cresson, 1863)	2
Osmia	*georgica*	(Cresson, 1878)	1
Peponapis	*pruinosa*	(Say, 1837)	8
Perdita	*swenki*	(Crawford, 1915)	1
Pseudopanurgus	*albitarsis*	(Cresson, 1872)	8
Sphecodes	*davisii*	(Robertson, 1897)	1
Sphecodes	*mandibularis*	(Cresson, 1872)	1
Sphecodes	*pimpinellae*	(Robertson, 1900)	1

Figure 1.1. Conceptual path diagram of all hypothesized links among local management, landscape context, local floral communities and bee communities across the restored prairie plantings. “Management variables” include local aspects of restoration management such as restoration age, presence of bison, and prescribed burning as well as landscape context such as % forest and % prairie within 500 m, and are hypothesized to affect bee abundance and richness either through a direct connection or through their effects on floral abundance and richness.

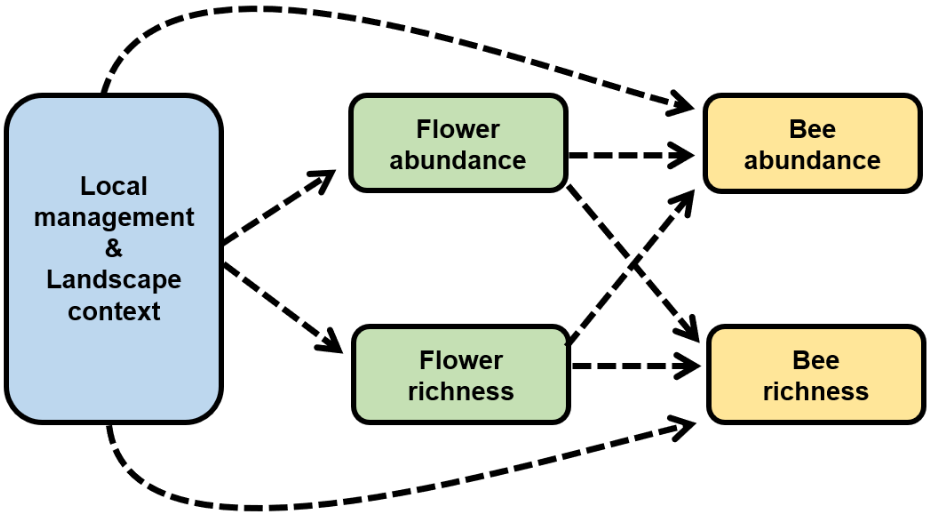


Figure 1.2. Map of the Nachusa Grasslands. Land within the boundary lines is composed of a mix of remnant tallgrass prairie, restored prairie, oak savannah, wetlands, forest, and other habitats. The dotted area indicates the area over which bison are able to roam, as of 2017. Dark grey patches indicate prairie restoration plantings used in our study.

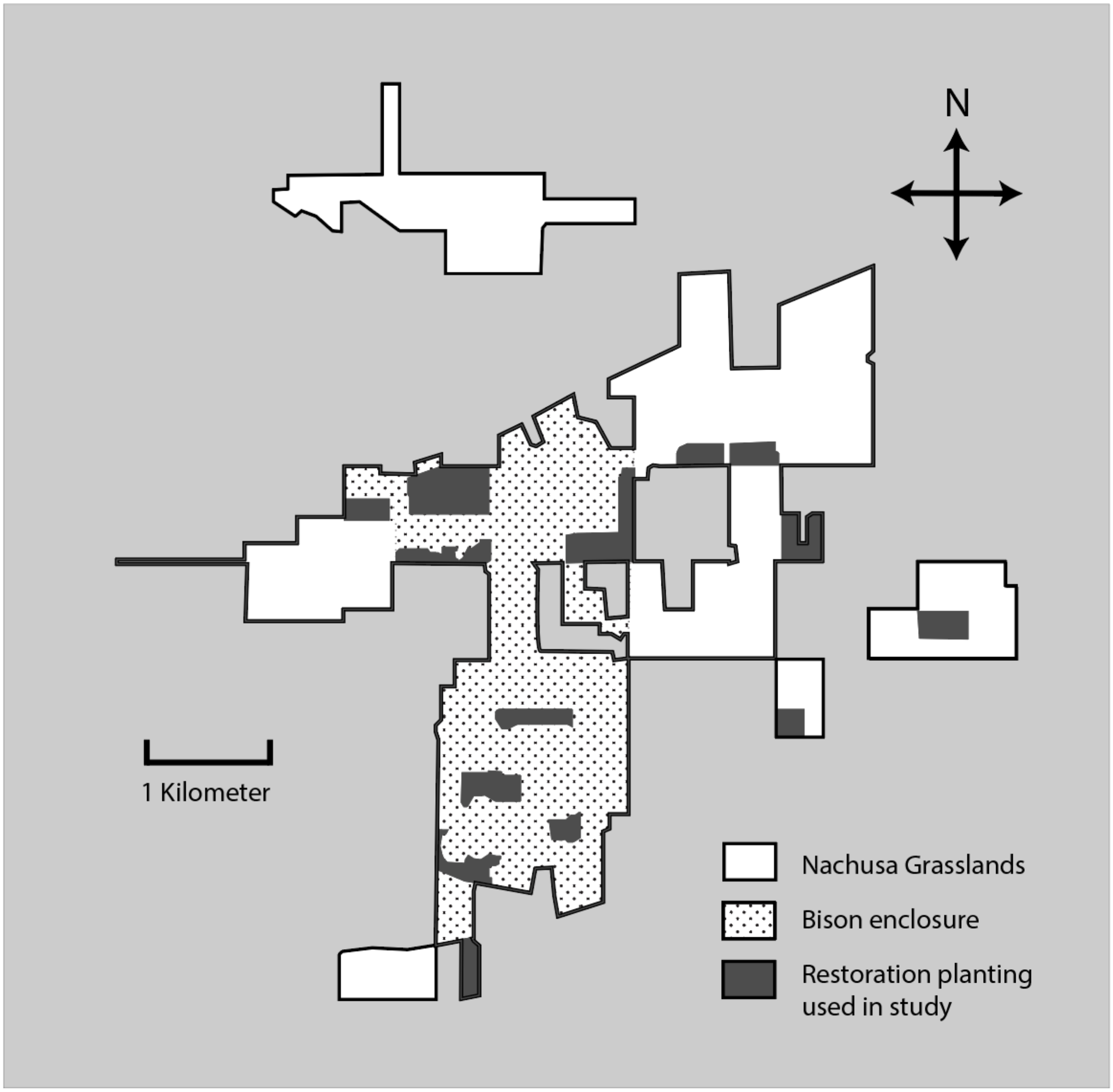


Figure 1.3. Pictures of traps for passively collecting bees across each restoration plot. a) Elevated pan trap array, composed of three bowls (fluorescent yellow, fluorescent blue, and white) attached to PVC pipe for stability and mounted on metal rebar to the height of local dominant vegetation. b) Blue-vane trap, hung from a clothes-hanger and mounted on metal rebar directly above the local dominant vegetation.

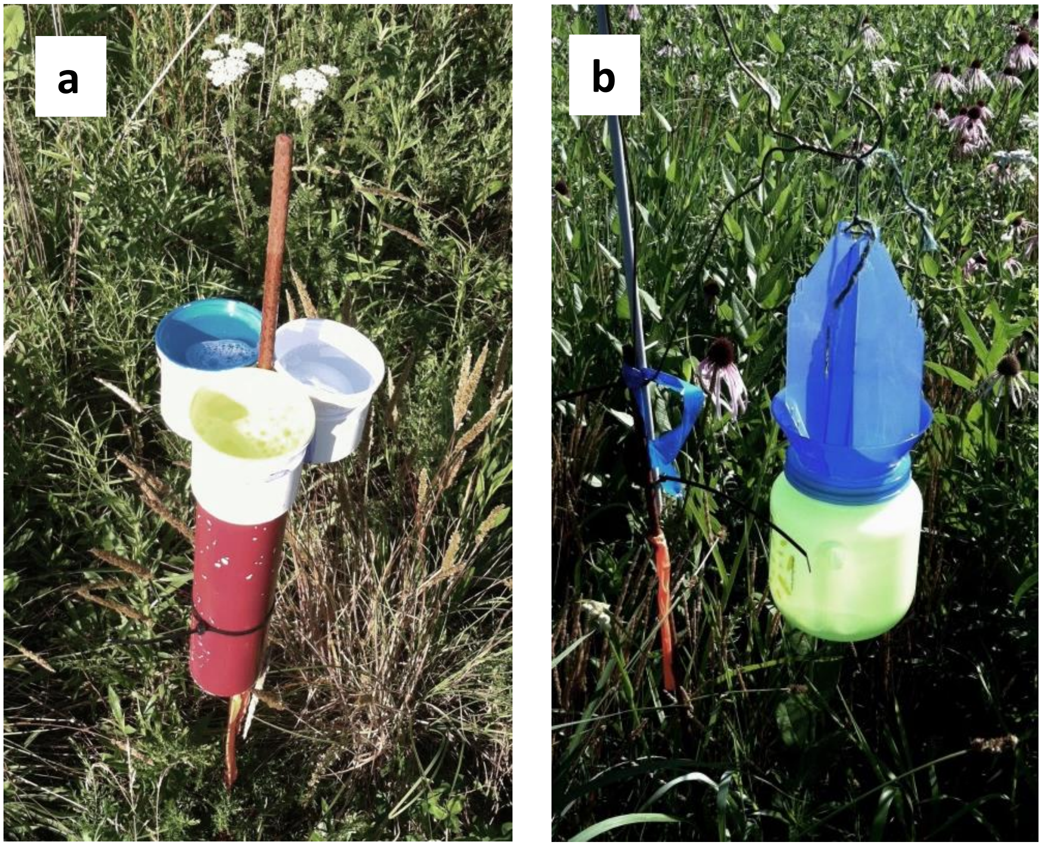


Figure 1.4. Best-fit path diagram of the links between management variables, landscape context, local flower communities, and bee communities retained in our best-fit piecewise structural equation model. Arrows indicate directionality of connections between variables, with black arrows indicating a positive effect and red arrows indicating a negative effect. Arrow thickness indicates the significance level of the path.

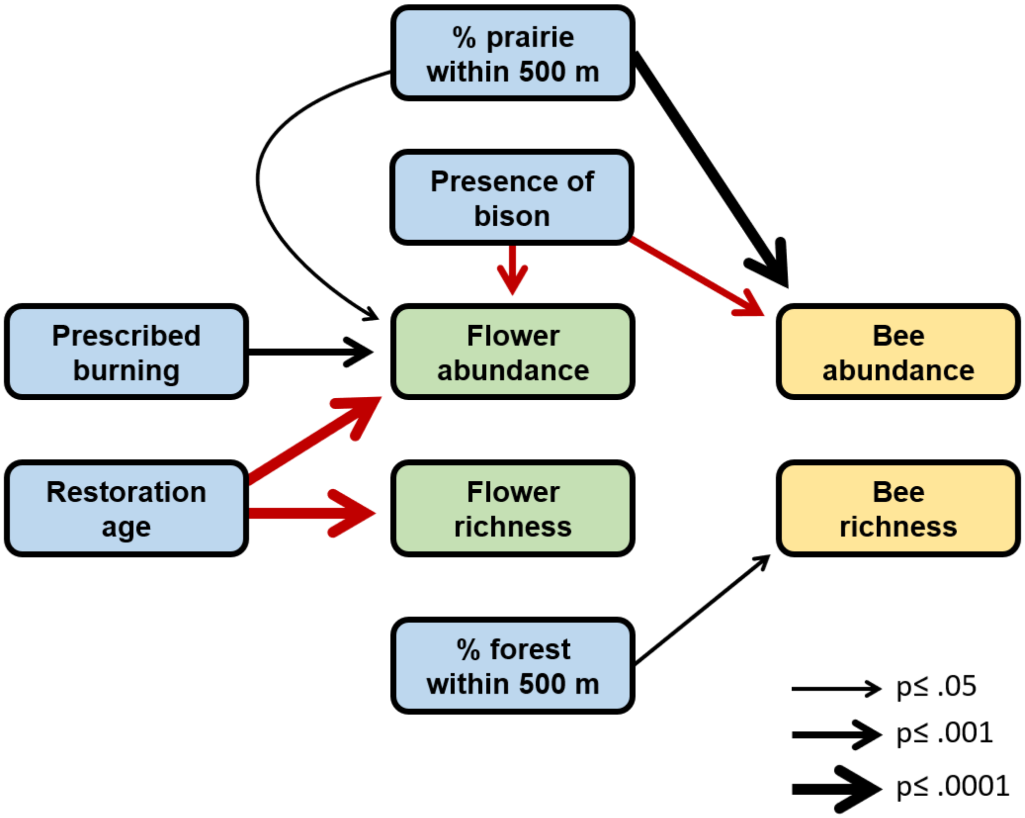


Figure 1.5. The relationships between bee abundance, bee richness, and management variables included in the best-fit structural equation model. a) Number of bee individuals per transect per year plotted against % prairie within 500 m from the sampling points, and b) Number of bee species per transect per year plotted against % forest within 500 m from the sampling points. Trend lines were predicted from models with only % prairie and % forest within 500 m included as fixed effects, respectively. c) Arithmetic means (± SE) of number of bee individuals per transect per year in restoration plots vs. bison treatment.

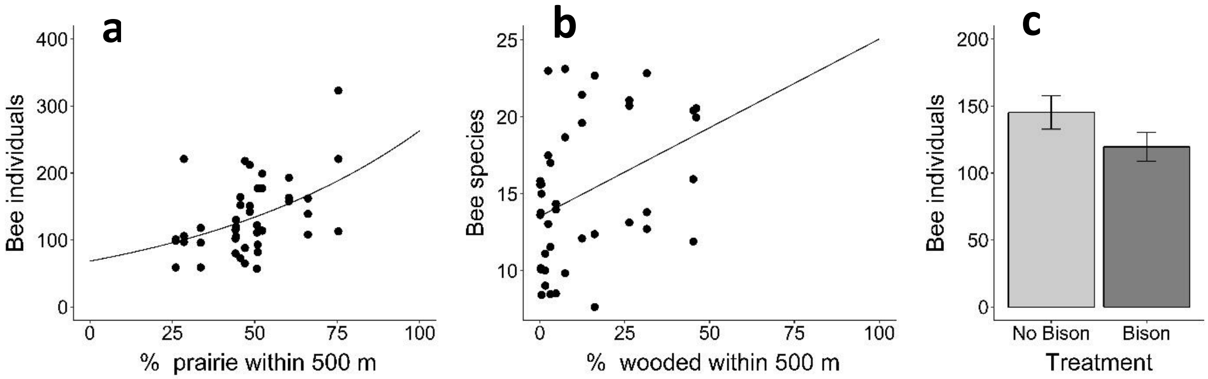
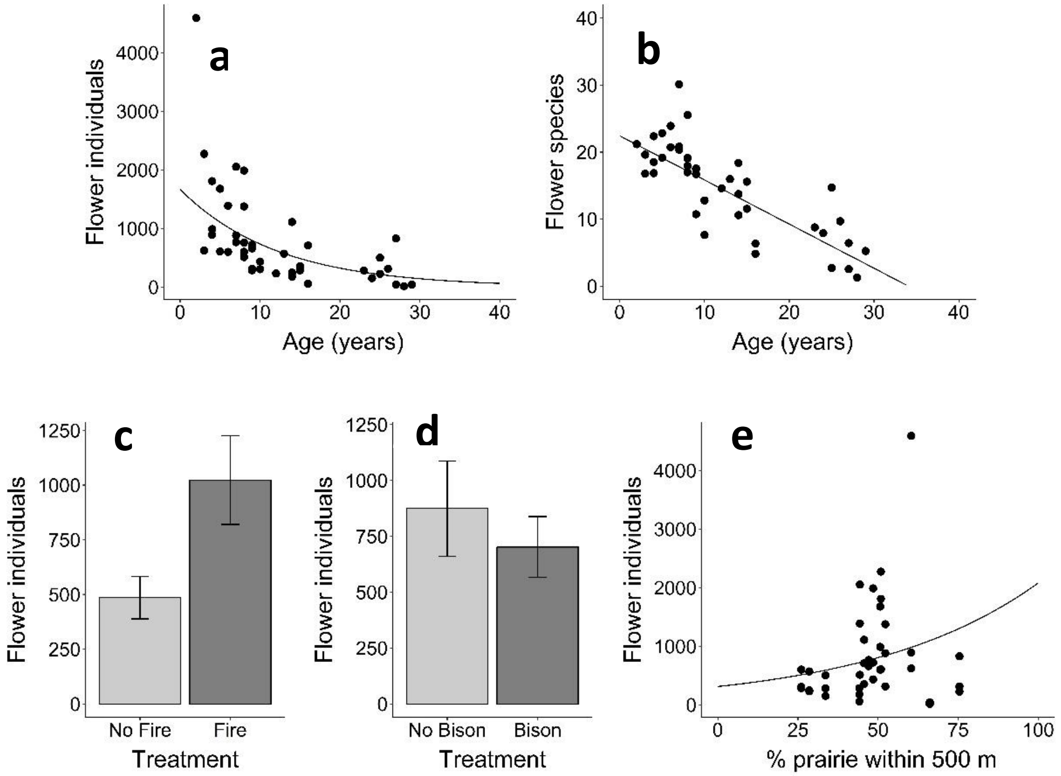


Figure 1.6. The relationships between plant abundance, plant richness, and management variables included in the best-fit structural equation model. a) Number of flowers per transect per year and b) number of flower species per transect per year plotted against age of the restoration plot. Trend lines were predicted from models with only age included as a fixed effect. c) and d) arithmetic means (± SE) of number of flower species per transect per year in restoration plots vs. fire and bison treatments, respectively. e) Number of flowers per transect per year plotted against % prairie within 500 m from the sampling points. The trend line was predicted from a model with only % prairie within 500 m included as a fixed effect.



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CHAPTER TWO

Connectivity and edge effects increase bee colonization in an experimentally fragmented landscape

Abstract

Though landscape corridors increase dispersal of many animals and plants, it remains unknown whether these positive effects extend to the process of colonization and establishment of new populations in fragments. Working in experimentally fragmented landscapes, we tested how two aspects of habitat fragments altered by corridors- connectivity and edge-to-area ratio- determine patterns of colonization by a solitary, cavity-nesting bee (*Megachile rotundata*). We found that though connectivity initially affected rates of nest-site occupation, edge-to-area ratio ultimately drove the final patterns of patch occupation and nest building, likely due to habitat selection by our focal species. These responses were independent of floral resource availability, indicating that spatial aspects of a habitat have a greater effect on the process of colonization than food resources. In sum, our results provide evidence of the importance of considering both connectivity and edge effects on population dynamics in habitat-based conservation.

Introduction

Habitat fragmentation, which decreases habitat amount while simultaneously increasing habitat isolation, is among the most serious threats to animals and plants worldwide (Haddad et al. 2015; Hanski 2015). At a landscape scale, fragmentation causes declines in metapopulation persistence by reducing the survival of local populations and providing a significant barrier to immigration between habitat patches (Gonzalez et al. 1998). Colonization is a key driver of these metapopulation dynamics; increasing rates of recolonization in habitats where local populations have been lost can increase metapopulation viability across fragmented landscapes (Hanski 1998). Theory predicts that colonization rates may be affected by spatial aspects of fragments such as connectivity to other habitat patches, patch shape, and patch area (Weins 1997; Klok and DeRoos 1998; Moilanen and Hanski 1998; Moilanen and Nieminen 2002; Ewers and Didham 2007). However, there has been little study of how habitat-focused conservation practices, like corridors, alter the ability of dispersing organisms to establish local populations.

One justification for the use of landscape corridors (thin strip of habitat used to connect isolated habitat fragment) is that they are expected to promote colonization of fragments and thereby increase population viability. Increased connectivity due to corridors has been shown to effectively increase the movement of plants and animals across landscapes (Gilbert-Norton et al. 2010; Resasco 2019). In turn, higher dispersal caused by corridors increases abundance (Haddad and Baum 1999; Resasco et al. 2014) and species richness in connected fragments (Gilbert et al. 1998; Damschen et al. 2006). However, though the higher dispersal caused by landscape corridors is expected to positively affect rates of colonization, few studies have explicitly studied the effects of corridors on population-level processes like establishment (Beier and Gregory 2012).

In addition to their primary function of restoring connectivity, corridors can have an unintended consequence: they increase patch edge-to-area ratio of fragments (Haddad and Baum 1999; Tewksbury et al. 2002). Higher edge-to-area ratio due to the long, thin shape of corridors may alter abiotic conditions within the connected patches themselves (Menz et al. 2011; Evans et al. 2012; Damschen et al. 2014) and increase the amount of suitable habitat for edge-associated species (Ewers and Didham 2007; Haddad et al. 2014). The addition of edgy habitat may also raise the probability that dispersing organisms will encounter habitat edge when moving through the landscape (Ewers and Didham 2006, 2007). Though both connectivity and edge effects of landscape corridors have been found to affect movement of organisms between fragmented habitats, their relative impacts on colonization are unknown.

Habitat-based conservation is commonly used for one group, the wild bees, with little understanding of how spatial factors may affect the ability of bees to establish new populations. Wild bees have been devastated by the destruction of natural habitat and other human activities (Potts et al. 2010; Winfree 2010; Goulson et al. 2015), causing these important pollinators to become a major focus of conservation efforts worldwide (Byrne and Fitzpatrick 2009; Wilson et al. 2017). Conservation practices generally center on the creation of high-flowering habitats (Winfree 2010; Scheper et al. 2015; Vaudo et al. 2015; Williams et al. 2015), with the hope that bees will quickly colonize these new habitats and diverse bee communities will persist over time. Thus, strategies that increase the likelihood and success of bee colonization may be vital for meeting conservation goals.

Landscape corridors and other linear habitat elements can increase bee dispersal and movement across landscapes (Haddad et al. 2003; Townsend and Levey 2005; Cranmer et al. 2012) and may therefore be a useful conservation strategy for bees. Foraging bumblebees follow hedgerows and other linear habitat elements through agricultural fields, essentially using them as corridors (Cranmer et al. 2012). In addition, higher connectivity between habitats leads to increased movement of pollen carried by bees and other pollinators (Tewksbury et al. 2002; Townsend and Levey 2005; Geert et al. 2010; Cranmer et al. 2012; Kormann et al. 2016). However, these and other studies of bee response to landscape fragmentation generally measure foraging bees rather than dispersers (Pyke 1984; Palladini and Maron 2014), and thus do not reveal whether corridors positively affect bee populations and population processes like colonization.

To test the effects of connectivity and edge-to-area ratio on habitat colonization by bees, we conducted a large-scale release experiment with solitary, cavity-nesting bees within a set of experimentally fragmented landscapes. The bees were not previously found in our landscapes, so any bees we observed originated from the location of our releases. We tested for effects of patch connectivity and edge-to-area ratio on three aspects of solitary bee colonization: initial bee occupation of nests, nest-building, and reproductive output. We also included floral availability as a covariate in our analysis based on the known, positive relationship between bee abundance and floral abundance (Potts et al. 2003; Roulston and Goodell 2011; Hopwood et al. 2015; Herrmann et al. 2017). Due to previous findings that corridors increase pollen transport and pollinator movement (Tewksbury et al. 2002; Haddad et al. 2003; Townsend and Levey 2005), we hypothesized that corridors would increase bee colonization across our fragmented landscapes and that these effects would be primarily driven by connectivity rather than edge effects.

Methods

Experimental landscapes

To test the effects of corridors and habitat edges on patterns of bee colonization, we used a long-established set of experimentally fragmented landscapes: the Savannah River Site Corridor Project. These experimental landscapes were created in winter 2000 and 2007 and are maintained by the US Forest Service at Savannah River Site (SRS; Figure 2.1a), a National Environmental Research Park in Aiken and Barnwell Counties in South Carolina, USA (33.20N, 81.40W).

Within this experiment, each of seven replicate landscapes (hereafter “blocks”) (Figure 2.1b) is composed of five equally-sized (~1 hectare) patches of open longleaf pine savanna habitat within a surrounding matrix of pine forest. Each block has one center patch surrounded by four peripheral patches. A single peripheral patch is connected to the center patch by a 150 m long by 25 m wide habitat corridor of more open longleaf pine savanna habitat (“connected” patch). Three unconnected patches in each block are the size of the connected patch plus the corridor. The additional area is added either as blind-ended corridors (“winged” patches) or an extended footprint (“rectangular” patches), to control for the added area of the corridors (Figure 2.1c). In this experimental design, comparison between connected and winged patches provides a test of the effect of connectivity because the two patch types have similar shapes and edge-to-area ratios but differ in connectivity. Comparison between winged and rectangular patches provides a test of the effect of edges, because both are disconnected but winged patches have ~50% more edge than rectangular patches. Finally, comparison between connected and rectangular patches provides a test of the additive effects of connectivity and edge, because connected patches have more edge and connectivity than rectangular patches. All habitat patches are being restored to longleaf pine savanna through prescribed burning and hardwood removal, and contain a diversity of flowering forbs that serve as floral resources for bees.

Experimental bee release

We studied the Alfalfa Leafcutting Bee (*Megachile rotundata*), a small solitary bee commonly managed as a pollinator of alfalfa and other commercial crops. Though this bee originated in Europe, its current range extends throughout the entire United States (Strange et al. 2017). *M. rotundata* was an ideal model organism for our landscape-level experiment for several reasons.

First, because this bee is commercially available and overwinters as larvae within cocoons made from leaves, it was possible to obtain sufficient numbers of bees and carefully control their emergence timing for our experiment (Pitts-Singer and Cane 2011). Second, as *M. rotundata* is a cavity-nesting species, we were able to manipulate nesting resources across each block to study patterns of dispersal and fragment colonization (McCorquodale and Owen 1997). Third, though this bee is found in the region surrounding SRS, to date it has never been found within our sites, likely due its sensitivity to hot summer conditions (Szabo and Smith 1972; Barthell et al. 2002). Therefore, we have high confidence that any *M. rotundata* found within our study sites originated from our release points. Finally, because body size predicts foraging range and dispersal ability in bees (Greenleaf et al. 2007), *M. rotundata* is a relevant model for the dispersal and colonization behavior of other small-bodied, solitary bees. *M. rotundata* also exhibits similar cavity-nesting behaviors to native Megachile, a common group in our study region (Horn and Hanula 2004).

In early April 2018, we obtained *M. rotundata* cocoons from a commercial bee provider, JWM Leafcutters Inc. We stored all cocoons at 4°C until late April when spring temperatures and floral resources at the experimental blocks were approaching suitable levels for *M. rotundata*. We then incubated the cocoons at 30° C for ~17 days until the first of the male bees started emerging, after which we placed 40 grams of cocoons (approximately 500 individuals) into each release box. Release boxes consisted of white cardboard boxes (10.16 by 10.16 by 5.08 cm) with small holes cut in one side, placed inside empty wooden nest boxes and mounted on heavy metal fenceposts one meter above the ground. Wooden boxes were built using 1.5 cm pine plywood, and were 36 cm tall, 24 cm wide and 18 cm deep, with an extra 6 cm overhang to protect bee nests from rain and direct sun. Four of these wooden nest boxes were placed in the center patch of each experimental block (36.77 m from the two closest edges), for a total of approximately 2000 *M. rotundata* individuals released per block. Because female bees generally make up about 1/3 of all *M. rotundata* (Pitts-singer and James 2005), our releases constituted approximately 667 reproductive female bees released per experimental block. All cocoons in release boxes were placed in the field on May 10th and 11th, and full emergence likely occurred within one to two weeks following this initial placement.

Prior to bee release, we placed standardized nesting resources in all peripheral patches to allow observation of bee colonization and brood rearing. Within nest boxes (identical to the wooden boxes described above) we used two types of nesting resources: bundles of *Phragmites* reeds ~0.8 cm diameter and 15.24 cm length, and a polystyrene block with drilled holes 0.6 cm diameter and 7.62 cm length. Two bundles of 30 reeds were zip-tied to the sides of each nest box, and one 15.24 by 15.24 cm polystyrene nesting block with approximately 210 holes was glued to the back of each nest box (Figure 2.2). Nest boxes were provisioned with this overabundance of nesting resources to increase our chances of colonization and so nesting resource availability was not limiting to bee colonization. We placed four nest boxes in each peripheral patch, positioned in every corner 14.85 m from the two closest edges. Because our questions related to edge context, we placed our nest boxes at standard distances from the edge, while recognizing that because of important features of our experimental design needed to test for edge effects, two corners of rectangular patches were 37.5 m further from the center patch than in connected and winged patches. Nest boxes were mounted on heavy metal fence posts approximately one meter from the ground and placed facing the south-east to standardize directionality and allow for morning sun exposure. Chicken wire was also attached to the front of all nest boxes as protection from disturbance by birds and mammals.

Bee dispersal and colonization

To study initial *M. rotundata* occupation of habitat patches, we counted individual bees within nest boxes soon after the release. To do this, we visited nest boxes at night when all occupying bees were present in their nests. Shining a bright flashlight into the reeds and nest blocks, we counted every bee present in nests and permanently marked the nest location using a colored paint pen. We were able to reliably distinguish *M. rotundata* from other species of wood nesting *Megachile* by their small size and white scopa. We started our first round of observation on May 20, 10 days after release, to give female bees adequate time to emerge, mate, and search for nests. We continued to visit all nests throughout the flight season to check for *M. rotundata* presence, and once no additional nesting *M. rotundata* were found in nest boxes (June 13th, 34 days after release), we removed all nest materials from the field.

In the lab, we dissected all reeds and occupied nesting blocks to count total number of nests and brood cells produced. As a measure of nests produced by *M. rotundata,* we counted all nesting resources (individual reeds plus holes in nest block) that had one or more fully formed brood cells. To obtain a measure of cells per nest, we counted the number of fully formed cells within each individual nest, pulling apart attached cells to gain an accurate count.

Floral resources

To measure floral resources available to nesting bees during our experiment, we counted flowers within the four peripheral patches of each experimental block. We created 50m transects at three distances from the nearest edge (1 m, 14.85 m, and 36.77 m), parallel to two sides extending from a randomly selected corner of the patch such that each transect was composed of two 25 m transects that met at a right angle. Within 1 m of each side of the transect, we counted all flowering plant individuals per species and the number of inflorescences per plant for 10 selected individuals per species. We then multiplied the average inflorescences for these 10 individuals by the number of individuals of that species to obtain an estimated number of inflorescences per species per transect. We added together the estimated number of inflorescences for all species in a transect and all transects in a patch to obtain a relative count of inflorescences per patch. We conducted two rounds of floral sampling, with the first round between May 25-29 and the second between June 10-13.

Analysis

We used generalized linear mixed models to examine the effects of patch type and floral resources on three measures of *M. rotundata* colonization: initial nest occupation, number of nests created, and number of cells per nest. We calculated all response variables per patch by combining counts from both types of nesting materials (reeds and nest blocks) and each of the four nest boxes within a patch. For each of our three models, we used patch type (connected, winged, and rectangular) and floral abundance as our main effects and included block as a random effect. We only used floral abundances from the first round of sampling for the initial nest occupation model to better match the timing of floral counts to the response variable. We summed floral abundances from both rounds of floral sampling for the other two models. For the cells per nest response variable, we removed one block from the model because bees did not build any nests in that block. We used GLMMs with Poisson distribution (package lme4; Bates et al. 2014) for all response variables. Test statistic (χ^2 value) and significance for fixed effects in all models were estimated using type III ANOVAs. We then conducted Tukey-Kramer contrasts (package lsmeans; Lenth 2016) to determine the effect of patch type. To confirm that our results for rectangular patches were not confounded by the additional 37.5 m distance of the further two boxes from the center patch, we repeated all analyses with only the subset of nest boxes in each peripheral patch closest to the center patch (2 boxes per patch). All analyses were performed in R (R Core Team, 2013).

Results

In our first survey, we observed 88 nesting female *M. rotundata*. We found an effect of patch type (χ^2=7.29, df=2, p=.03) on initial occupation rates. Connected patches had higher, albeit non-significant, initial occupation than winged patches (35% more bees, Figure 2.3) and significantly higher initial occupation than rectangular patches (111% more bees, Figure 2.3), indicating an additive effect of connectivity and edge-to-area ratio. We found no effect of floral resources (χ^2=0.42, df=1, p=.52) on initial occupation rates.

After the nest tubes were removed from the field at the end of the flight period, we counted a total of 168 *M. rotundata* nests containing 494 sealed brood cells. We found that the number of nests in patches was strongly affected patch type (χ^2=18.03, df=2, p=.0001), as bees in connected and winged patches produced similar numbers of nests, but those patch types had 122-159% more nests than rectangular patches (Figure 2.4a). Nest number was not related to floral resources (χ^2=1.32, df=1, p=.25, Figure 2.5). The number of cells per nest was not affected by patch type (χ^2=0.44, df=2, p=.80, Figure 2.4b) or floral resources (χ^2=0.21, df=1, p=.65, Figure 2.5).

When we repeated all analyses with a sub-sample of our data including only the two nest boxes in each peripheral patch closest to the center patch, we found similar trends to those using the full dataset. Patch shape marginally affected initial bee occupancy (χ^2=5.49, p=0.06) and cells per nest (χ^2=5.55, p=0.06), and significantly affected nest production (χ^2=10.8, p=0.005). These findings confirm higher bee occupancy and nest building in connected and winged patches than in rectangular patches. Further, these results indicate that our initial results with the full dataset were not driven by the fact that the furthest two nest boxes in rectangular patches were 37.5 m further from the center than those in the other peripheral patch types.

Discussion

By introducing a new species to experimentally fragmented landscapes and studying its occupation of empty habitat patches, we show that landscape corridors increase patch colonization and likely increase metapopulation viability. Bee colonization of fragmented habitats responded strongly to both spatial aspects altered in our experimental design by corridors, increased connectivity and edge-to-area ratio. There was a temporal component to these effects: landscape corridors increased initial bee occupation of nest boxes in connected habitats due to the additive effects of increased connectivity and increased edge-to-area ratio, whereas higher edge-to-area ratio was the ultimate determinant of nest establishment across habitat fragments. Thus, landscape corridors affect not only individuals' movement and dispersal behaviors as previously shown (Resasco 2019), but also population-level processes within fragmented habitats.

The effects of corridors on colonization observed in our study may have important consequences for metapopulation dynamics. Metapopulation persistence across landscapes is a function of both local population viability and colonization rates of unoccupied habitats, because persistence in the long-term depends on the overall size of the metapopulation and its ability to replace extirpated local populations (Hanski 1998). To date, population-focused work with corridors has largely focused on local population viability. Previous studies have shown that higher connectivity can increase dispersal between habitat fragments (Gilbert-Norton et al. 2010; Resasco 2019) and increase population sizes (Gonzalez et al. 1998; Hudgens and Haddad 2003; Resasco 2019). Our study instead focused on the second factor that affects metapopulation persistence, colonization, and found positive effects of both increased connectivity and edge. Thus, higher rates of metapopulation survival in landscapes connected by landscape corridors (Gonzalez et al. 1998) may be attributable to both increased local population viability and higher rates of recolonization. Further, these population-level responses can be expected to scale up to affect entire communities, providing a possible mechanistic explanation for increased species richness in habitat fragments connected by landscape corridors (Damschen et al. 2006).

Our study shows that increases in insect movement that have been observed in response to corridors (Townsend and Levey 2005) result in more rapid colonization of patches. Connectivity had an additive effect on initial rates of nest-site occupation, with the highest initial occupation of nest boxes occurring in patches connected by a landscape corridor. Corridors likely channeled dispersing bees from the center patches of the experimental blocks towards connected patches, allowing them to quickly find suitable nesting habitats and establish new populations. Such behavior may have been due to edge-following from release patches into the corridors (Haddad 1999; Joyce et al. 1999; Berggren et al. 2002; Holzschuh et al. 2009; Cranmer et al. 2012) and a preference for flying through the open habitat of the corridors rather than through the forest matrix. An effect of connectivity was evident in our fragmented habitats separated by only 150 m; differences in colonization rates may be expected to be even more pronounced when fragments are more isolated or smaller in size.

Although connectivity initially affected occupation of nest-sites, edge-to-area ratio had an additive effect on these occupation rates and ultimately drove patterns of nest building. Almost double the number of nests were produced in high-edge connected and winged patches than in low-edge rectangular patches. Because most nests were incomplete during the final count, we believe that most female bees only produced one nest each within our study; thus, patterns of nest building were likely driven by dispersal by individuals rather than differences in reproductive success between patches. One explanation for our findings is that dispersing bees may have eventually been able to fly anywhere at the scale of our experimental blocks, and preferentially settled in high-edge patches. Such habitat selection for high edge habitat seems likely given that the bee used in our experiment, *M. rotundata*, is a cavity-nesting species that would naturally nest in holes in trees and is therefore an edge-associated species (Pitts-Singer and Cane 2011). Dispersing bees may also prefer the abiotic conditions of high-edge habitats, which are cooler than rectangular patches (Evans et al. 2012). Bees are known to be sensitive to temperature in terms of their foraging (Rader et al. 2013) and nesting (Potts and Willmer 1997; Weissel et al. 2006), so differences in temperature between patches differing in edge:area ratio could affect bee brood production and nesting. Further, *M. rotundata* are predominantly northern in distribution and known to be prefer cooler environmental conditions (Szabo and Smith 1972; Barthell et al. 2002). Another explanation for these patterns is that the corridors in the connected patches and blind-ended corridors (wings) of the winged patches may have functioned to intercept and channel dispersing bees towards the interior of the habitat patches. High-edge habitats are known to have higher rates of colonization than more compact, low-edge fragments due to the increased chance of encounters by dispersing organisms (Hamazaki 1996; Bevers and Flather 1999; Collinge and Palmer 2002; Ewers and Didham 2006, 2007), but this effect has rarely been shown in relation to habitat corridors (Tewksbury et al. 2002, Levey et al. 2005, Townsend and Levey 2005, but see Fried et al. 2005).

Once bees settled in patches, female fecundity did not vary across patch types. Although landscape corridors allow foraging pollinators greater access to connected habitats (Townsend and Levey 2005), this increased access did not translate into higher reproduction for bees in our study. This may have been due to the small foraging range of our focal bee species *M. rotundata* (Greenleaf et al. 2007), which may have foraged solely in the patch in which they nested, making connectivity with other patches irrelevant from a foraging perspective. Alternatively, floral resources within patches may have been sufficiently abundant to support bee reproduction, regardless of patch type.

We found no effect of floral abundance on bee colonization or reproductive success, indicating that colonization by *M. rotundata* is driven more by spatial characteristics of the landscape than quality of a given habitat patch. This is surprising given the well-known association between bee abundance and floral resources (Steffan-Dewenter et al. 2001; Potts et al. 2003; Hopwood 2008; Palladini and Maron 2014). Our finding could be due to the difficulty of dispersing across fragmented landscapes and the limited availability of suitable nesting resources; once a dispersing bee finds an adequate nest-site, they may decide to settle regardless of local food resource availability. Flowers may also simply not be a limiting factor across most of our experimental blocks, as reflected by the fact that floral abundance had no effect on bee reproductive success. Future studies are needed to further elucidate the relationship between floral resource availability and bee colonization.

Conservation efforts for bees generally focus on improving the quality of food and nesting resources within new and restored habitat patches (Winfree 2010; Scheper et al. 2015;

Vaudo et al. 2015; Williams et al. 2015), but our results show that spatial aspects of habitats should also be an important consideration for bee conservation. We show that bee colonization of pollinator habitats may be facilitated through increases in habitat connectivity and edge. Given the importance of colonization for metapopulation persistence, habitat-based conservation practices such as landscape corridors that increase these qualities should be implemented widely to support bee populations in fragmented landscapes.

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APPENDIX

Figure 2.1. a) Savannah River Site (SRS) in South Carolina; b) Location of experimental landscape blocks within SRS; c) Diagram of one of seven experimental blocks. Four bee release boxes were placed in the center patch of each block (36.77 m from two near edges), and four nest boxes were placed in the corners (14.85 m from two near edges) of each peripheral habitat patch.

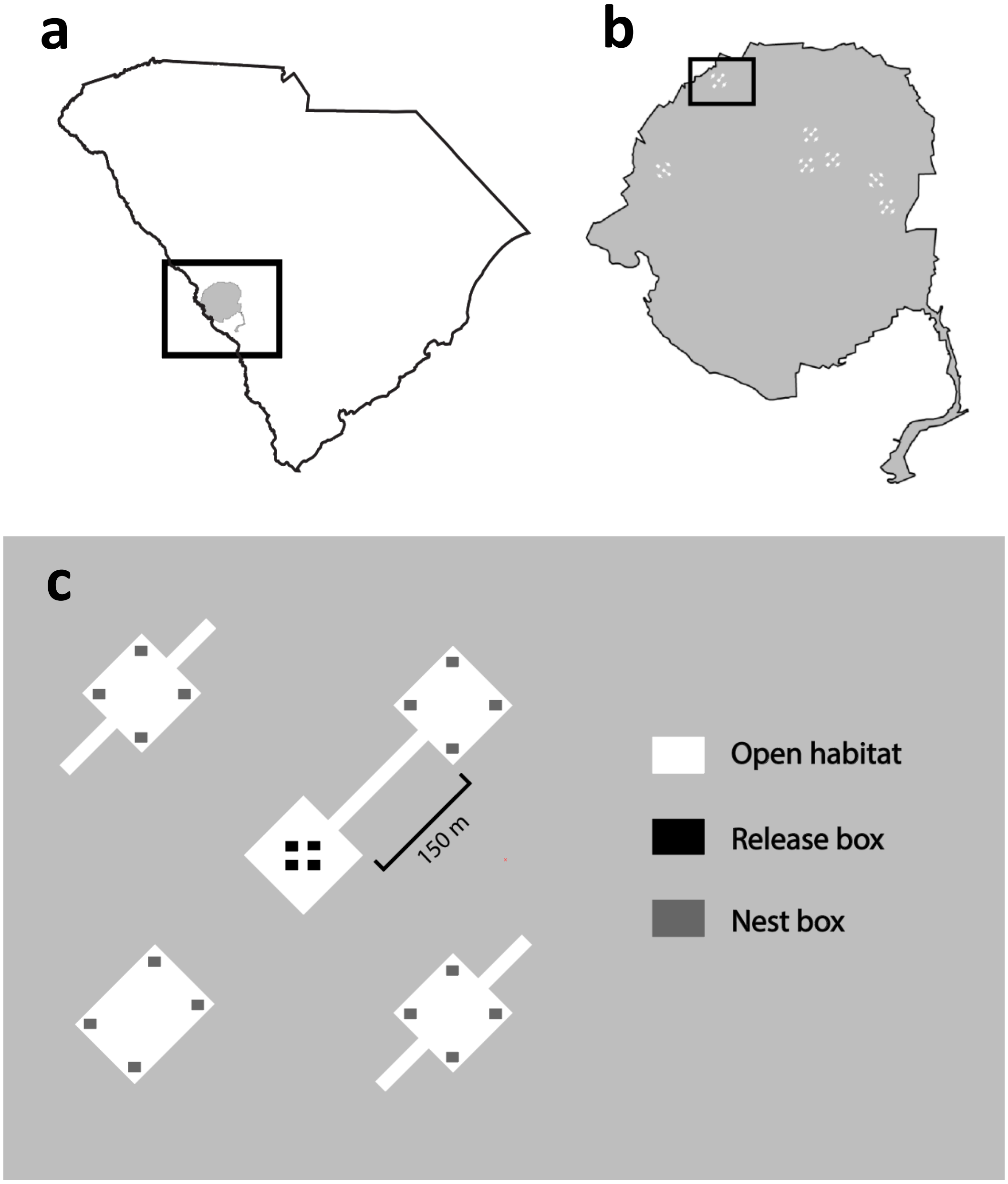


Figure 2.2. a) *M. rotundata* release box. b) Nest box, with two types of nesting resources, phragmites reeds and polystyrene nest block with holes for *M. rotundata* nesting. Reeds were ziptied to the box above polystyrene nest block. c) Dissected reeds with *M. rotundata* brood cells.

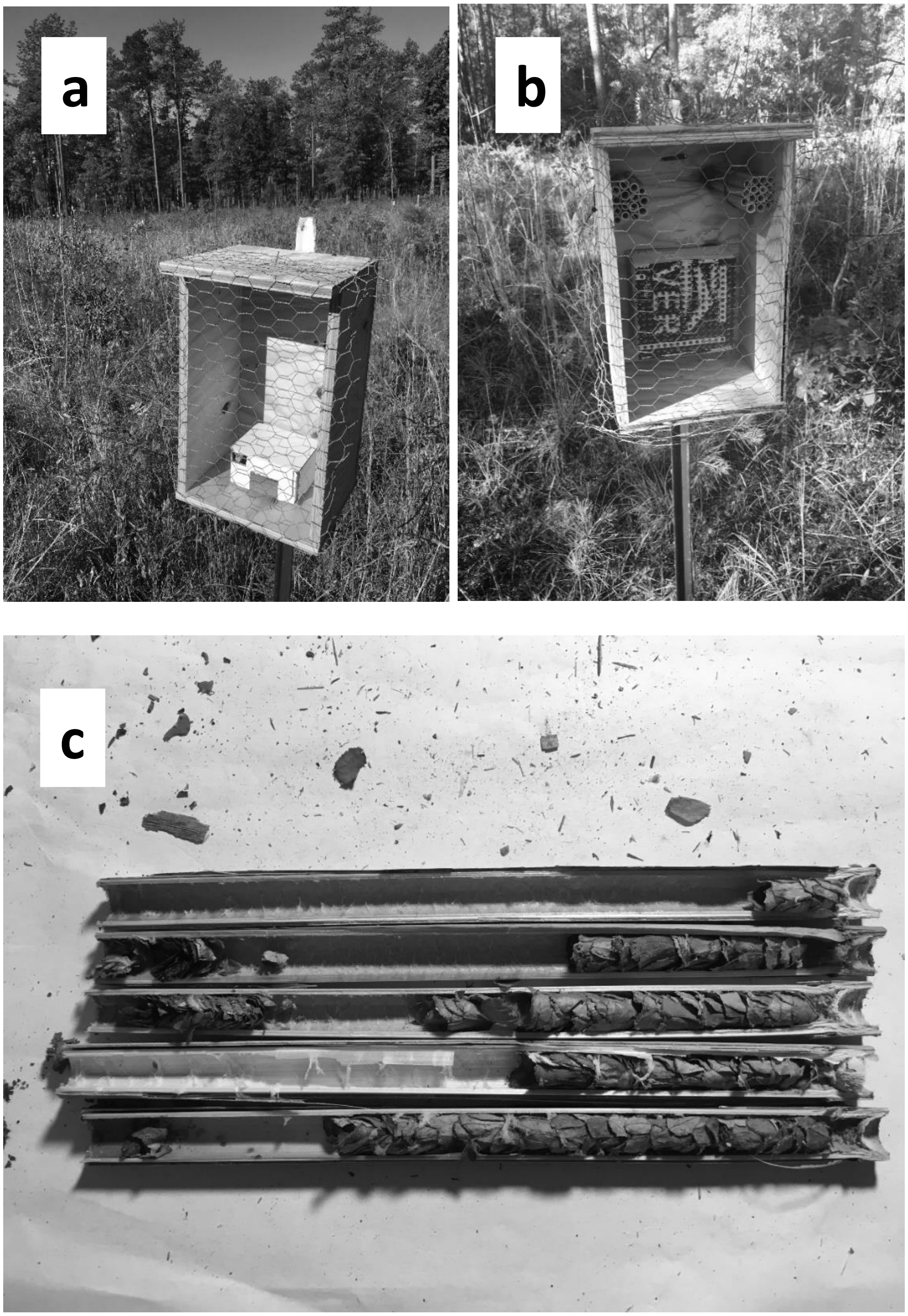


Figure 2.3. Arithmetic means (± SE) of counts of *M. rotundata* bees initially occupying nesting resources in each patch type. Letters denote significant differences (p<.05) between patch types, calculated using Tukey-Kramer contrasts on generalized linear mixed models. Connected patches had significantly higher bee occupation than rectangular patches, though bee occupation of winged patches was not significantly different from either connected or rectangular patches.

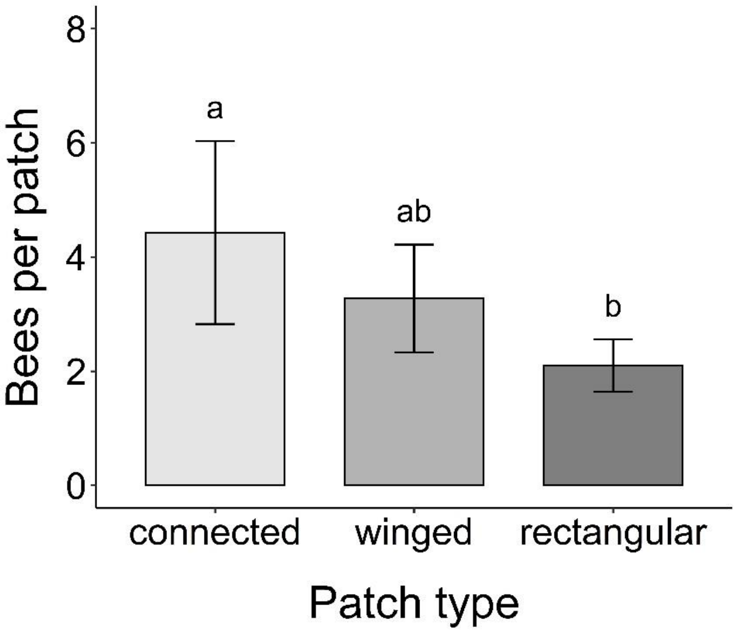


Figure 2.4. The effect of patch type on *M. rotundata* nesting and brood production, measured through nest dissection at the end of the flight period. Arithmetic means (± SE) of a) *M. rotundata* nests produced in each patch type and b) *M. rotundata* cells created per nest. Letters denote significant differences (p<.05) between patch types, calculated using Tukey-Kramer contrasts on generalized linear mixed models. Nest production in connected and winged patches were significantly different from rectangular patches but not from each other. Bees produced a similar number of cells per nest in each patch type, indicating no effect of patch type on brood production.

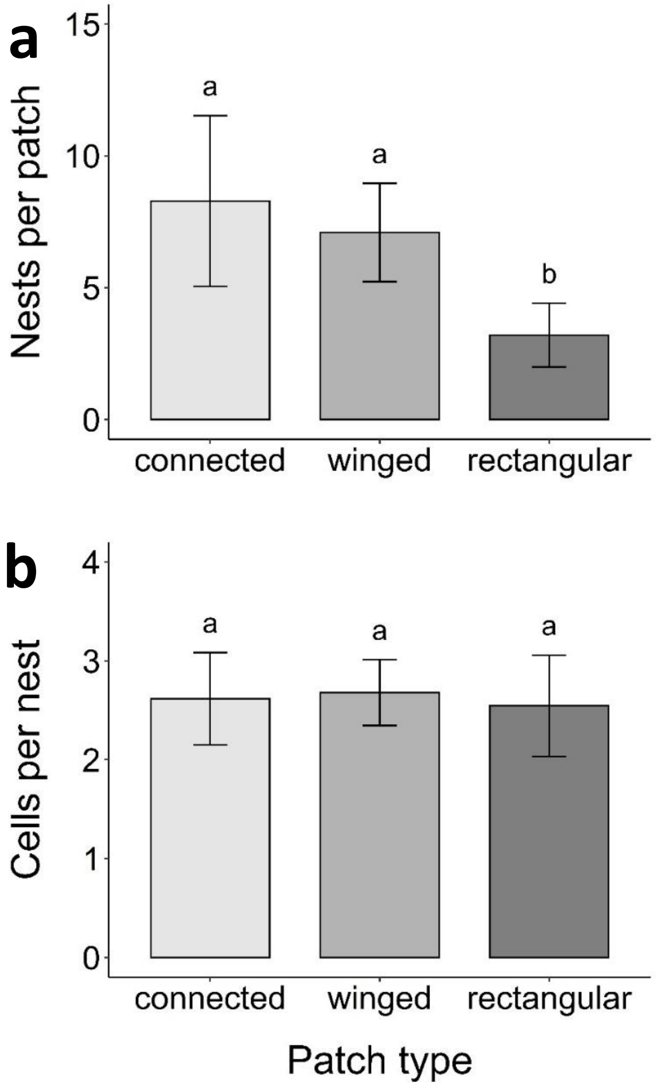
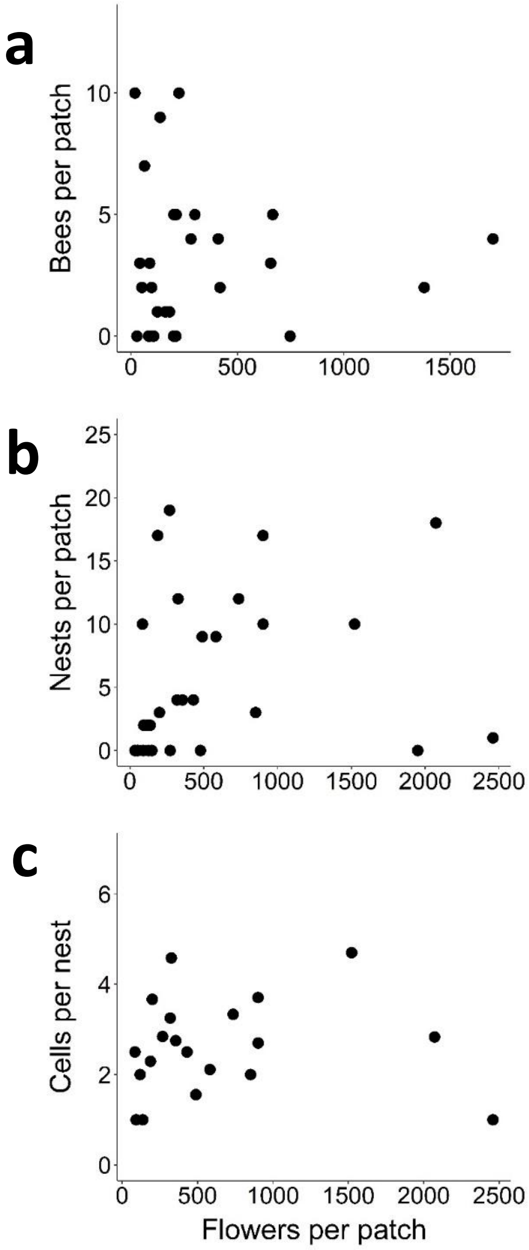


Figure 2.5. Effects of patch-level floral resources on *M. rotundata* colonization. a) Initial nest site occupation vs. floral resources at each patch (peripheral patches only; 1st round of floral resource surveys). b) Total number of nests created per patch vs. total floral resources at each patch (peripheral patches only; sum of 2 rounds of floral resource surveys). c) Cells created per nest vs. total floral resources at each patch (peripheral patches only; sum of 2 rounds of floral resource surveys).



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CHAPTER THREE

Bee dispersal and colonization across agricultural landscapes is determined by patch size and local flowering resources

Abstract

Effective conservation of threatened organisms across agricultural landscapes requires an understanding of how spatial factors interact with land-use to affect population-level processes like dispersal and colonization. We tested the effects of land-use on colonization by solitary bees, a group experiencing serious declines worldwide. We examined four aspects of bee colonization and demography (bee dispersal, nesting, reproductive success, and survival) by conducting an experiment in which we released *M. rotundata* bees in experimental landscapes composed of identically sized patches of high- and low-flowering habitat. To test the effects of spatial scale and patch size on bee colonization, we repeated this experiment using two experimental landscapes, one with 0.11-ha patches and one with 0.91-ha patches. *M. rotundata* did not respond to habitat type when patches were small, but they showed a strong preference for high-flowering habitats when patches were large. The response in larger patches is likely due to the higher distance required to travel from low-quality patches to forage. Thus, bee colonization was strongly dependent on patch size, with increased habitat heterogeneity of the small-patch fields leading to equal bee establishment across high- and low-flowering habitat types. We show that in addition to increasing landscape heterogeneity generally, conservation and restoration efforts for bees should emphasize reduced agricultural field sizes and incorporating patches of high-quality habitat into diversified working landscapes.

Introduction

Agricultural intensification has led to the destruction of natural habitats, increases in crop-field sizes, and the widespread homogenization of agricultural land (Tilman et al. 2001; Benton et al. 2003). These uniform agricultural landscapes are inhospitable to many organisms, and the resulting habitat loss and fragmentation from agricultural intensification is a leading cause of biodiversity declines worldwide (Benton et al. 2003; Haddad et al. 2015; Newbold et al. 2015). Despite ongoing efforts to protect natural habitats and restore degraded land, ubiquitous habitat conservation is unrealistic due to the need for large-scale agriculture to support human society (Foley et al. 2005; Tscharntke et al. 2005). Many threatened organisms may therefore rely on conservation measures incorporated into existing agricultural landscapes (Tscharntke et al. 2005; Kremen and Merenlender 2018), such as restoring landscape heterogeneity through embedding small patches of natural habitat within the agricultural matrix (e.g. Morandin and Kremen 2013; Schulte et al. 2017). Increasing landscape heterogeneity has been shown to increase the abundance and diversity of many taxa (Gonthier et al. 2014; Schulte et al. 2017). However, less is known about the effects of such conservation actions on population-level processes like dispersal and colonization, processes that we study here.

For working agricultural landscapes to support biodiversity, organisms must be able to recolonize these areas following extirpation (Tscharntke et al. 2005; Ponisio et al. 2019). Agricultural intensification and the resulting habitat loss disrupt population processes by decreasing food and shelter for organisms and increasing habitat isolation (Opdam et al. 1993; Tscharntke et al. 2005; Steffan-Dewenter and Schiele 2008). Therefore, conservation measures that reintroduce important resources may be expected to increase rates of colonization and population establishment in agricultural lands (Hanski 1998; Moilanen and Hanski 1998;

Fleishman et al. 2002). Spatial aspects of agricultural landscapes such as patch size, isolation from natural habitats, and distance to source populations may further affect colonization rates through the ability of organisms to locate and utilize suitable habitats (Gutzwiller and Anderson 1992; Hanski 1998; Ewers and Didham 2007). Needed are tests of the interacting effects of land-use and spatial factors on colonization.

Wild bees are sensitive to land-use change and show a strong negative response to high-intensity agriculture (Garibaldi et al. 2011; Kennedy et al. 2013). Bees are exhibiting dramatic declines worldwide due to habitat loss and other human impacts (Potts et al. 2010; Goulson et al. 2015), with the greatest regional declines experienced by the subject of our study, solitary bees (Powney et al. 2019). Given the role of bees as important pollinators of both natural habitats (Ollerton et al. 2011) and crop systems (Klein et al. 2007; Garibaldi et al. 2013), bee declines have caused considerable concern. This has stimulated growing interest in viable methods of conservation, especially across agricultural lands (Byrne and Fitzpatrick 2009; Winfree 2010).

An increasingly popular conservation strategy for pollinators is the creation of flower-rich habitats including floral plantings, small-scale restorations, and linear strips of habitat such as hedgerows and unmanaged field margins (Winfree 2010; Morandin and Kremen 2013). These high-flowering habitats provide important nectar and pollen resources for bees across otherwise resource-poor agricultural areas, and have been shown to successfully increase bee abundance and diversity around agricultural fields (Carvell et al. 2007; Morandin and Kremen 2013; Williams et al. 2015; Ponisio et al. 2016, 2019; Schulte et al. 2017; M'Gonigle et al. 2018). The addition of high-flowering habitats also increases the movement of bee foragers across agricultural landscapes (Cranmer et al. 2012; M'Gonigle et al. 2018; Ponisio et al. 2019) and can even have positive spill-over effects for adjacent pollinator-dependent crops (Morandin and

Kremen 2013; Blaauw and Isaacs 2014; Dainese et al. 2017). However, there has been little study of the population-level effects of high-flowering habitats on bees and how these effects are mediated by spatial factors such as habitat isolation and landscape scale (Murray et al. 2009). An understanding of bee dispersal and colonization would give insight into how to design landscapes to best encourage rapid establishment and high population viability.

We tested the interacting effects of land-use and spatial scale on solitary bee colonization by conducting a managed release of bees in experimental agricultural landscapes with replicated patches of high- and low-flowering habitats. To test the effects of spatial scale on these dispersal and colonization processes, we repeated this study across two experimental landscapes, one with small patches (0.11 hectares) and one with large patches (0.91 hectares). We examined four aspects of colonization and demography (dispersal, establishment, reproduction, and offspring survival) to ask the following questions: 1) How does land-use influence bee colonization of habitats within an agricultural landscape? 2) How does patch size affect the colonization process? Based on the known, positive association between bees and flowers (Potts et al. 2003; Roulston and Goodell 2011), we hypothesized that bees would preferentially colonize high-flowering habitats and would experience greater reproductive success and offspring survival in these high-flowering habitats. Further, due to flight distance constraints and the energetic requirements of foraging (Gathmann and Tscharntke 2002; Greenleaf et al. 2007), we hypothesized that these effects would be stronger in the landscape with large patches where bees in low-flowering habitats would need to travel further to access resources.

Methods

Experimental landscapes

To test the effects of land-use and patch size on bee colonization, we worked in two existing agricultural experiments at the W.K. Kellogg Biological Station in Hickory Corners, MI (42°24′N, 85°24′W). These two experiments are similar in structure in that they are both comprised of patches of land-use treatments ranging from monocrop agriculture to conservation lands, but differ in patch size and details such as number and identity of treatments. The two experimental landscapes included the smaller Biofuel Cropping Systems Experiment (hereafter Small Patch Landscape), and the larger Long Term Ecological Research Main Cropping System Experiment (hereafter Large Patch Landscape). The Small Patch Landscape was established in 2008 to study potential biofuel crops, and is composed of 50 0.11 ha plots divided into five spatially randomized replicate blocks (Figure 3.1a). The Large Patch Landscape was established in 1988 to study the long-term effects of various agricultural practices, and is composed of 42 0.91 ha plots divided into six spatially randomized replicate blocks (Figure 3.1b).

To study parallel treatments across studies, we identified two high-flowering and two low-flowering treatments per landscape. Our two high-flowering treatments were identical between the two experimental landscapes: “Oldfield,” which were never seeded and remain generally unmanaged, and “Early successional,” which were previously filled with poplar trees that were harvested in spring 2018 to allow early successional plants to grow naturally throughout spring and summer 2018. Both high-flowering treatments had naturally-developed plant communities composed of a high-blooming mix of goldenrods, asters, and other forbs, and resembled conservation lands with natural plant communities, high abundance of floral resources (see Table 3.1 and Figure 3.2), and minimal management. Our low-flowering treatments differed in identity between the two landscapes but were comparable in that they represented high-intensity agriculture with low plant diversity and low abundance of floral resources (see Table 3.1 and Figure 3.2). In the Small Patch Landscape, the two low-flowering treatments were “Native grasses” and “Switchgrass” plots, which were seeded with a mix of native grasses and switchgrass, respectively, and were densely structured with very low floral abundance. In the Large Patch Landscape, the two low-flowering treatments were “Bare field” plots, which had been cleared of vegetation in fall 2017 and were regularly managed for weeds throughout the 2018 season through mowing, and “Soy” plots, which were composed of planted soybean crops that had largely finished blooming.

We used 5 plots of each high- and low-flowering treatment in the Small Patch Landscape for a total of 20 plots, and 6 plots of each high- and low-flowering treatment in the Large Patch Landscape for a total of 24 plots. Hereafter, the two high-flowering and the two low-flowering treatments for each experimental landscape have been lumped in the analyses. Both experimental landscapes have other crop treatments interspersed through our landscape and that we ignored. Treatments in both experimental landscapes are separated by lanes of mown grass, 15 m wide in the Small Patch Landscape and 8 m wide in the Large Patch Landscape.

Experimental bee release

We used a model bee species for our study: the Alfalfa Leafcutting Bee *Megachile rotundata*. This small, solitary Eurasian bee was ideal for our experiment for several reasons. First, it is commercially reared for use in agricultural pollination and was therefore available in large quantities. Second, overwintering larvae develop in individual brood cells made from leaves and emerge as adults based on known temperature cues (Pitts-Singer and Cane 2011). This allowed us to release precise numbers of individuals and carefully control their emergence timing using incubation. Third, *M. rotundata* nest in existing cavities above ground (Pitts-Singer and Cane 2011), meaning that we could manipulate their nesting resources across our experimental landscapes. Though *M. rotundata* are found in our region, there were likely no nesting *M. rotundata* in our study plots prior to the release experiment due to the lack of wood and other nesting materials.

Within each experimental landscape, we conducted a separate managed-release of *M. rotundata* to study their colonization of nest boxes placed in treatment plots. We selected five points to release bees within each landscape, chosen to maximize coverage of the landscape (Figures 3.1a and 3.1b). For each release point, we placed a single wooden nest box attached to a metal fence post about 1.5 m off the ground and facing southeast. Boxes were built using 1.5 cm pine plywood and were 36 cm tall, 24 cm wide and 18 cm deep, with an extra 6 cm overhang for protection against direct sun and precipitation. Each of these wooden boxes contained 6 smaller, stacked white cardboard boxes ("release boxes;" Aviditi M752 Corrugated Mailer; 17.78 X 12.7 X 5.08 cm) with ~3 cm entrance holes cut in one side to allow exit by newly emerged bees (see Figure 3.3a for photo).

We used florescent dye powder to study the distance and location traveled by bees from release boxes to study plots. Each release box was divided in half using a short cardboard divider, such that we could place unemerged bee cells on one side and a thin layer of colored fluorescent dye on the other side, thus forcing newly emerged bees to walk through the dye before exiting the box. Each release point was given a different color of dye so that we could track the origin of dispersing bees (see Figures 3.3b and 3.3c for photos).

Nest boxes (wooden boxes as described above) were each provisioned with one 29.85 by 14.92 cm polystyrene nest block (Beaver plastics) with about 450 holes (potential nests) of 0.6 cm diameter and 7.62 cm depth. All polystyrene blocks were affixed to a heavy cardboard backing using screws and firmly attached to the back of the nest boxes with construction glue and Velcro. We placed two nest boxes in each plot, for a total of 40 boxes in the Small Patch Landscape and 48 boxes in the Large Patch Landscape. In the Small Patch Landscape, we placed boxes 12 m from the western edge and 12 and 20 m from the northern edge of each plot (Figure 4.3a). In the Large Patch Landscape, we placed boxes 40 m from the western edge and 40 and 50 m from the northern edge of each plot (Figure 4.3b). We mounted each box on a heavy metal fence post approximately one meter from the ground or immediately above the dominant structural vegetation. We positioned nest boxes facing southeast to maximize morning sun exposure. We also attached chicken wire to the front to protect nests from disturbance by animals. Nest boxes in high-flowering patches were surrounded by flowers, but boxes in low-flowering patches were an average of 36.7 m from the nearest high-flowering patch in the Small Patch Landscape and an average of 59.5 m from the nearest high-flowering patch in the Large Patch Landscape.

We obtained *M. rotundata* cells in May 2018 from a commercial bee provider (JWM Leafcutters Inc.) and stored all cells at 4°C until the start of our experiments. Once floral resources were blooming across our study plots, we incubated the cells at 30°C for ~17 days until we observed emergence by male bees. We then placed ~50 grams of unemerged bee cells (approximately 500 individuals) into each cardboard release box, for a total of ~3,000 bees per release point and ~15,000 bees per landscape. We placed bees in the field during peak bloom for each experimental landscape, such that we released bees on August 3rd in the Small Patch Landscape and August 17th in the Large Patch Landscape.

Measurements of bee colonization

We conducted weekly sampling to study patterns of bee colonization over time, from August 10th to September 24th in the Small Patch Landscape (six rounds), and from August 24th to September 25th in the Large Patch landscape (five rounds). We visited nest boxes at night and used a flashlight to count and identify nesting bees. We were able to differentiate *M. rotundata* females in the field by their distinctive morphology, and other species of cavity-nesting bees were rare due to the late placement and small hole size of nesting blocks. During the second round of sampling, we used an ultraviolet flashlight to count the number of fluorescent marks of each color on each box, passively deposited by marked bees moving into and around nesting holes. Though observations of florescent powder could not be used to determine the absolute number of dispersing bees due to the possibility of a single bee creating several marks, these data provided a standardized index of initial bee dispersal to nest boxes from each release point.

After field observations, we collected all nesting materials and brought them back to the lab for dissection. We dissected nesting blocks to count the total number of nests and brood produced by pushing *M. rotundata* cells out of the blocks using a thin metal rod. We counted a nest as any hole within the nesting block that contained one or more fully formed brood cells and counted the cells per nest by gently pulling apart attached cells. Each nest was then individually stored in floral tubes, placed in ventilated plastic containers according to their source box, and stored at 4°C. After approximately 6 months of cold storage, cells were incubated at 30°C until all bees emerged (approximately 1 month). At this time, we counted emerged bees to measure overwintering survival. We also determined the ratio of emerged female to male bees, because this ratio has been shown to be an indicator of surrounding resource availability and habitat quality for mother bees at the time of egg-laying (Peterson and Roitberg 2006).

Floral surveys and vegetation structure

To determine floral abundance used above to confirm difference in flowering between high- and low-flowering patches, we conducted detailed floral surveys for each of our study patches. In the Small Patch Landscape, we created one 60 m transect within each experimental patch along a standardized sampling path which started at the south end of each patch and took 4 right-angle turns before going through both nesting box locations to end at the north edge of the patch (Figure 3.4a). We also established one 60 m transect within each experimental patch of the Large Patch Landscape, but each of these paths went straight down the patch through the two nest box locations, with no turns due to the overall large size of the patch (Figure 3.4b). Within 1 m to each side of the transects, we identified and counted every individual flowering plant, and counted the total number of flowers per individual plant for 10 individuals per species. This allowed us to calculate an estimated total number of flowers per species per transect for each patch. We then summed these estimates for each patch to determine the number of flowers per patch. The only exception to this method was with the soy plants within the "Soy" treatment of the Large Patch Landscape, in which we estimated floral abundance by counting the total number of flowers per m^2 for 10 $1m^2$ locations per field and multiplying the average by the total number of m^2 for the 60m transect (120 total). We conducted two rounds of floral counts per experimental landscape: in August 10-13 and August 29-Sept. 3 in the Small Patch Landscape and in August 23-24 and Sept. 11-13 in the Large Patch Landscape.

Because visibility could affect the ability of bees to find and colonize nest boxes, we measured light penetration through the vegetation (shade) as a measure of density of the vegetation obscuring individual boxes. Using a ceptometer as close to solar noon as possible (times ranged from 12:00 to 15:00) on clear, sunny days, we took light measurements at each of the nest boxes in our study. To measure light at each point, we took three measurements: two perpendicular light measurements at the focal point with the sensor held at 1 m parallel to the ground, and one control measurement with the sensor held parallel to the ground but above the level of the vegetation to allow full sun. Thus, shade at each point was estimated by averaging the two perpendicular measurements and subtracting the total from the control measurement.

Analysis

To test the effect of habitat type on dispersal distributions of released bees, we fit probability density functions to counts of fluorescent marks at nest boxes across each experimental landscape. Because each release point in an experimental landscape was assigned a different color of fluorescent dye, we were able to determine the origin point and distance traveled for every fluorescent mark in a nest box, giving us a count of fluorescent marks at distances across each landscape. We fitted several common density functions (exponential, normal, lognormal, Student’s t, gamma and Weibull) to the data for high-flowering and low-flowering plots for each experimental landscape using maximum likelihood estimation (package fitdistrplus; Delignette-Muller and Dutang 2015). We selected the best-fitting distribution using AIC corrected for small sample size (package AICcmodavg; Mazerolle 2019). To statistically test differences between the dispersal distributions of high and low-flowering plots in each landscape, we used a Mann-Whitney U test to the compare the average distance traveled to nest boxes and a Kolmogorov-

Smirnov test to compare the overall distance distributions in high and low-flowering patches (stats package; R Core Team 2013).

Separately for each landscape, we used generalized linear mixed models to examine the effects of habitat type on six measures of *M. rotundata* colonization and demography: fluorescent marks indicating initial dispersal, nest box occupation by females, number of nests created per nest box, number of cells produced per nest, % offspring that were female, and % offspring survival. For models of fluorescent marks, the response variable (counts of each color) was calculated per box, and we used habitat type (high- and low-flowering), distance to source box (determined by dye color), and box location (near or far from edge) as fixed effects. We also included shade per nest box as a fixed effect, because shade and height of vegetation surrounding nest boxes could potentially alter local abiotic conditions and box visibility for dispersing bees. Dye color was included as a random effect. Both models (Small and Large Patch Landscapes) used GLMMs with negative binomial distribution (package glmmadmb; Fournier et al. 2012).

For nest box occupation by females, number of nests, and number of cells per nest, the response variables were calculated per nest box. We used habitat type, shade per nest box, distance to nearest source box, and box location as fixed effects, with block included as a random effect in all models. We also included sample round as a fixed effect for models of nest occupation by females to examine changes in bee abundance throughout the flight season, and patch nested within block as a random effect for models with cells as a response variable. We used a negative binomial distribution for models of nest occupation by females and number of nests, and quasipoisson distribution for models of cells per nest (package glmmadmb; Fournier et al. 2012).

For % female offspring (% females out of all emerged offspring) and % offspring survival (% emerged bees out of all counted cells), response variables were calculated per patch. Therefore, we only used habitat type and average shade within a habitat patch as fixed effects, with block included as a random effect. Both models (Small and Large Patch Landscapes) used GLMMs with beta distribution (package glmmadmb; Fournier et al. 2012). For all models, test statistic (χ^2 value) and significance were estimated using type III ANOVAs. We then conducted Tukey-Kramer contrasts ('glht' function in the multcomp package; Hothorn et al. 2008) to determine the effect of patch type for our models. All analyses were performed in R (R Core Team, 2013).

Results

Dispersal

In the Small Patch Landscape, we counted a total of 524 fluorescent marks. We found no effect of habitat type (Figure 3.5a) or nest box location, but there were more fluorescent marks of each color at nest boxes closer to release boxes of the same color (χ^2=129.25, p<0.001; Table 3.2) and with more shade (χ^2=6.57, p=0.01). In the Large Patch Landscape, we counted a total of 274 fluorescent marks. We found an effect of habitat type (χ^2=4.00, p=0.046), with about 88% more marks in high-flowering habitats than low-flowering (Figure 3.5b). We also found more marks at nest boxes closer to release points of the same color (χ^2=61.35, p<0.001), but no effect of shade or box location. Bees traveled up to 736 m to visit nest boxes, but we did not find any differences in the shapes of dispersal distributions or average distance travelled to nest boxes between high and low-flowering plots in either landscape (Figure 3.6).

Overall dispersal distances within the Small Patch Landscape ranged from 17.79 to 352.54 m, with a median distance of 96.91 m and a mean of 116.38 m. Dispersal to nest boxes in high-flowering patches ranged from 25.85 to 352.54 m with a median range of 93.76 m and mean of 127.30 m (Figure 3.6a), and dispersal to nest boxes in low-flowering plots ranged from 28.44 to 280.50 m with a median range of 113m and a mean of 116.65 m (Figure 3.6c). Within the Large Patch Landscape, overall dispersal ranged from 64.03 to 736.02 m, with a median of 157.49 m and a mean of 236 m. Dispersal to nest boxes in high-flowering patches ranged from 66.03 to 736.02 m with a median range of 160.05 m and mean of 252.45m (Figure 3.6b), while dispersal to nest boxes in low-flowering patches ranged from 66.94 to 661.22 m with a median range of 153.94 m and mean of 212.05 m (Figure 3.6d). The best-fitting density functions for three of the four datasets (high-flowering Small Patch, high-flowering and low-flowering Large Patch) were best described by lognormal distributions, while low-flowering Small Patch was best fit by a Weibull distribution.

We found no difference between the dispersal distributions of high-flowering and low-flowering patches of either the Small Patch (Figures 3.6a and 3.6c) or Large Patch Landscape (Figures 3.6b and 3.6d). In the Small Patch Landscape, there was no difference between high-flowering and low-flowering patches in terms of average dispersal distances (MWU-test: Z=773, p=0.80) and distance distributions (KS-test: p=0.72). Similarly, in the Large Patch Landscape, there was no difference between high-flowering and low-flowering patches in terms of average dispersal distances (MWU-test: Z=288, p=0.82) and distance distributions (KS-test: p=0.86).

Establishment

In the Small Patch Landscape, we counted 241-907 nesting females per sample round over a six-week observation period. Females did not respond to habitat type (Figure 3.7a), local shade, or nest box location, but more females were found in patches closer to source release boxes (χ^2=34.35, p<0.001; Table 3.2). In the Large Patch Landscape, we counted 216-912 nesting per sample round over a five-week observation period. Females responded strongly to patch type (χ^2=47.37, p<0.001; Figure 3.7b), with about 106% more females on average in the high-flowering patches at the peak of occupation (sampling round 2). More females were found in nest boxes closer to source points (χ^2=54.37, p<0.001), but bees did not respond to local shade or box location (Table 3.2).

In the Small Patch Landscape, we found a total of 1,374 nests when the nest blocks were collected and dissected. The number of nests in patches was not affected by habitat type (Figure 3.8a), local shade, or box location, but more nests were produced in patches closer to source points (χ^2=8.65, p=0.003; Figure 3.9a). In the Large Patch Landscape, we found a total of 1,395 nests. The number of nests produced in patches was highly affected by habitat type (χ^2=18.59, p<0.001; Figure 3.8b) and distance to release boxes (χ^2=18.96, p<0.001; Figure 3.9b), with about 130% more nests found in high-flowering than low-flowering patches.

Reproduction

In the Small Patch Landscape, the number of cells per nest was not affected by habitat type (Figure 3.8c) or any other habitat factors (Table 3.2). In the Large Patch Landscape, about 17% more cells were produced per nest on average in high-flowering habitat (χ^2=5.11, p=0.02; Figure

3.8d). Nests also had more cells closer to release points (χ^2=5.31, p=0.02). % female offspring was not affected by habitat type (Figures 3.10a and 3.10b) or any other factors.

Offspring survival

Survival rates were not affected by habitat type (Figures 3.8e and 3.8f) or average shade per patch at landscapes of either patch size (Table 3.2).

Discussion

Colonization and persistence of solitary bee populations across working agricultural landscapes may be best supported by the creation of many small, highly interspersed habitats rather than fewer large, more isolated habitats. In our study, bees released across a landscape with large patch sizes preferred high-flowering habitats and had lower dispersal, population establishment, and reproductive success in low-flowering habitats. These findings indicate that bee colonization is likely severely reduced in homogeneous agricultural landscapes. In contrast, smaller, interspersed patches of high- and low-flowering habitats allowed bees to equally colonize patches with and without floral resources. Our results add to the growing body of evidence showing the many conservation benefits of creating high-flowering habitat patches for bees in agricultural landscapes.

Using a model bee species allowed us to gain significant insight into bee dispersal and colonization, processes which have been largely understudied despite their importance to bee conservation. Due to the difficulty of tracking dispersing bees across landscapes, most previous studies have focused on community-level colonization patterns with no information about the origin or dispersal behavior of individuals (e.g. Gathmann et al. 1994; Steffan-Dewenter 2003;

Steckel et al. 2014). By creating a model system in which we introduced both *M. rotundata* bees and nesting resources into two landscapes that initially had zero nesting *M. rotundata*, we were able to more directly study how the design and composition of working landscapes affect bee population-level processes. Though *M. rotundata* is not a threatened species, it can serve as a relevant model for other solitary bees of higher conservation value (Pitts-Singer and Cane 2011), especially cavity-nesters and species with similarly generalized diets. Further, body size determines dispersal ability and foraging range in bees (Greenleaf et al. 2007), meaning that *M. rotundata* dispersal distributions and response to patch size may reflect those of other small-bodied bees. Thus, our findings not only inform bee conservation broadly, but may also be useful for estimating rates of colonization, fecundity, and survival for demographic models of other, at-risk solitary bees.

Dispersal

Dispersing bees in the landscape with larger patch sizes were more likely to visit nest boxes in high-flowering patches than in low-flowering patches, indicating that bees respond to the presence of local food resources even at the initial stages of colonization. This may be due to females actively searching for nest sites; female bees may preferentially search for and investigate suitable nesting locations in habitats with ample floral resource and ignore habitats with sparse resource. Alternatively, such patterns may arise from a more passive process, in which newly-emerged female bees are initially drawn to high-flowering habitats to find food, and are therefore more likely to encounter nest boxes in those habitats even without preference for nest location. Regardless, this pattern was apparent only in the landscape with larger patches, revealing an interaction between habitat quality and patch size on bee movement and dispersal.

We were able to directly examine the initial stages of dispersal by marking newly emerged bees with fluorescent dye and counting marks on nest boxes across our experimental landscapes. Though fluorescent dye has been previously used to study foraging bees and pollen movement (e.g. Adler and Irwin 2006), our study is the first to use this method to track bee dispersal. As is typical of most animals (Sutherland et al. 2000), dispersal distributions were highly left-skewed. However, we also found evidence of bees traveling the furthest distances available in each of our landscapes at even the initial stages of dispersal, up to 736 meters in the larger landscape. This is intriguing given the small body size and presumed limited foraging range of our focal species, *M. rotundata* (Greenleaf et al. 2007). Bees are thought to be physiologically capable of long-distance travel (Murray et al. 2009), but most previous studies of bee movement have focused on foragers rather than directly studying dispersal behavior as we do here (Greenleaf et al. 2007; Palladini and Maron 2014). Some bees exhibit significant genetic relatedness across large spatial scales (Jha and Kremen 2013; López-Uribe et al. 2016), but it remains unclear the extent to which such patterns are due to dispersal from natal areas, especially by female bees. Further study is needed to determine maximum dispersal distances, the frequency and drivers of long-distance bee dispersal, and population-level consequences for this behavior.

Establishment

Occupation and nest-building by female bees closely followed patterns of initial dispersal, with high-flowering habitats experiencing greater establishment than low-flowering habitats in the landscape with larger patch size. Bee diversity and abundance are known to decrease with increasing distance from natural areas (Garibaldi et al. 2011; Kennedy et al. 2013), and our results indicate that this may be due in part to reduced colonization outside a certain range from floral resources. Bees are central place foragers, meaning that female bees travel back and forth from their nests when foraging (Murray et al. 2009; Zurbuchen et al. 2010). Though bees are considered highly mobile and able to forage over large distances, many bee species will preferentially forage near their nests (Gathmann and Tscharntke 2002; Greenleaf et al. 2007), and can experience reduced reproductive success at greater distances from resources (Peterson and Roitberg 2006). Further, flight ability is known to be correlated with body size in bees (Gathmann and Tscharntke 2002; Greenleaf et al. 2007), and *M. rotundata* are small bees with relatively limited foraging ranges (Peterson and Roitberg 2006; Greenleaf et al. 2007). Thus, bees in our study may have nested only within their optimum flight range to flowering resources.

Reproduction

Though bees nesting in low-flowering habitats were only ~60 m from floral resources in the landscape with larger patch size, bees in these habitats experienced a cost in terms of lower fecundity. The reduced cell production in low-flowering habitats likely reflects energetic costs of foraging over greater distances, such as higher energy expenditure, increased travel time, and increased search time for flowers while foraging (Zurbuchen et al. 2010). However, increased isolation from floral resources did not decrease the percentage of female offspring produced in our study, as has been previously found (Peterson and Roitberg 2006). Regardless, reduced fecundity will make it more difficult for bees to establish new populations in low-flowering habitats. These findings underscore the importance of local food resources for increasing bee abundance in agricultural landscapes.

Offspring survival

We found that offspring survival did not respond to local habitat type across landscapes of either patch size. In our study, the primary cause of offspring mortality was parasitism. The most common parasite of our bees were chalcid wasps (*Pteromalus venustus*), one of the worst agricultural pests for *M. rotundata* (Pitts-Singer and Cane 2011). It remains unclear the degree to which parasites drive bee population dynamics in natural systems (Steffan-Dewenter and Schiele 2008; Palladini and Maron 2014), but one of the few studies of brood parasitism of cavity-nesting bees found that parasitism was higher in habitats with higher abundance of native floral resources (Palladini and Maron 2014). We found no such effect across our experimental landscapes, indicating that though the larger ~1 ha patches were large enough to affect bees, this spatial scale did not affect the movement of their parasites. Our results may have also been impacted by our method of storing nests in the same incubator, which possibly led to further parasitism within the incubator itself. Further study is needed to understand the role of brood parasitism in bee population dynamics.

Concluding thoughts

Bee conservation across agricultural lands is essential for protecting bee diversity and pollination services in the face of widespread bee declines. Our study shows that one common conservation method, the introduction of high-flowering plantings like restorations and hedgerows, can have strong positive effects on the establishment of new bee populations. We also found that small, interspersed patches of flowers can increase the usability of agricultural lands themselves for bee nesting. Thus, conservation plans for bee population recovery should emphasize reduced crop field sizes and the use of floral plantings interspersed across agricultural landscapes.

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APPENDIX

Table 3.1 Arithmetic means (± SE) of counts of total flowers per treatment in each experimental landscape (Small Patch Landscape and Large Patch Landscape).

	Flowering	**Treatment**	**Flowers**
Small Patch	*High*	*Oldfield*	28,428.2± 9019.2
	High	*Early successional*	141,543.0± 29,772.2
	Low	*Switchgrass*	3,942.8± 1,739.4
	Low	*Native grasses*	178.8± 58.5
Large Patch	*High*	*Oldfield*	212,339.5± 29,997.0
	High	*Early successional*	259,161.2± 52,468.8
	Low	*Soy*	3,344.0± 508.5
	Low	*Bare ground*	4,064.0± 1,381.9

Table 3.2. Chi squared and p values for fixed effects included in models for the Small Patch and Large Patch Landscapes. Rows represent the models for each separate response variable. Significant fixed effects are in bold.

	Response	**Flowering**	**Shade**	**Closest source**	**Box location**
Small Patch	*Fluorescent marks*	χ^2=0.02 p=0.89	**χ^2=6.57** **p=0.01**	**χ^2=129.25** **p<0.001**	χ^2=3.3 p=0.07
	Female occupation	χ^2=0.16 p=0.69	**χ^2=4.11** **p=0.04**	**χ^2=34.35** **p<0.001**	χ^2=0.02 p=0.88
	Nests produced	χ^2=0.04 p=0.84	χ^2=2.01 p=0.16	**χ^2=8.65** **p=0.003**	χ^2=0.00 p=0.96
	Cells per nest	χ^2=0.87 p=0.35	χ^2=1.68 p=0.19	χ^2=0.01 p=0.93	χ^2=0.70 p=0.40
	% female	χ^2=0.00 p=0.97	χ^2=0.36 p=0.97	*NA*	*NA*
	% survival	χ^2=0.31 p=0.58	χ^2=0.07 p=0.79	*NA*	*NA*
Large Patch	*Fluorescent marks*	**χ^2=4.00** **p=0.045**	χ^2=0.07 p=0.78	**χ^2=61.35** **p<0.001**	χ^2=0.64 p=0.43
	Female occupation	**χ^2=47.37** **p<0.001**	χ^2=0.06 p=0.81	**χ^2=54.37** **p<0.001**	χ^2=2.34 p=0.13
	Nests produced	**χ^2=18.59** **p<0.001**	χ^2=0.00 p=0.95	**χ^2=18.96** **p<0.001**	χ^2=0.76 p=0.38
	Cells per nest	**χ^2=5.11** **p=0.02**	χ^2=1.47 p=0.23	**χ^2=5.31** **p=0.02**	χ^2=0.00 p=1.0
	% female	χ^2=2.91 p=0.09	χ^2=0.16 p=0.69	*NA*	*NA*
	% survival	χ^2=0.03 p=0.31	χ^2=0.03 p=0.87	*NA*	*NA*

Figure 3.1. Diagrams of the two experimental landscapes used in the study. Blue represents high-flowering habitats ("Oldfield" and "Early successional") and yellow represents low-flowering habitats ("Native grasses" and "Switchgrass" for the Small Patch Landscape, "Bare ground" and "Soy" for the Large Patch Landscape). a) "Small Patch Landscape," the Biofuels Cropping Systems Experiment (BCSE) run through the Great Lakes Bioenergy Research Center. Each plot in this landscape was 0.11 hectares in size. b) "Large Patch Landscape," the KBS Long Term Ecological Research project. Each plot in this landscape was 0.91 hectares in size.

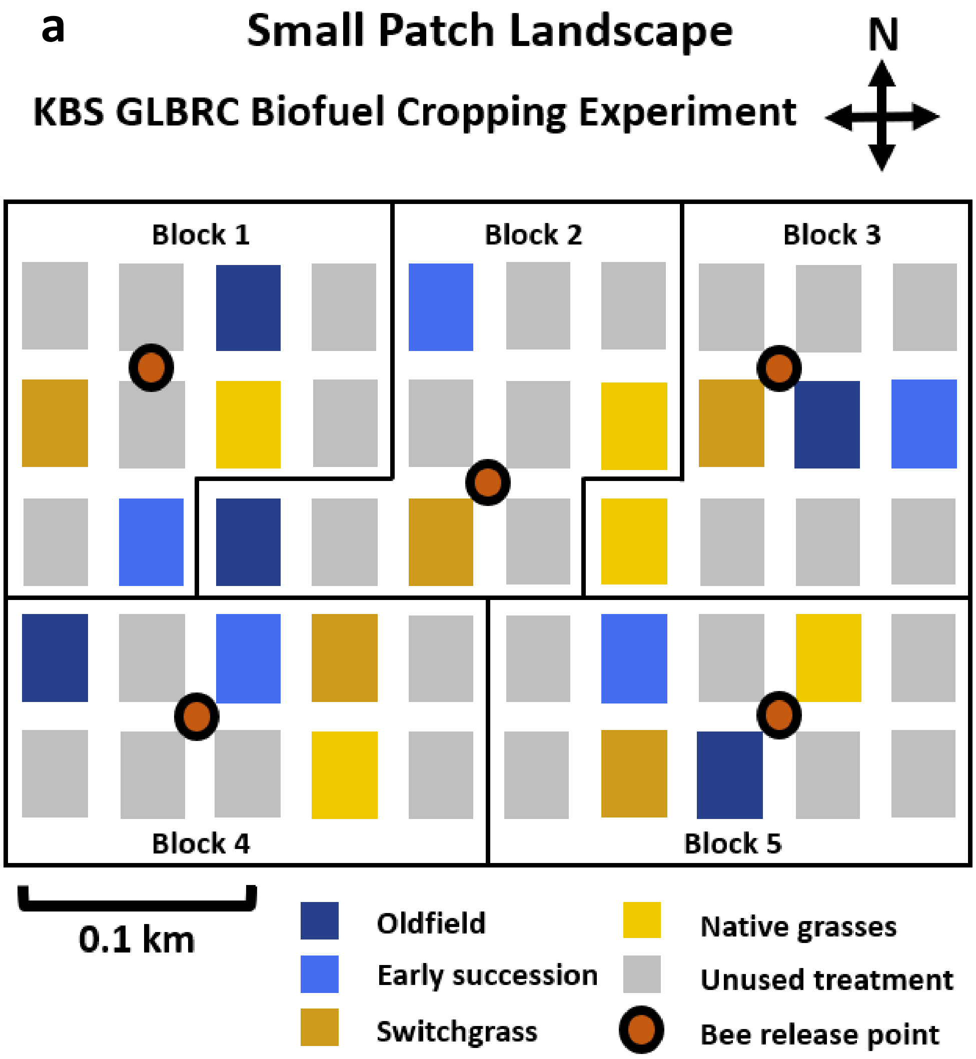


Figure 3.1 (cont'd)

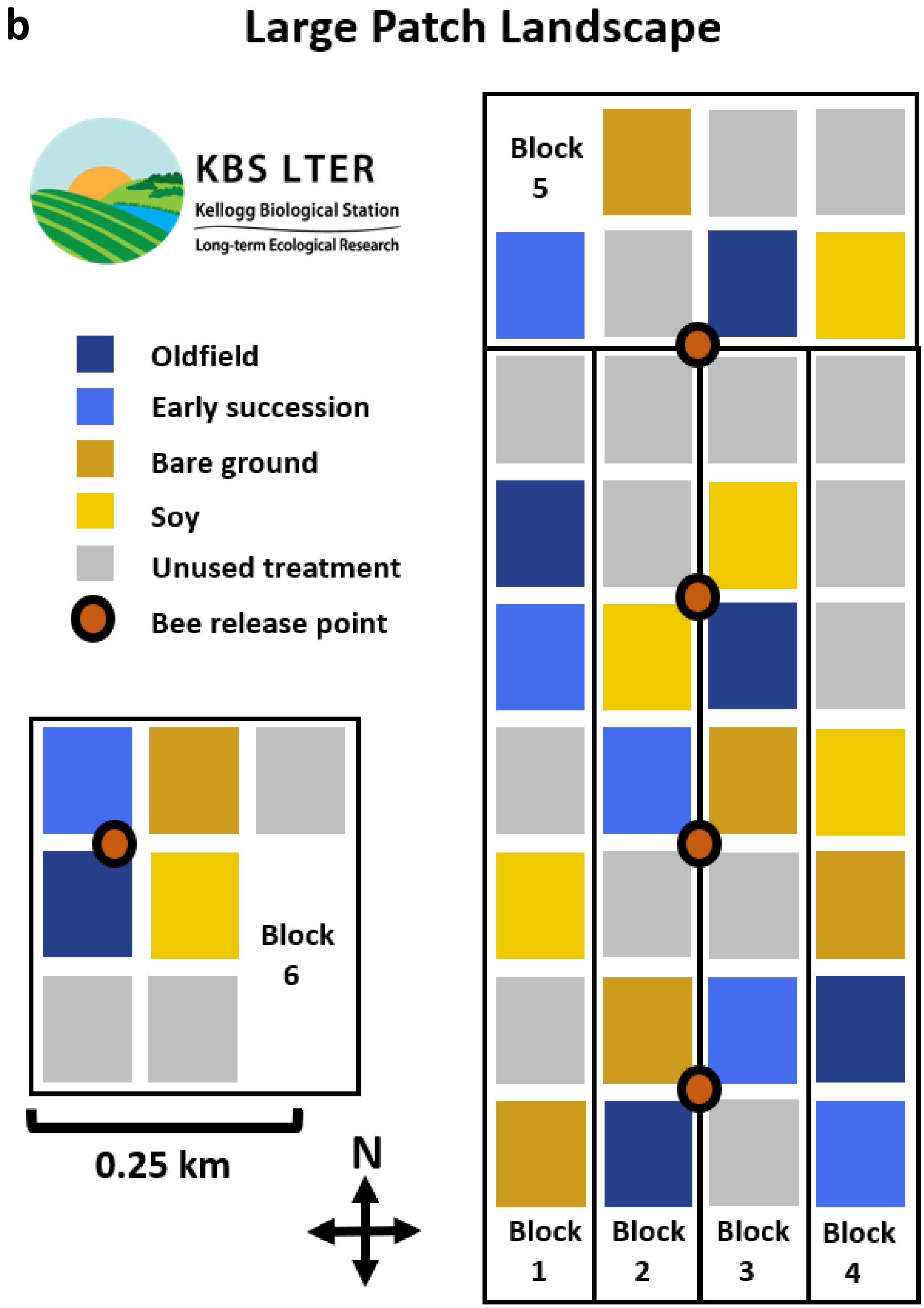


Figure 3.2. Arithmetic means (± SE) of counts of total flowers (a, Small Patch Landscape and b, Large Patch Landscape) in each patch type. Asterisks denote significant differences calculated using Tukey-Kramer contrasts on generalized linear mixed models (n.s.= p>0.05, * = p≤0.05, **=p≤0.01, ***=p≤0.001).

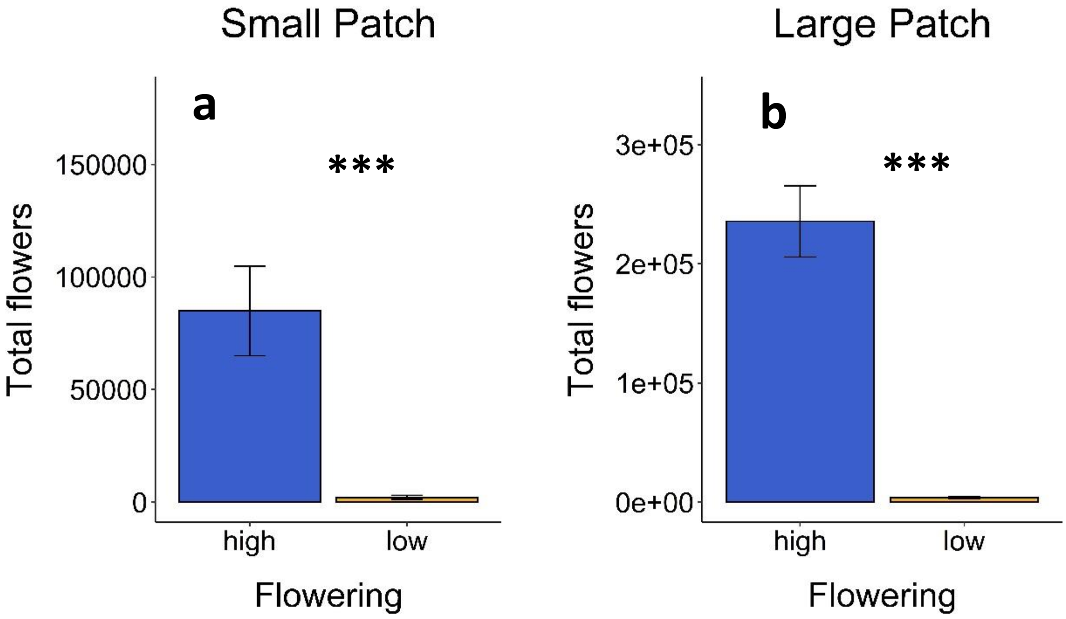


Figure 3.3. a) Preparing a cardboard *M. rotundata* release box for placement. Each box contained ~500 cocoons separated from fluorescent dye by a short partition of cardboard. Newly emerged bees were forced to walk through the fluorescent dye while exiting the release box, thus picking up dye on their bodies. b) *M. rotundata* covered in dye. c) Bee release box, containing a total of 3,000 cocoons. Each release box in a landscape was given a different color of fluorescent dye, so the origin point of fluorescent marks on nest boxes could be identified.

Figure 3.4. Diagrams of a sample plots from a) the Small Patch Landscape and b) the Large Patch Landscape. Two nest boxes were placed in each plot: at 12 m from the western edge and at 12 and 20 m from the northern edge for plots in the Small Patch Landscape, and at 40 m from the western edge and 40 and 50 m from the northern edge for plots in the Large Patch Landscape. A 60 m transect was created in each plot for floral counts and shade measurements.

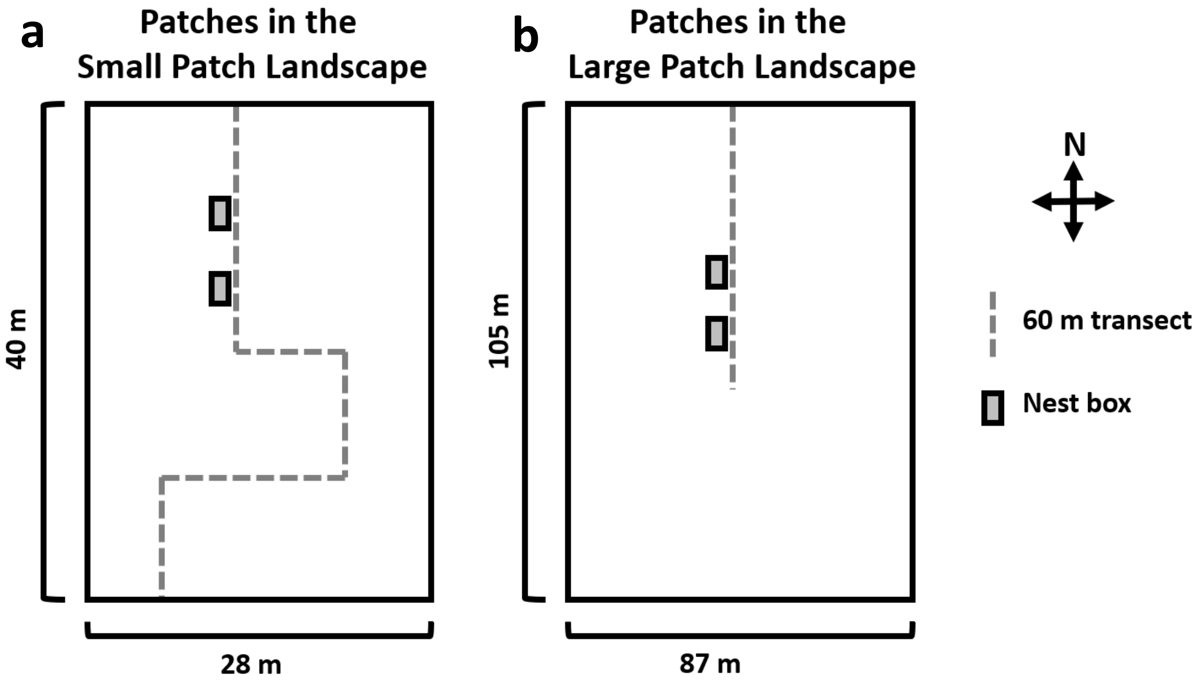


Figure 3.5. Arithmetic means (± SE) of counts of fluorescent marks on boxes left by dispersing bees, per dye color per box, in the a) Small Patch Landscape and b) Large Patch Landscape. Asterisks denote significant differences calculated using Tukey-Kramer contrasts on generalized linear mixed models (n.s.= p>0.05, * = p≤0.05, **=p≤0.01, ***=p≤0.001).

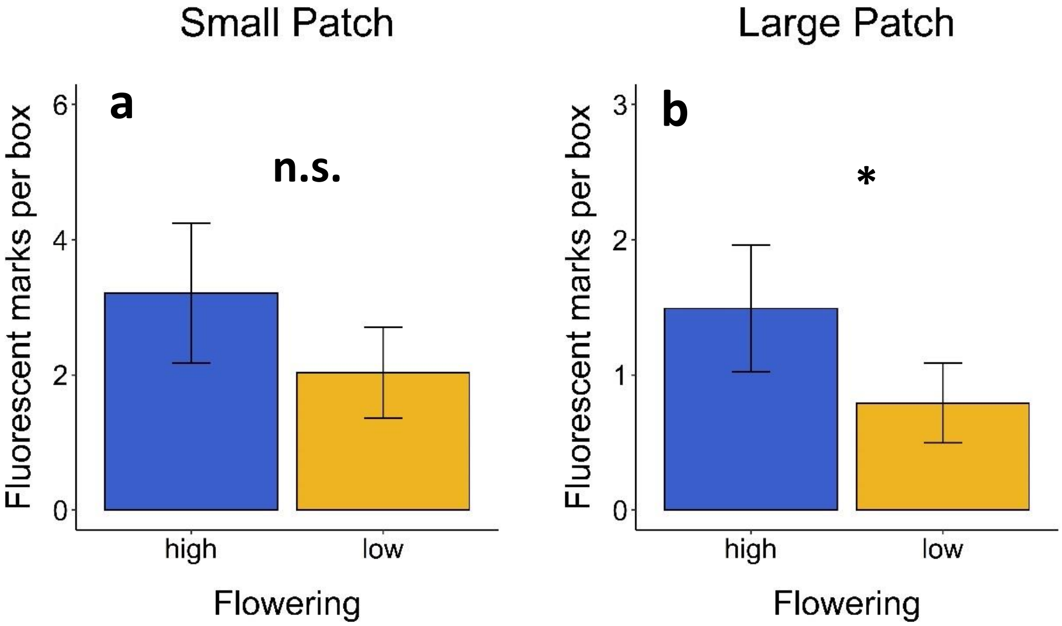


Figure 3.6. Dispersal distributions of bees in a) high-flowering habitats in the Small Patch Landscape, c) low-flowering habitats in the Small Patch Landscape, b) high-flowering habitats in the Large Patch Landscape, and d) low-flowering habitats in the Large Patch Landscape. Bars represent frequency distributions of fluorescent marks on nest boxes across the range of possible distances within the experimental landscapes.

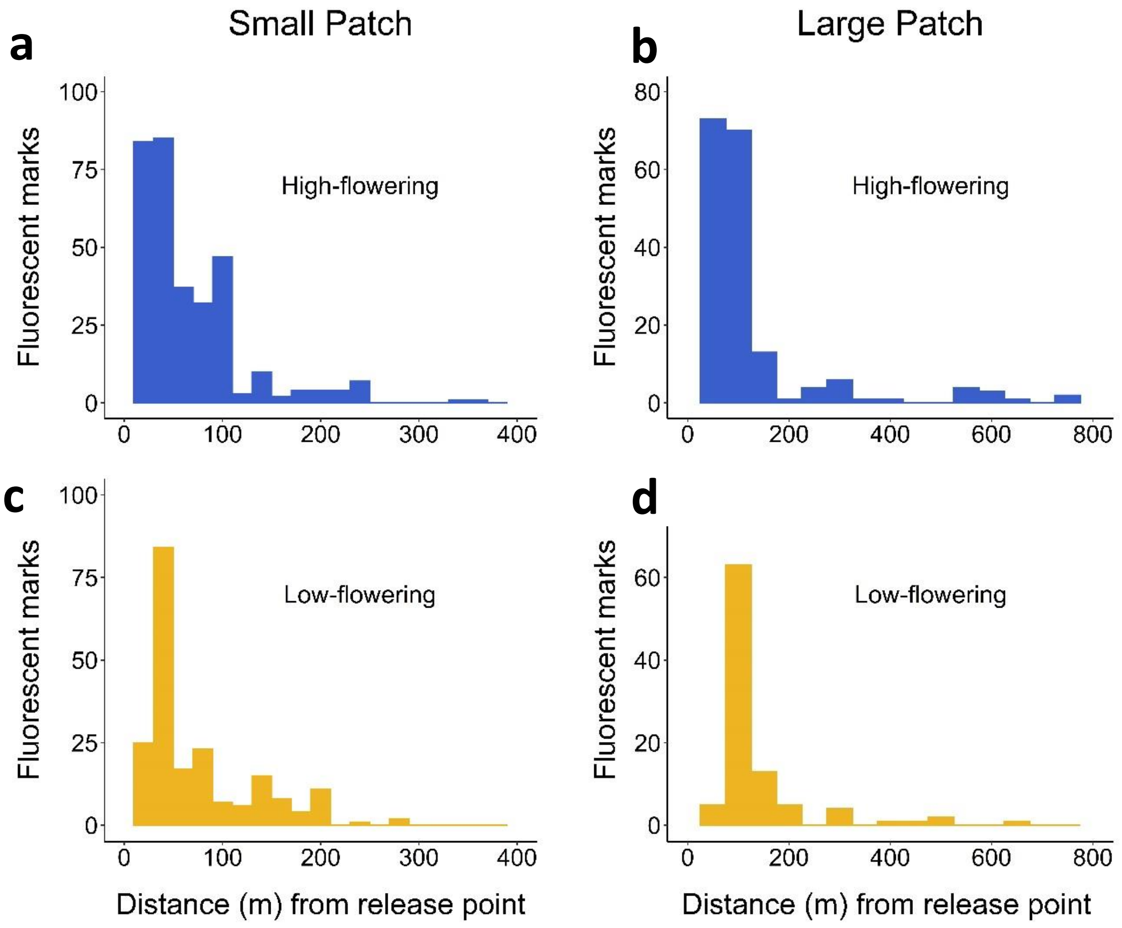


Figure 3.7. Mean counts (±SE) of *M. rotundata* females occupying nest boxes during weekly sample round throughout the flight season in the a) Small Patch Landscape and b) Large Patch Landscape.

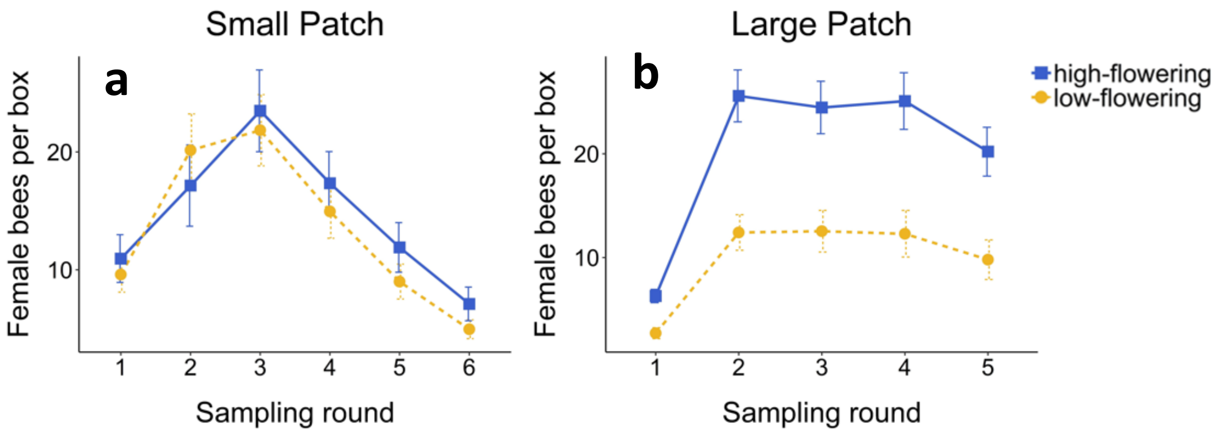


Figure 3.8. Arithmetic means (± SE) of counts of total nests (a, Small Patch Landscape and b, Large Patch Landscape), cells per nest (c, Small Patch Landscape and d, Large Patch Landscape), and % survival (e, Small Patch Landscape and f, Large Patch Landscape) in each habitat type. Asterisks denote significant differences calculated using Tukey-Kramer contrasts on generalized linear mixed models (n.s.= p>0.05, * = p≤0.05, **=p≤0.01, ***=p≤0.001).

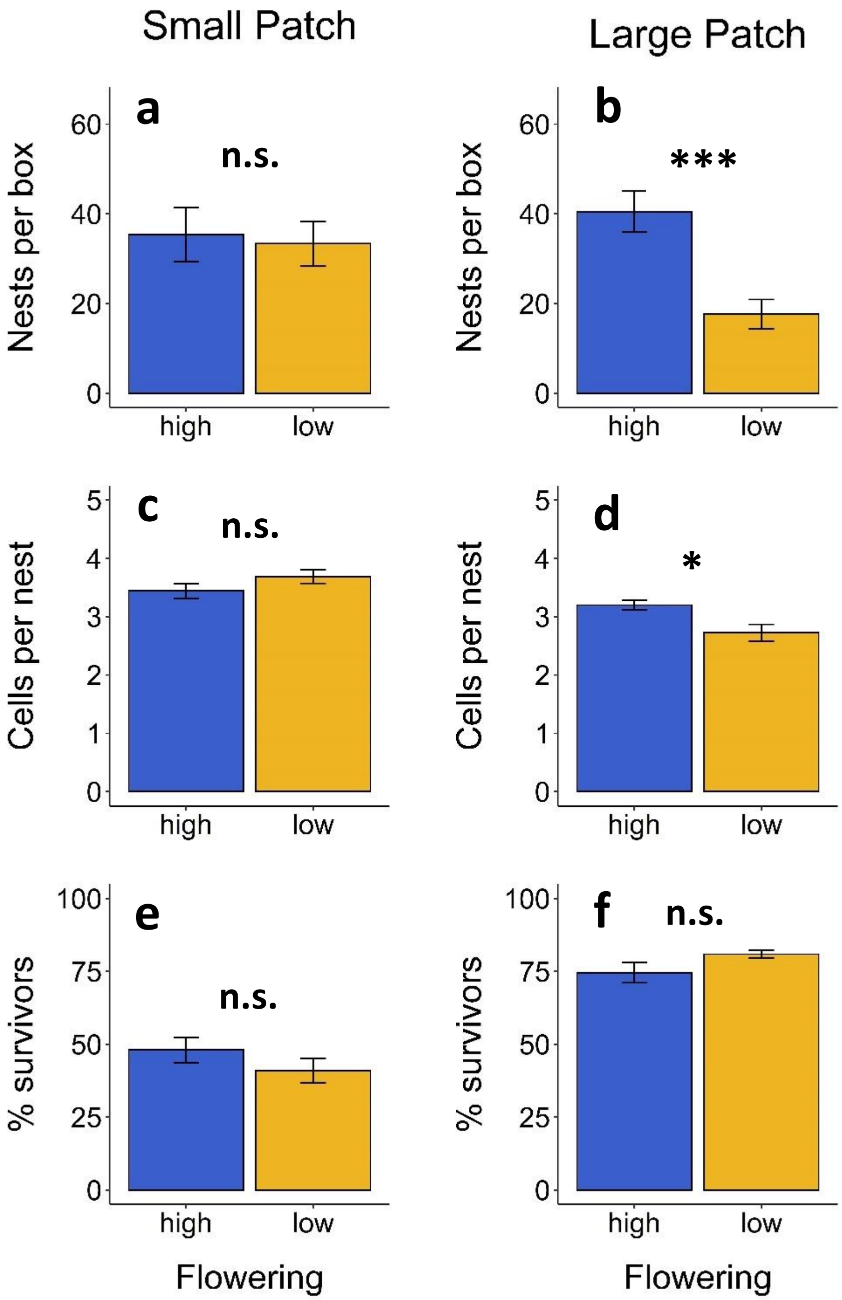


Figure 3.9. Counts of total nests in each nest box plotted against their distance from the nearest bee release point (source location) for the a) Small Patch Landscape and b) Large Patch Landscape. The line was generated from a model with only distance from release point included as a fixed effect.

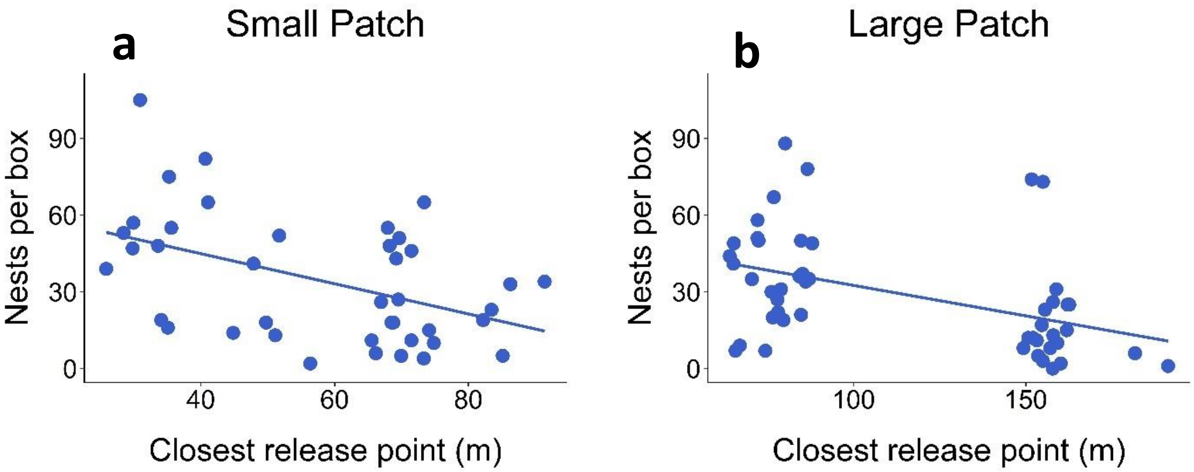
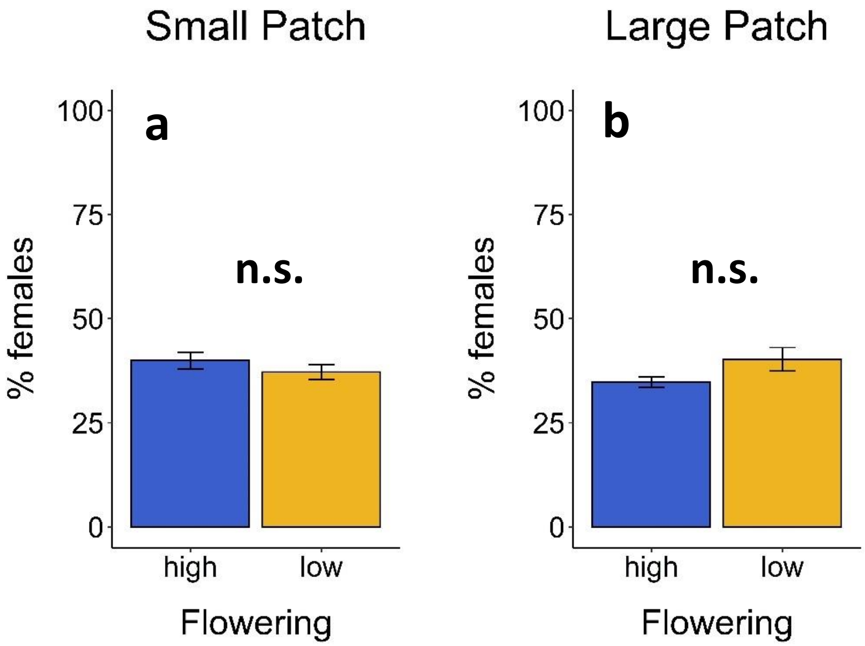


Figure 3.10. Arithmetic means (± SE) of % of offspring that were female in each habitat type (a, Small Patch Landscape and b, Large Patch Landscape). Asterisks denote significant differences calculated using Tukey-Kramer contrasts on generalized linear mixed models (n.s.= $p>0.05$, * = $p\leq0.05$, **=$p\leq0.01$, ***=$p\leq0.001$).



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CONCLUDING REMARKS

Habitat restoration is an important tool for conserving wild bees, yet little is known about how restoration management and design affects bees within newly available habitats. Within this dissertation, I addressed existing gaps in the pollinator conservation literature by conducting three studies focused on identifying ways to improve restoration for bees. Specifically, I examined the effects of plant-focused restoration management and spatial aspects of the habitat such as connectivity and distance between natural habitats. The results of these studies will help inform restoration managers towards better design and management of restored habitats for native bees.

The most significant, overarching finding throughout my three chapters is the importance of considering landscape context and spatial factors in restoration design. In my first chapter, I found that bee abundance and richness in restoration plantings was driven primarily by the percentage of prairie and forest habitat in the landscape, rather than local flower communities or other management factors. When I examined bees at the population level in my second and third chapters by studying their colonization of new habitats, I found that bee colonization behaviors were highly affected by spatial aspects of the habitat. Specifically, in my second chapter I found that increased habitat edge increased bee colonization of isolated habitat patches, indicating that linear or high-edge habitat elements may be useful for establishing bee populations. In my third chapter, I found that the size and configuration of habitat patches dramatically affected the ability of bees to colonize habitats within an agricultural landscape. Thus, landscape and spatial factors appear to play an extremely important role in shaping both bee populations and communities across fragmented landscapes.

Restoration efforts generally focus on increasing local floral resources available to bees, but I found that landscape and spatial factors can interact with floral resource availability and in some cases be even more important for determining wild bee response to restoration. Wild bees are dependent on flowers as their main source of food and thus require their presence for successful establishment, but their response to the flowering community may be dependent on the landscape context. For example, in my first chapter I found no links between bees and local floral communities. Instead, bees appeared to respond to floral availability at a larger spatial scale and exhibited greater abundance in response to higher proportions of surrounding prairie, likely due in part to increased floral resources within the greater landscape. Similarly, in my third chapter I found that bee response to patches of floral resources depended on the size and isolation of habitat patches. Bees preferentially colonized high-flowering habitats when patches were large and spatially separated but were able to equally colonize patches of high- and low-flowering habitat when patch sizes were small and flowering resources were highly interspersed within the landscape. These results indicate that restoration efforts should extend beyond just increasing local floral resources to a more holistic view of restoration that considers spatial aspects of habitat.

By examining the population-level processes of dispersal and colonization in my second and third chapters by releasing a model species of bees across experimental landscapes, I was able to gain new insight into bee natural history and population biology. Most notably, rather than being a passive process, I found that bee colonization responds to aspects of the habitat such as patch shape, distance to resources, and distance from source locations. Thus, restorations must take such factors into account when designing new habitats, in order to encourage rapid establishment of bees in restored habitats. Further, I was able to use novel methods to track bee movement across experimental landscapes, thus allowing me to measure the occurrence of long-distance dispersal events and create bee dispersal distributions. This has never before been done, and thus represents an important step forward in our understanding of bee dispersal biology.

Collectively, my findings have several implications for restoration practice. Most importantly, restoration efforts should prioritize increasing the amount of natural, flowering habitat within degraded landscapes and reduce isolation between habitat patches. Based on my studies, such efforts may increase overall bee colonization and abundance across the greater landscape. Increasing floral availability broadly, rather than focusing efforts on increasing the quality or size of individual floral patches, may lead to higher bee abundances and increased persistence across entire landscapes. These restoration efforts may take the form of hedgerows, prairie strips, or other small-scale restorations, and may be most effective within low-intensity agricultural lands or degraded natural lands that do not have disturbances such as plowing or pesticide application that would disrupt nesting bees. Such scattered, small-scale patches of floral resources may allow bees to establish and nest even within surrounding low-flowering lands, thus increasing overall metapopulation size and population viability across these landscapes.

Further, to encourage rapid bee establishment across fragmented landscapes, practitioners should consider the shape and landscape context of individual restoration plantings. In my second chapter, I found that habitat edge promoted bee colonization and establishment. Thus, high-edge habitats may be preferable when rapid bee establishment is key to restoration goals. In addition, in my third chapter I found that habitats closer to source populations will have significantly higher bee visitation and colonization success, thus encouraging population establishment within restored lands. Finally, in my first chapter, I found that habitat diversity surrounding habitat patches increased bee diversity within the patches themselves. Thus, restorations created near different types of habitats or within diversified landscapes may encourage higher bee diversity.

Though the research presented in this dissertation addresses several key gaps in the conservation literature, there remain many exciting avenues for future research. In particular, there are very few mechanistic studies of bee response to restoration. Observational studies have linked bee community abundance, richness, and composition to restoration and landscape management, but there is little understanding of the underlying drivers of bee population dynamics that lead to such observations. Further experiments using model species or bees with observable nesting behaviors to examine the effects of specific restoration methods on bee demographics and long-term population dynamics would provide many helpful insights into methods for bee restoration. In addition, bee dispersal and habitat colonization remain understudied given the importance of these processes for restoration goals such as establishment in restored habitats and bee metapopulation persistence across degraded landscapes. Thus, future study is needed to understand the frequency and drivers of long-distance dispersal events, bee dispersal and colonization distances in natural systems, and bee population connectivity across fragmented landscapes. Landscape genetic methods may be especially helpful for understanding these population-level processes across real-world landscapes.

Through this dissertation, I have examined bee response to restoration methods at both the community and population levels with the goal of improving restoration for bees. Given the importance of landscape context and spatial aspects of restored habitats demonstrated in these studies, I hope that my findings encourage restoration practitioners to consider these factors when planning new restoration efforts. With careful habitat design and management planning, restoration can be a powerful tool for promoting wild bees across fragmented landscapes.