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**POLLINATOR COMMUNITY COMPOSITION AND ECOSYSTEM
SERVICE PROVISION ACROSS HUMAN-ALTERED
LANDSCAPES**

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**POLLINATOR COMMUNITY COMPOSITION AND ECOSYSTEM
SERVICE PROVISION ACROSS HUMAN-ALTERED
LANDSCAPES**

by

Sarah Jane Cusser

Dissertation

Presented to the Faculty of the Graduate School of

The University of Texas at Austin

in Partial Fulfillment

of the Requirements

for the Degree of

Doctor of Philosophy

The University of Texas at Austin

May 2018

Dedication

Dedicated to putting small drops in a big bucket.

Acknowledgements

This dissertation is the product of a tremendous amount of help from a number of fine people. First and foremost, I'd like to thank my advisor, Shalene Jha, for her constant support, patience, and encouragement. Her feedback has greatly improved both my graduate experience, as well as this dissertation itself. Further, I would like to thank my committee for providing insight and pushing me to improve my work every year. Thank you to the growers, land owners, and Texas State Parks that allowed me to sample on their lands, welcomed me into their homes, and supported my work. Without them none of this would have been possible. In addition, the help of Texas A&M extension agents, independent crop consultants, The Welder Wildlife Refuge, and the Hill Country Fruit Counsel, was invaluable. In particular, Roy Parker, Stephen Biles, Lee Hutchins Jr., Micky Eubanks, Nabil Nassari, Olivia Kost, Katie LeVan, Kenneth Hanslik, Taylor Shaw, Terry Blankenship, and Selma Glasshook were very helpful. Thanks to the Jha lab and others for helpful feedback and general support, including Kim Ballare, Megan O'Connell, Nathaniel Pope, Antonio Castillo, Hollis Woodard, Alison Northup, Nathan LeClear, Laurel Trevino, Nick Ivers, Elinor Litchenberg, Nicole Vojnovich, Rebecca Ruppel, Clare Glinka, Alan Ritchie, and Will Swanson. Thank you to Jack Neff and Eduardo Almeida for help with bee identification. Thank you to our collaborators and friends in Brazil, including Carolina Grando, Margarita Lopez-Urbe, and Maria Zucchi. Thank you to Insu Koh for help with InVEST. Thank you to the UT EEB support staff for all the help over the years, especially Tamra Rogers, Sylvia Moore, Frances Lemear, and Theresa Barnes. Lastly, and most importantly, thank you to my family, friends, and mental health network. I am very grateful to my parents, siblings, cousins, the GC, the Posse, and many, many more.

POLLINATOR COMMUNITY COMPOSITION AND ECOSYSTEM SERVICE PROVISION ACROSS HUMAN-ALTERED LANDSCAPES

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The University of Texas at Austin, 2018

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Ecosystem services and the underlying biodiversity that support them are critical to the functioning of natural and human-dominated landscapes. However, across the globe, land cover change is rapidly altering the availability of semi-natural habitats in these ecosystems, with unknown consequences for biodiversity and services. This is particularly worrisome given the multiple temporal and spatial scales that likely drive successional change following a disturbance. Though both contemporary and historic processes likely mediate biodiversity and ecosystem service patterns, most studies focus primarily on contemporary and local factors. Identifying how both the current and historic land cover drive ecosystem services in human-altered landscapes across spatial scales and agro-ecosystems may reveal key governing principles that transcend a single region, target taxon, or type of human disturbance.

Ecosystem services provided by mobile organisms, such as pollinators, may respond to land cover change through two major mechanisms. First, land cover change may alter ecosystem services through changes in pollinator community composition (Chapters 1, 2, 3, 4). Second, land cover change may alter ecosystem services through changes in pollinator behavior (Chapter 5). To examine these mechanisms, we conducted an extensive survey of the wild pollinators of the peach (Chapter 1) and cotton agro-ecosystems across

Texas, USA (Chapter 2) and conducted a comparative study of cotton in Mato Grosso, Brazil (Chapter 3). We also examined the consequent changes in ecosystem services in cotton due to land cover and community change (Chapter 4). Lastly, to understand how land cover alters pollinator foraging behavior, we built quantitative plant-pollinator networks, focused on the two most common cotton pollinator species in the region (Chapter 5).

Our results reveal that the overall composition of the wild pollinator community is closely related to the abundance and heterogeneity of semi-natural land cover both currently and historically (Chapter 1, 2, 3). Further, changes in community composition were closely related to ecosystem service provision in the cotton agro-ecosystem (Chapter 4). Lastly, beyond composition, pollinator behavior was found to respond to land cover through changes in generalization (Chapter 5).

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Chapter 1: Pollinator extinction debt in exurban landscapes

ABSTRACT

For the first time in more than a century, people across the planet are migrating en masse from cities to rural areas. In this process of ‘exurbanization’ humans are rapidly converting natural and agricultural regions into low-density housing. Despite the scale of this exurban development and its potential negative impact on biodiversity, little is known about how this specific type of land conversion impacts wild pollinators. In this study, we conduct an extensive survey of the wild pollinators of the peach agro-ecosystem and investigate the impact of current and historic land-use at multiple spatial scales on pollinator community composition within agro-ecological landscapes that have recently undergone exurban development. We reveal that the overall composition of the wild pollinator community is significantly associated with current local agricultural and natural land cover. Specifically, local agricultural land-use was associated with ground-nesting bee community composition, while natural lands were associated with cavity-nesting bee composition, revealing that nesting materials drive community composition for these two groups in exurban habitats. In contrast, community composition for butterflies and flies, which made up the smallest proportion of our communities, were not strongly associated with any particular land-use type, likely due to their non-central-place foraging strategy. Most interestingly, our results indicate that historic land-use remains a significant factor impacting the current abundance of all pollinators in the peach agro-ecosystem, offering the first evidence of extinction debt in this rapidly expanding exurban landscape.

Cusser, S., Neff, J. L., & Jha, S. (2015). Land use change and pollinator extinction debt in exurban landscapes. *Insect Conservation and Diversity*, 8(6), 562-572.

S. Cusser was responsible for designing research, performing research, analyzing data, writing the dissertation and writing the manuscript.

INTRODUCTION

Rural America is undergoing a dramatic transformation. For the first time in more than a century, people are migrating en masse from cities to rural areas (Johnson, 1998; Rudzitis, 1999) driving changes in land-use across the nation (Brown *et al.*, 2005). Termed “exurban development”, the construction of low-density housing in historically natural and agricultural regions is now one of the fastest growing forms of land conversion in the U.S. (Brown *et al.*, 2005) and internationally (Scott *et al.*, 2011; Woods, 2011) and is likely having major impacts on biodiversity. While habitat loss (e.g. Fahrig, 2003) and increased impervious cover (e.g. Jha and Kremen, 2013a) resulting from human activity are well established as major threats to biodiversity, the specific impacts of exurban development have only recently become a topic of conservation priority.

Recent studies have revealed that exurban development can negatively impact biodiversity through changes in habitat availability and the alteration of ecological processes and biotic interactions (reviewed by Hansen *et al.*, 2005). Studies examining a range of taxa, from bird and rodent community composition (Blair, 1996; Racey and Euler, 1982, respectively), to large carnivore population ecology (Mladenoff *et al.*, 1995), have indicated the negative impacts of exurban development on biodiversity (also see Temple and Cary, 1988; Hansen *et al.*, 2005). In fact, exurban development may have more devastating impacts on biodiversity than many other types of land-use change because: 1) large areas of land are currently affected by exurbanization (Brown *et al.*, 2005), 2) pristine regions are often the target for conversion (Czech *et al.*, 2000; Marzluff and Ewing, 2001), and 3) exurbanization is occurring at a rapid rate (Johnson, 1998; Rudzitis, 1999). This last factor is especially important to recognize given that many taxa respond slowly to land-use change, and the resulting slow extinction and emigration may delay the development of a new equilibrium (Krauss *et al.*, 2010). These delayed extinctions, or so called ‘extinction debts’ (Tilman, 1994), are critical to consider from a conservation perspective given

that delayed extinction following land-use change may lead to overly optimistic assessments of the status of biodiversity. Tilman (1994) suggests that even very abundant species are not immune to the effects of extinction debt. In the presence of extinction debt, land managers may overestimate species abundance and richness in habitats that cannot support species in the long-term (Hanski and Ovaskainen, 2002; Helm *et al.*, 2006).

Empirical studies on the topic have amassed evidence of extinction debt following land-use change in a variety of taxa including lichens and fungi (Berglund and Jonsson, 2005), mammals (Cowlshaw, 1999), and vascular plants (Ellis and Coppins, 2007; Gustavsson *et al.*, 2007; Cousins *et al.*, 2007). Despite these efforts, the impacts of land-use change remain to be assessed for a wide range of taxonomic groups. In particular, insects, which constitute more than half of the world's terrestrial species pool and are responsible for a wide-range of ecosystem services (Losey and Vaughan, 2008), only constitute a small fraction of studies on extinction debt (but see Sang *et al.*, 2010; Bommarco *et al.*, 2014). Even less is understood about how insect communities experience extinction debt following the ever-expanding process of exurban development.

Insect pollinators, including bees (Hymenoptera: Anthophila), flies (Diptera), and butterflies (Lepidoptera), are an important and taxonomically diverse group of animals united by the common behavior of floral visitation and pollen transfer. As a whole, pollinators are thought to respond rapidly to habitat loss, reaching a new equilibrium in remnant habitat patches within a few years of disturbance due to their relatively short generation times, high mobility, and ability to track resources in the new environment (Kuussaari *et al.*, 2009; Krauss *et al.*, 2010). However, within this larger group of wild pollinators, distinct pollinator communities are likely to respond differently to habitat loss and at different spatial scales depending on two factors unique to each community: 1) their degree of nest resource specialization, and 2) their foraging ability.

First, given that some pollinators exhibit very specific nesting preferences (e.g. Michener, 2007) it is possible that nest-resource availability drives response to land-use change for these communities. For example, cavity-nesting bees require pre-excavated holes in which they provision their young, whereas ground-nesting bees depend on exposed soil in which they excavate tunnels. If both of these substrates are removed as the habitat is destroyed, both cavity and ground-nesting bees may exhibit declines or may go locally extinct (Potts *et al.*, 2005). In contrast, pollinators that do not require specific nesting material but rather depend on plants for oviposition and larval substrate, such as some butterflies and flies (e.g., Scott, 1992; Sadeghi and Gilbert, 2000), may remain in the habitat, even if it is severely altered, until those host plants have gone extinct (Bommarco *et al.*, 2014).

Second, different pollinator communities are also expected to vary in their susceptibility to habitat loss depending on their foraging ability. Wild bees are central-place foragers and require suitable floral resources within their flight range, which may be limited, given that some of the smallest bees in our region forage only a few hundred meters from their nesting habitat (Greenleaf *et al.*, 2007). Unlike bees, most flies and butterflies are not central-place foragers, but instead require that ovipositing and larval substrates be distributed throughout their habitat (Schweiger *et al.*, 2005). Thus, limited foraging abilities may prevent bees from quickly emigrating out of unsuitable habitat while insects that can move freely across landscapes during foraging bouts, including flies and butterflies (Stevens *et al.*, 2010; Jauker *et al.*, 2009), may not be as likely to remain in low-quality habitats during their lifetime. Previous research on a variety of pollinator taxa, including lepidopteran larvae (Roland and Taylor, 1997), hoverflies (Jauker *et al.*, 2009), and hymenoptera (Steffen-Dewenter *et al.*, 2002), offer evidence that taxa with different life histories tend to respond to land-use change at scales related to taxa-specific resource needs and physical constraints. Thus, while movement-limited pollinator taxa may respond to local resource densities,

more vagile pollinators may be more limited by connectivity between patches and may respond to landscape changes at a larger spatial scale (Steffen-Dewenter *et al.*, 2002).

In this study, we investigate the impact of current and historic land-use on the wild pollinators of the peach agro-ecosystem to determine the factors and spatial scales that drive community composition and to identify the potential existence of extinction debt following recent exurban development. Specifically, we examine three main hypotheses: 1) Given the constraints of pollinator flight range and the importance of the local landscape to pollinators in other agro-ecological systems (Williams and Kremen, 2007; Steffan-Dewenter *et al.*, 2002; Concepción *et al.*, 2012), we hypothesize that local land-use is more predictive of pollinator community composition than regional land-use, 2) Because pollinator food and nesting resources are often most available within remnant natural areas (Williams and Kremen, 2007), we hypothesize that natural land-use will have the largest and most positive impact on pollinator communities, and 3) Given that exurban land-use change is often rapid (Brown *et al.*, 2005), we hypothesize that extinction debt exists across pollinator communities, with historic land-use serving as a better predictor of pollinator abundance and richness than current land-use. To address these hypotheses, we survey a number of wild pollinators that visit peach orchards in a rapidly urbanizing Texas peach-growing region, and we measure the relative impact of local (200m) vs. regional (2km) and current (2006) vs. historic (1992) land-use on pollinator community composition, richness, and abundance.

METHODS

Study System

Peach orchards are a common agro-ecological matrix found in humid subtropical climates and currently cover more than 45,680 ha in the U.S. (Perez and Pollack, 2003) and 1,500 ha in Central Texas alone (USDA, National Agricultural Statistics Service, 2009). Many peach varieties

benefit from pollination service, setting more fruits, that are larger, and ripen more quickly, than those not visited by pollinators (Langridge *et al.*, 1977; Nyéki *et al.*, 1998). In addition, peach blossoms are known to attract a wide range of visitors, which have the potential to act as pollinators (Langridge *et al.*, 1977; Lizhong *et al.*, 1997). In Texas, peaches bloom in very early spring (February), when very few other plants are in bloom. Because of the large, dense floral displays, and the relative lack of competition from other flowering plants, it may be safe to assume that during the brief period of bloom, peach orchards attract a majority of active pollinators in the immediate region.

In Texas, the peach agro-ecological landscape has changed rapidly in the last few decades, with land long-used for agriculture being converted into rural subdivisions, “ranch-ettes”, and other types of dispersed housing (Margo, 1992; Brown *et al.*, 2005). We focused our sampling efforts on nine landscapes between Stonewall and Fredericksburg (30.235044, -98.663117 to 30.275376, -98.871315) in Gillespie County, Texas. This region is, both currently and historically, the largest peach-producing area in the state, with over 600 ha of peaches currently in commercial production, and accounts for over 40% of the Texas peach crop every year (TAMU Extension, 2013). While the region has a long history of agriculture and ranching (Wilkins *et al.*, 2003), between 1992 and 2007, overall agricultural land-use decreased by over 600 hectares (Gillespie County, USDA, National Agricultural Statistics Service, 2009) and the number of residential properties increased by 26.7%, leading to increased land fragmentation and reduced average property size (Wilkins *et al.*, 2003).

Current and Historic Land-use at Local and Regional Scales

Within each of the nine landscapes, we selected a central study site within a peach orchard for insect and floral resource surveying. Three 10m x 50m long parallel transects were established at a distance of 25, 50, and 75 meters from the central point of each study site. We calculated the

proportion of land-use surrounding a central point of each transect at the local scale (200m diameter) and an averaged central point at the regional scale (2km diameter) using land-use data from the USGS National Land Cover Database layers (NLCD) within the program qGIS (Quantum GIS Development Team, 2015). Current land-use information was obtained from 2006 NLCD layers, and historic land-use information from 1992 NLCD layers. Land cover was grouped into four broad categories of interest: ‘agricultural’, ‘developed’, ‘natural’, and ‘rangeland’. ‘Agricultural’ land included the NLCD categorizations of cultivated crops, row crops, small grains, orchards, and vineyards. ‘Developed’ included open-, low-, medium- and high-intensity residential, commercial, industrial, and transport land types. ‘Natural’ included deciduous forest, evergreen forest, mixed forest, scrubland, grassland, and herbaceous land types. ‘Rangeland’ included the pasture and hay land types. Other land-use types accounted for less than 0.01% of our landscapes and were not included in our analysis.

Pollinator and Floral Sampling

Each transect included between five and eight blooming peach trees and was sampled three times over the course of the peach bloom in 2013 (08-Mar to 12-Mar, 16-Mar to 20-Mar and 20-Mar to 25-Mar). To record the number of open peach blossoms per transect, hereafter called ‘bloom density’, we counted the number of open peach blossoms on a single branch and multiplied it by the number of blooming branches per tree. To ensure that this was an accurate estimate of blossoms per tree, we counted the total number of blooms on five trees and compared it to our estimated counts. Given that estimates were within ~500 blooms of the actual counts, we felt comfortable using these estimates as a measure of floral blossom count per tree for the remainder of the study. To sample the pollinator community, we walked transects for 20 minutes collecting pollinators found actively foraging on peach blossoms. Only actively foraging insects, making contact with floral reproductive parts, were collected as they are the most likely to act as

pollinators, transferring pollen between flowers. The European honey bee (*Apis mellifera*), while abundant, was not included in this study as they are managed by farmers in the region and fluctuations in their populations are not likely the result of changes in land-use. Wild pollinators were collected into individual wax paper cones and placed into jars with ethyl acetate. Time was stopped while pollinator specimens were being processed to ensure that all transects were sampled for the same duration.

Statistics

To address our first and second hypotheses, concerning the importance of scale and land-use in determining pollinator community composition, we tested for the influence of current and historic land-use types at local and regional scales using Non-metric Multi-Dimensional Scaling (NMDS). We ordinated the entire pollinator community across sites to create a two-dimensional representation of pollinator community composition based on relative Bray Curtis distances (R vegan package, Oksanen *et al.*, 2011). We used 999 iterations with random starting positions, accepted stress less than two and instability less than 0.0003. NMDS uses rank distance for ordination and sites with similar composition fall out close together in the plot (Legendre and Legendre, 1998). Thus, the physical distance between sites in ordination space shows the relative similarity of their pollinator composition (McCune *et al.*, 2002). As one site pair may have been close enough for pollinators to fly between (2.1 km apart), we checked for spatial autocorrelation between community composition and physical distance using a Mantel test (999 permutations) and found no significant autocorrelation (Mantel statistic $R=0.01129$, $p\text{-value}=0.439$).

We then used the results of the NMDS to test for the relation between current and historic land-use at local and regional scales, bloom density, and pollinator community composition using a multivariate correlation analysis (R vegan function “*envfit*”, Oksanen *et al.*, 2011). This approach provides a metric of the effect of the predictor variables on community composition in which longer vectors indicate stronger correlations between the explanatory variables (i.e. land-use and

bloom density) and the dependent variables (i.e. community composition). We ran 999 iterations of envfit to assess the significance of predictors. Significance was determined at the p -value = 0.05 level.

To address our third hypothesis, we indirectly tested for extinction debt using historic and current land-use data. Given that long-term datasets are rarely available, various indirect approaches have been suggested for detecting extinction debt (Kuussaari *et al.*, 2009). One such approach is to compare historic and current land-use to current community composition; if current species abundance or richness is better described by historic than by current landscape variables, then the presence of an extinction debt is likely (Paltto *et al.*, 2006; Piha *et al.*, 2007). To determine the importance of historic and current land-use we used conditional inference trees, also called regression trees, which use a conditional inference framework to estimate the relative importance of explanatory variables through binary recursive partitioning. First, the regression trees test whether explanatory variables are independent of one another and independent of the response variable. If independent, then an algorithm is used to select the explanatory variable that has the strongest association to the response. This association is measured by a p -value corresponding to a test for the partial null hypothesis of a single explanatory variable and the response, and the response is split by that selected input variable. The algorithm repeats itself until there are no explanatory variables significantly associated with the response. In this way, regression trees estimate regression-type relationships in a non-parametric way and thus do not assume linearities in response variables. We used the R package ‘party’ to build our regression trees using historic and current land-use at local and regional scales and bloom density as explanatory variables to explain the responses in pollinator richness and abundance (Hothorn *et al.*, 2006).

Separate trees were made using local and regional data as explanatory variables, to explain responses in both total insect richness and total insect abundance across sites. At the local scale, insect samples were grouped over the three sample periods to produce a single measure of

pollinator abundance and richness for each transect. At the regional scale, insect samples were grouped over the three transects to produce a single measure of pollinator abundance and richness for each site. Given that we examined abundance and richness at the transect level at the local scale, and to avoid biases related to potential non-independence of transects (De'ath and Fabricius, 2000), we also used the package 'coin' (Hothorn *et al.*, 2008) to examine the relationship between the response variable and the explanatory variables given study site-level stratification. The null distribution of the test statistic was calculated using univariate p -values and these 'site-stratified' p -values were calculated for the first node of each tree and presented in the text below as 'site-stratified p -values'.

RESULTS

Current and Historic Land-use at Local and Regional Scales

Historically (1992), local scale (200m) land-use surrounding sites averaged 25.1% (SE 3.2 %) agriculture and 73.0% (SE 3.3%) natural areas. Development and rangeland, on the other hand, historically covered 2.5% (SE 0.5%) and 0.1% (SE 0.001%) of the local landscape. On a regional scale (2km), land-use surrounding sites averaged 23.4 % (SE 1.8 %) agriculture, 73.3% (SE 1.7%) natural area, 2.5% (SE 0.2%) development, and 0.6% (SE 0.01%) rangeland (Figure 1.1).

Current (2006) local land-use surrounding our sites was comprised of 19.3% agriculture (SE 3.2%), 64.7% natural land (SE 4.0%), 13.6 % development (SE 0.9 %), and 3.2% rangeland (SE 0.06%). Regionally, sites were comprised of 10.3% agriculture (SE 1.0 %), 77.8% natural (SE 1.4%), 10% developed (SE 0.6%), and 2.1% rangeland (SE 0.2%).

Pollinator Sampling

We captured a total of 821 insect specimens of 61 species. The specimens were of three orders: Hymenoptera (37 species), Diptera (9 species), and Lepidoptera (14 species). Small

Lasioglossum specimens of 12 species comprised 54% of the specimens. Another 15% of the sample was accounted for by the solitary bee species *Agapostemon texanus*, *Halictus ligatus*, and *Osmia subfasciata*. Of the remaining species, 32 were singletons and 5 were doubletons (see chapter 1 Appendix for entire species list). Species accumulation curves (Figure 1.2) suggest that sampling effectively captured most species present at some of our sites, those reaching an asymptote. However, it seems that many of our sites were under sampled, not reaching an asymptote. Because of the short bloom, and temperamental weather of the early spring period, including high winds and frequent cold temperatures, it was not feasible to sample again during the peach bloom.

Statistics

Of the explanatory variables, our NMDS plot illustrates that only current natural and agricultural variables at the local scale, shown as solid lines, were significant predictors of community composition at the p -value = 0.05 level (p -value = 0.01 and p -value = 0.04, respectively) (Figure 1.3). While not statistically tested, there do exist some correlations between predictors of community composition and aspects of nesting habitat. Based on the ordination of the entire pollinator community, there is evidence to suggest that sites with a high proportion of current local agriculture tend to have more ground-nesting bees, including *Agapostemon*, *Dialictus*, and *Halictus* species, (shown as grey squares which tend to be aligned with the local agriculture area axis). Sites with more current local natural area tend to have more cavity-nesting bees (shown as black circles which align more with the local natural area axis), including *Osmia*, *Megachile*, and *Ashmeadiella* species. Non-central place foragers, Diptera and Lepidoptera (open circles), were not associated with any particular land-use type. Bloom density was initially included in models but was found not to be significant (p -value > 0.5). As a result, bloom density was not included as an explanatory variable in our models.

On a local level, regression trees indicate that pollinator abundance was greater in transects with greater amounts of historic local agricultural area (p -value = 0.012). Specifically, transects historically surrounded by greater than 1.27 ha (40.5%) local agricultural land cover averaged significantly more insects per transect (Figure 1.4). Approximate general independence tests show that pollinator abundance stratified by site was also significantly greater in sites with greater than 1.27 ha historic agricultural land cover ($z = -1.46$, p -value = 0.03). Pollinator richness was significantly greater in transects with greater current local natural area (p -value = 0.004). Specifically, those transects with more than 2.51 ha (80.0%) of local natural area had between 1 and 4 more species per transect. Approximate general independence tests show that pollinator richness stratified by site was greater in sites with greater than 2.51 ha of natural area ($z = 1.5127$, p -value = 0.038). We found that no regional land-use values, neither current nor historic, were significant in our models.

DISCUSSION

In this study, we document the importance of spatial scale and the impact of current and historic land-use on pollinator community ecology in exurban landscapes. First, we reveal that local land-use is a better predictor of pollinator community composition in Central Texas peach orchards than regional land-use. Second, we show that both current agricultural and natural land-use types are important in shaping pollinator community composition. Finally, our analyses indicate that historic agriculture is an important factor affecting current pollinator abundance, offering indirect evidence of extinction debt in this rapidly expanding exurban landscape.

Spatial Scale

We found that local (200m) land-use is more predictive than regional (2km) land-use in determining pollinator community composition. In agreement with other studies, this result is

likely related to the constraints of the relatively short foraging distances that characterize many of the small-sized pollinators that we found in our study (Steffan-Dewenter *et al.*, 2002). From a management perspective, these findings may have important ramifications for the conservation of diverse and robust pollinating assemblages. Management practices, such as the addition or maintenance of local floral and nesting resources have been documented to increase insect abundance and richness and alter community composition even in degraded regional landscapes (Batary *et al.*, 2011; Munyuli, 2012; Williams and Winfree, 2013). Results from this study further support this claim, showing that local availability of habitat-related resources has a major impact on pollinator community composition.

Natural and Agricultural Land-use

We also found evidence supporting our second hypothesis, that natural land-use was highly predictive of pollinator community composition. To our surprise, local agricultural land was also an important factor in predicting pollinator community composition. This result is likely due to the direct relationship between the particular qualities of natural and agricultural lands in our region and pollinator nest-site availability. This finding is best evidenced by the divergent impacts on subsets of the pollinator community seen in pollinators with differing life history traits: ground-nesting bees, cavity-nesting bees, and non-central place foragers, including butterflies and flies.

We found evidence suggesting that ground-nesting bees were most diverse and abundant at sites with an abundance of local agricultural land, whereas cavity-nesting bees were most diverse and abundant at sites locally surrounded by an abundance of natural area. In our region, natural areas were predominantly comprised of deciduous, evergreen, and mixed forest, and thus provide the vegetative biomass and associated crevices for cavity-nesting bees. Other studies have confirmed this relationship, showing that the availability of nesting cavities directly impacts the richness and composition of local cavity-nesters (Potts *et al.*, 2005; Jha and Vandermeer, 2010).

Further, other studies have shown that forested natural areas are strongly associated with cavity-nesting bees (Tylianakis *et al.*, 2006), and that conversion of natural habitat to other land-use types can cause reductions in this group (Williams *et al.*, 2010). Agricultural land-use, on the other hand, offers a very different suite of nesting resources. In our study region, agricultural land is comprised of orchards, vineyards, and a few row crops. Thus, one of the most obvious impacts of agricultural development in the region is the clearance of forests and consequent exposure of bare ground, which remains largely untilled due to utilization for orchards and vineyards. Ground-nesting bees require this type of exposed and undisturbed soil in which they excavate tunnels to provision their young. Potts *et al.* (2005) found similar results, showing that nearly half of the variation in bee community composition and species richness was explained by the availability of nesting resources, which was closely related to land-use and disturbance regimes at a given site. Likewise, Williams *et al.* (2010) found that ground-nesting bee species richness did not suffer from human activities such as agricultural intensification, because disturbance improves access to bare soil. Further, this result is confirmed by other studies which have shown that some wild bees may actually benefit from agricultural development, such as those ground-nesting bees that use disturbed areas for nesting, pollinators that benefit from pollen-rich crop fields (Westphal *et al.*, 2003), or bees that benefit from ecosystems in which agricultural areas provide a greater diversity, continuity, or abundance of floral resources than original habitat types (Winfrey *et al.*, 2007; Winfrey *et al.*, 2011). The distinction of our study from others that have investigated ground nesting bees in agricultural habitats is that our system lacks the intense and destructive tillage practices that have the potential to disrupt ground nesting bees (Williams and Kremen, 2007).

Interestingly, the non-central place foragers, butterflies and flies, were not significantly associated with any particular land-use type. Unlike bees, butterflies and flies are not tethered to particular nest sites and may forage for longer distances than many bees (Jauker *et al.*, 2009). However, butterflies and flies require substrates for ovipositioning and larval development to be

distributed throughout the habitat (Schweiger *et al.*, 2005). While we did not measure host-plant availability, our results are congruent with past studies that suggest that butterfly and fly assemblages respond to different aspects of land-use (Bergman *et al.*, 2004; Jauker *et al.*, 2009) and larger spatial scales (Schweiger *et al.*, 2005) than bees.

Historic Land-use and Exurban Development

Lastly, we found evidence to support our third hypothesis, the existence of extinction debt in landscapes experiencing rapid exurbanization. We found that historic agricultural area was the most important variable in explaining pollinator abundance across groups and was more predictive than the presence or absence of current habitats. Given that agricultural lands offer important resources to wild pollinators, it follows that its conversion to residential/commercial human development and resulting increase in impervious cover has far-reaching detrimental effects on local pollinator fauna. Our findings resonate with studies that document negative impacts of impervious surfaces on bee dispersal in rapidly urbanizing areas (Davis *et al.*, 2010; Jha and Kremen, 2013a; Jha and Kremen, 2013b). Furthermore, our data reveal that current pollinator communities reflect not only current but historic land-use composition, providing evidence of possible extinction debt.

While one might not expect that pollinator abundance, rather than richness or diversity, would be the measure to best reflect extinction debt resulting from land-use changes, past studies have shown that pollinator abundance is often dependent, in part, on past floral resources. Crone (2013) found that solitary bees experienced demographic responses to pulsed resources in the following year. The study, in agreement with others (Minckley *et al.*, 1994; Williams and Kremen 2007), showed that in environments where resources change over time, increased floral resources in one year affect bee fecundity in that year and consequently the abundance of bees in the following year. While other studies have also hypothesized this type of delayed impact of floral

resources on bee abundance (e.g. Tepedino and Stanton, 1981; Potts *et al.*, 2003), only two studies thus far have documented the relationship (Inari *et al.*, 2012; Crone, 2013). Our study is the first to suggest that this type of relationship may persist over multiple years.

Future Directions and Conclusions

While more time-intensive, we suggest that future studies investigating extinction debt following exurban development should examine pollinator species composition before, immediately after, and more than 5 years after the disturbance. Given that we documented differences in pollinator community response to land-use periods separated by five years, we suggest this as a starting point for assessing extinction debt and posit that some pollinator communities may stabilize only after many more years. Additionally, we posit that pollinator surveys conducted across a time series would allow for the assessment of biodiversity changes as a result of the initial land conversion as well as the delayed impact of exurbanization over time. In conclusion, it is clear that exurbanizing areas have threatened a wide variety of organisms in the recent past (Beatley, 2000). Our study offers one of the first assessments of exurbanization impacts on wild pollinators, reveals the importance of local natural and agricultural lands to community composition, and provides evidence for potential pollinator extinction debt following exurban land development.

Table 1.1: Chapter 1 Appendix

Species List			
Hymenoptera	<i>Agapostemon texanus</i>	Lepidoptera	<i>Battus philenor</i>
	<i>Andrena cressonii</i>		<i>Colias eurtheme</i>
	<i>Andrena imitatrix</i>		<i>Colias eurytheme</i>
	<i>Andrena personata</i>		<i>Estigmene acrea</i>
	<i>Anthophora affabilis</i>		<i>Euptoieta claudia</i>
	<i>Ashmeadiella maxima</i>		<i>Hylephila/Phyleus sp.</i>
	<i>Augochloropsis metallica</i>		<i>Hyles lineata</i>
	<i>Ceratina shinneri</i>		<i>Junonia coenia</i>
	<i>Eucera belfragei</i>		<i>Lerodea eufala</i>
	<i>Halictus ligatus</i>		<i>Nathalis iole</i>
	<i>Halictus tripartitus</i>		<i>Papilio polytenes</i>
	<i>Heterosarus illinoiensis</i>		<i>Phyciodes phaon</i>
	<i>Lasioglossum (Dialictus) bruneri</i>		<i>Pontia prodtodice</i>
	<i>Lasioglossum (Dialictus) callidum</i>		<i>Pyrgus communis/aibescens</i>
	<i>Lasioglossum (Dialictus) coactum</i>		<i>Strymon melmys</i>
	<i>Lasioglossum (Dialictus) connexum</i>		
	<i>Lasioglossum (Dialictus) disparile</i>	Diptera	<i>Allograpta exotica</i>
	<i>Lasioglossum (Dialictus) hunteri</i>		<i>Allograpta obliqua</i>
	<i>Lasioglossum (Dialictus) semicaeruleum</i>		<i>Copestylum avidum</i>
	<i>Lasioglossum (Dialictus) sp. TX-03</i>		<i>Copestylum marginatum</i>
	<i>Lasioglossum (Dialictus) sp. TX-16</i>		<i>Eristalis arbustorum</i>
	<i>Lasioglossum bardum</i>		<i>Eupeodes americanus</i>

Table 1.1: Chapter 1 Appendix continued

	<i>Lasioglossum morrilli</i>		<i>Eupeodes volucris</i>
	<i>Megachile brevis</i>		<i>Toxomerus marginatus</i>
	<i>Megachile gentilis</i>		
	<i>Megachile montivaga</i>		
	<i>Megachile parallela</i>		
	<i>Megachile polcaris</i>		
	<i>Melissodes tepaneca</i>		
	<i>Osmia conjuncta</i>		
	<i>Osmia lignaria</i>		
	<i>Osmia subfasciata</i>		
	<i>Xylocopa micans</i>		
	<i>Xylocopa virginica</i>		

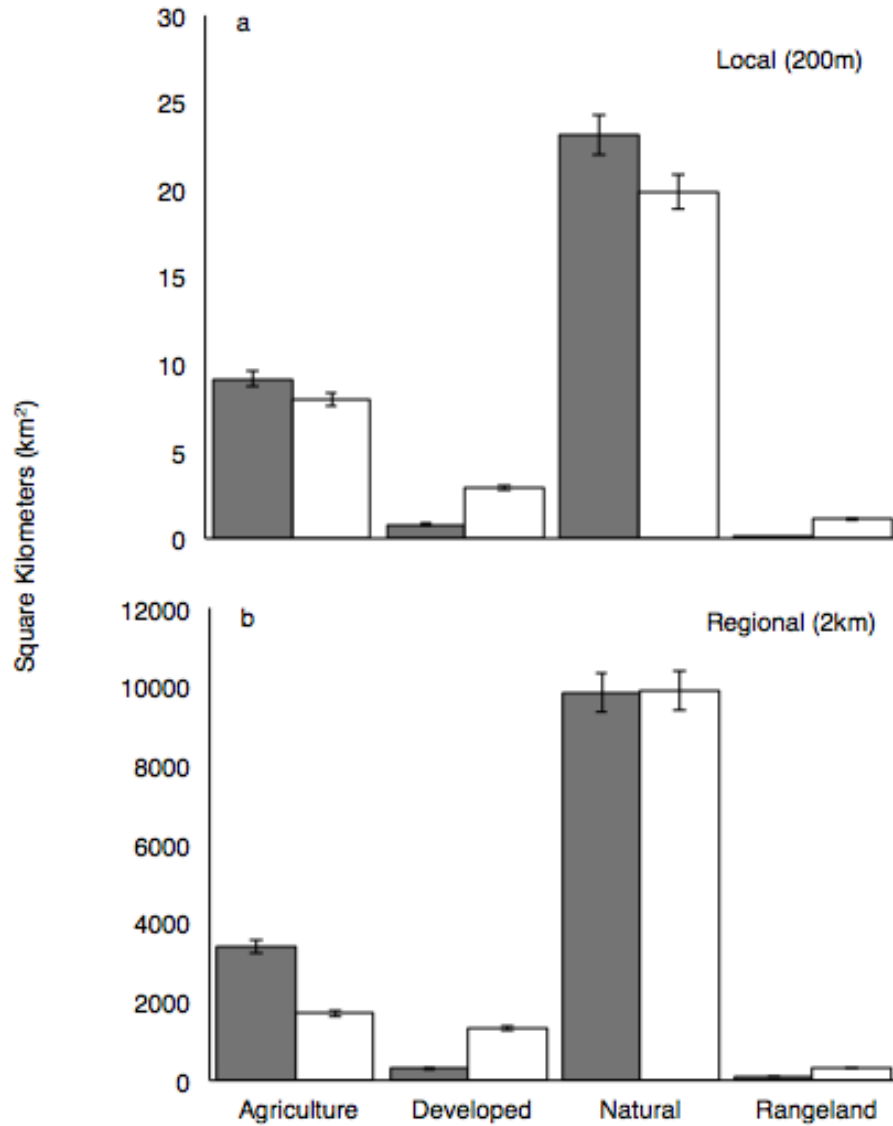


Figure 1.1: Land-use composition, describing the area of agriculture, development, natural, and rangeland surrounding study sites at (a) local (200m) and (b) regional (2km) scales in square kilometers. Historic land-use (1992) is shown as dark grey bars, current land-use (2006) is shown as light grey bars.

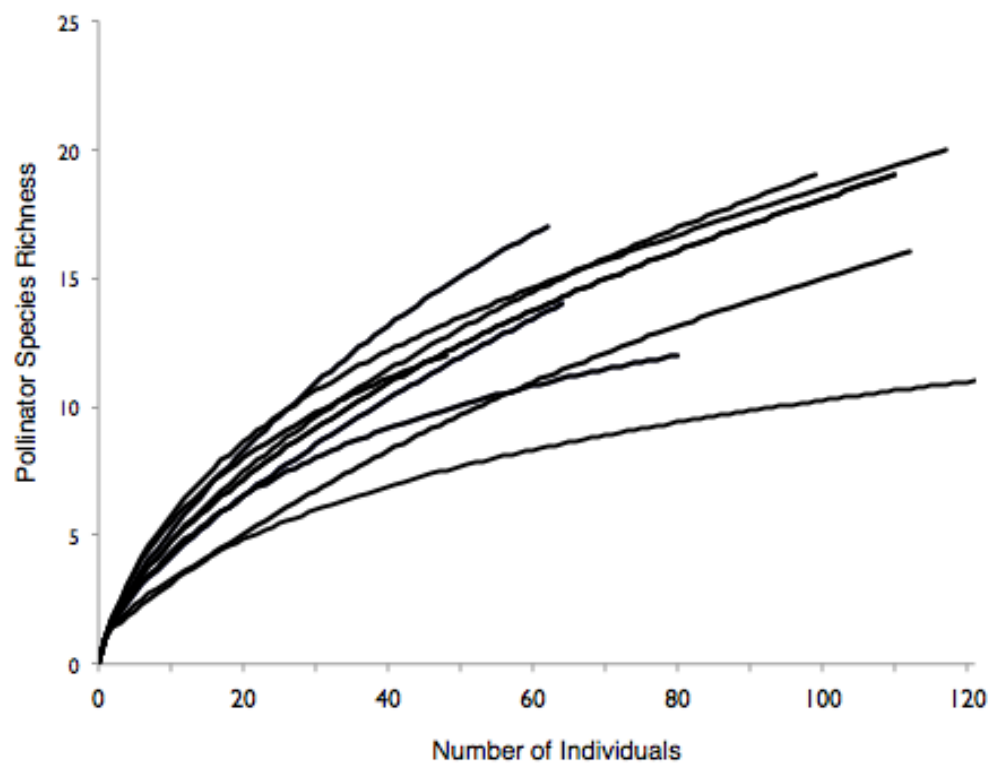


Figure 1.2: Species accumulation curves for each study site

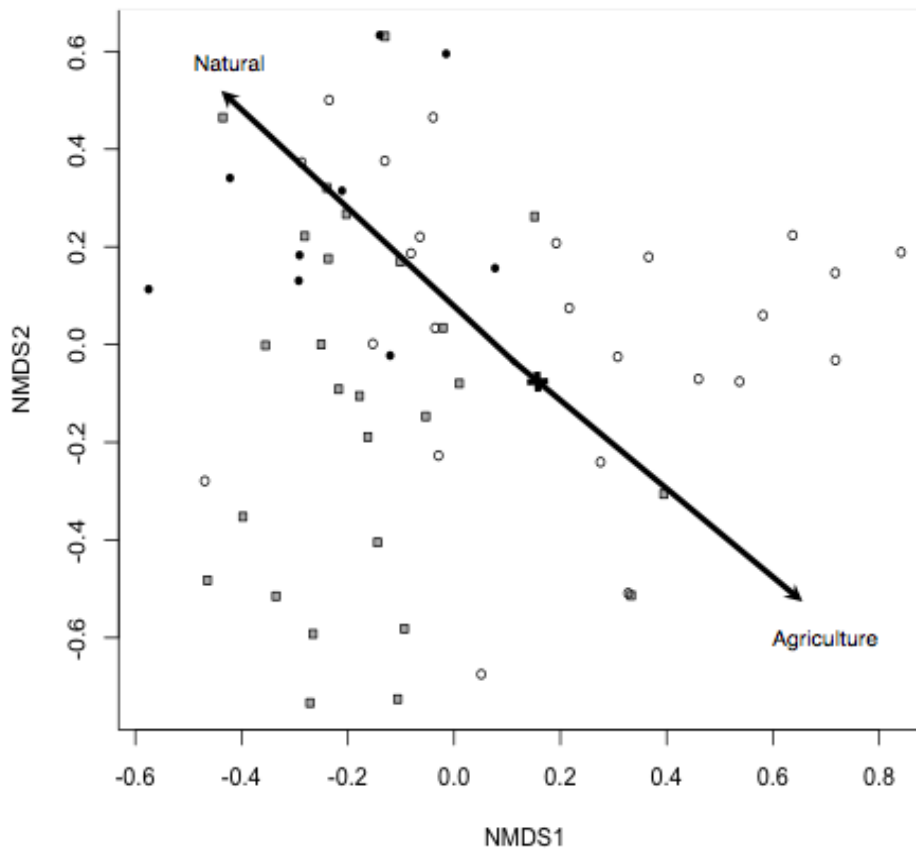


Figure 1.3: Non-metric multidimensional scaling (NMDS) of the pollinating insect community. Explanatory factors are projected to maximally correlate with corresponding species variables, with longer vectors indicating stronger correlations. Current local natural and agricultural variables were significant at the p -value = 0.05 level. Sites with a high proportion of local agriculture have more ground-nesting bees (shown as grey squares) and sites with more local natural area have more cavity-nesting bees (shown as black circles). Non-central place foragers, Diptera and Lepidoptera (open circles) were not associated with any particular land-use type.

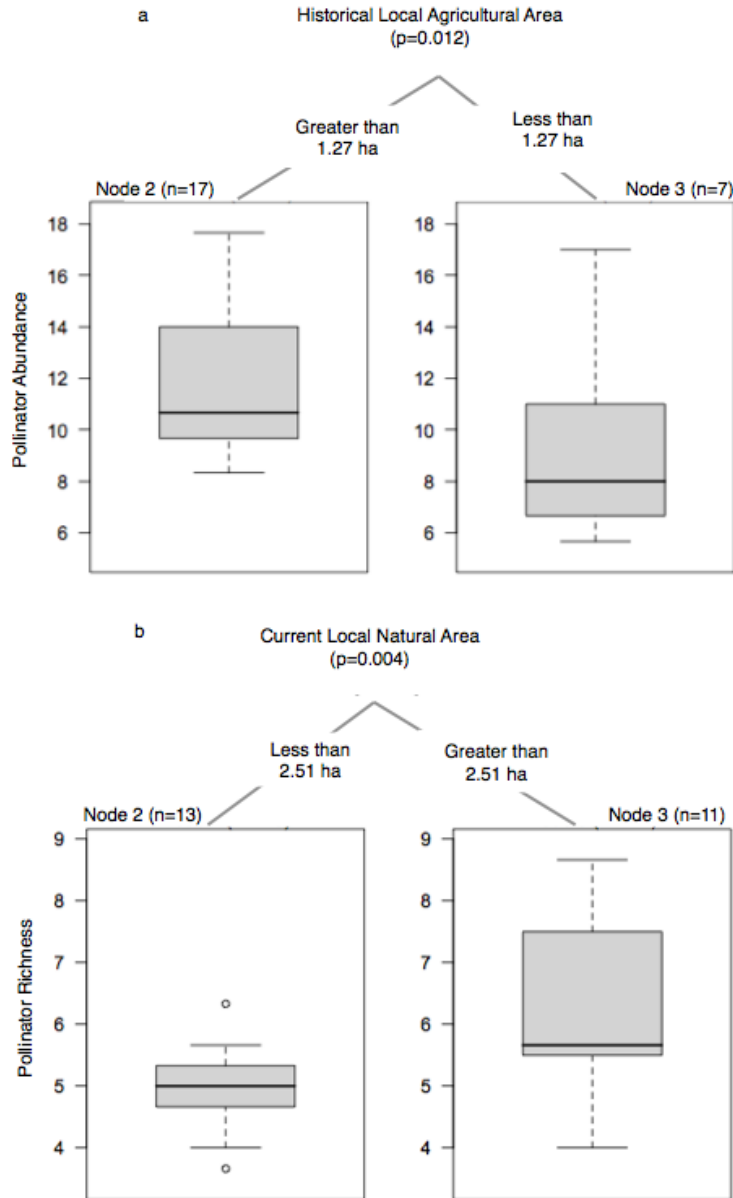


Figure 1.4: Regression trees for (a) pollinator abundance and (b) pollinator richness and local land-use explanatory variables. The p -values listed at each node represent the test of independence between the listed variable and the response variable. Box plots show the distribution of the abundance data within that branch. Boxes represent the inner-quartile range of the data, dark horizontal lines within the boxes represent the median, while whiskers represent the extent of data within the $1.5 \times$ inner-quartile range. Circles above and below the whiskers represent data points outside of this range. The number of transects that fall within each branch (n) are listed above the box plots.

Chapter 2: Land-use history drives contemporary pollinator community similarity

ABSTRACT

Habitat loss, especially within agriculture, can be a threat to biodiversity. However, biodiversity may respond slowly to habitat loss, taking time to undergo successional change following a disturbance. Despite the fact that historic processes often mediate current patterns of biodiversity, most studies focus only on contemporary factors. Our research examines how both contemporary and historic environmental factors impact current pollinator community similarity, or beta-diversity. We examine two hypotheses: H1) that contemporary land-use predicts community similarity, but also that land-use history has long-lasting effects on beta-diversity; H2) that the specific response to contemporary and historic environmental factors is explained by variation in pollinator species life-history traits. We sampled 36 pollinator communities over a three-year period across cotton fields differing in historic and contemporary land-use. Using multiple regression on distance matrices (MRDM), we investigate correlations between community similarity and differences in contemporary and historic environmental factors. First, we show that increased time between sampling events and the loss of semi-natural habitat over a 19-year period led to decreased community similarity. Interestingly, neither geographic distance nor contemporary environmental factors contributed to similarity. Second, we show that much of the variation in community similarity is due to variation in pollinator species life-history traits, such as foraging ability and diet breadth. Results indicate that land-use history has long-lasting effects on community composition, greater than effects exhibited by contemporary factors. These legacy effects are critical considerations for conservation as their omission may lead to overly optimistic assessments of biodiversity in recently disturbed habitats.

INTRODUCTION

Rapid habitat loss is among the greatest hazards to biodiversity across taxonomic groups and biogeographic regions and is identified as the main threat to 85% of species on the IUCN's Red List (IUCN, 2016). One of the leading causes of habitat loss, and subsequent biodiversity decline, is the conversion of natural landscapes into agriculture (Tscharniske *et al.*, 2005). Interestingly, this shift in land-use management does not necessarily address the ecological requirements of the focal crop as both agroecosystems and natural habitats benefit from the ecosystem services provided by biodiversity (e.g. pest control, erosion control, pollination services, and many others) (Ghermandi *et al.*, 2010; Loreau *et al.*, 2001; Tscharniske *et al.*, 2005). In fact, the long-term productivity of both cultivated and natural systems depends on management practices that preserve biodiversity and the ecosystem services it supports (Kremen, 2015). Thus, understanding how land-use change alters contemporary biodiversity patterns is essential for both the conservation of species as well as the preservation of critical ecosystem services across human-dominated and natural landscapes.

Humans value biodiversity across multiple temporal and spatial scales and also seek to understand drivers of this variation across a range of ecological conditions. To determine how best to conserve biodiversity across these temporal, spatial, and ecological scales, it is necessary to describe the mechanisms driving differences or similarity in composition across scales (Anderson *et al.*, 2011). For example, researchers often face the question: how do differences/similarities in composition measured locally scale up to regional biodiversity patterns (Gardner *et al.*, 2013)? The solution lies in our understanding of beta diversity, the component of regional biodiversity (gamma diversity) that accumulates due to differences between local species assemblages (alpha diversity) (Anderson *et al.*, 2011; Vellend 2010). In other words, understanding drivers of community similarity/dissimilarity is vital to our understanding of how regional or landscape diversity emerges from local species pools (Davis *et al.*, 2005). Across taxa, drivers of beta diversity are numerous, however a few groups of variables are consistently identified as important:

1) differences in time between when those communities were sampled, 2) the physical or geographic distance between those communities, and 3) the environmental context and history of each community (Schweiger *et al.*, 2005).

Contemporary factors, such as time, geographic distance, and environmental context are well known to play an important role in shaping community composition. Time is thought to alter community similarity through a number of processes, including succession (Lockwood *et al.*, 1997; Chase 2003), phenological shifts (Forrest and Miller-Rushing, 2010), patch dynamics (Leibold *et al.*, 2004), and stochastic environmental events (e.g. flooding, fire, or drought) (Ives and Carpenter, 2007). Further, long geographic distances may generate high species turnover between communities by decreasing the probability of species exchange through dispersal and colonization events (Tilman, 1982; Leibold *et al.*, 2004). Lastly, environmental factors, including the differences in habitat abundance or richness surrounding two communities can also lead to differences in community composition through environmental filtering (Rosenzweig, 1995; Ricklefs, 2004; Qian and Ricklefs, 2012).

Beyond contemporary environmental factors, historic land-use may have long-lasting effects on contemporary community composition. Especially in the last few decades, many natural landscapes have undergone rapid and drastic changes in which semi-natural habitats, such as shrubland, forest, and pastureland, have been converted into agricultural and urban development (De Groot, 2006). Many biological communities within these converted landscapes may respond slowly to land-use change, taking a relatively long time to undergo succession following disturbance events. These delayed reactions are critical to consider from a conservation perspective as they may lead to overly optimistic assessments of the status of biodiversity in recently disturbed habitats (Hanski and Ovaskainen, 2002; Helm *et al.*, 2006). Thus, research that omits land-use history when predicting contemporary community similarity may merely be capturing a snapshot of communities as they gradually turn over in composition towards a post-disturbance state.

Empirical studies investigating this topic have amassed evidence of delayed reactions or long-lasting effects in a wide variety of taxa including lichens and fungi (Berglund and Jonsson, 2005), mammals (Cowlshaw, 1999), and vascular plants (Ellis and Coppins, 2007). Despite these efforts, the long-term impacts of land-use change remain to be assessed for a wide range of taxonomic groups. In particular, insects constitute only a small fraction of studies on the legacy effects of land-use change despite the fact that past work indicates that historic land-use can have long lasting effects on contemporary insect communities (Sang *et al.*, 2010; Bommarco *et al.*, 2014; Cusser *et al.*, 2015).

Insects are often involved in ecosystem functions, pollination representing one particularly critical function across human-managed and natural landscapes. Both in ecological and economic terms, pollinators are extremely valuable, supporting the reproductive processes that contribute to 35% of global agricultural production (Klein *et al.*, 2007) and >80% of all flowering plant species (Ollerton *et al.*, 2011). Global declines in a number of pollinator species may threaten the important ecosystem services pollinators provide, making wild plant communities more vulnerable to extinction (National Research Council, 2007), and potentially placing crop pollination at risk within agricultural landscapes (Kremen *et al.*, 2002). While pollinator declines have been attributed to a wide variety of causes (e.g. agrochemicals, pathogens, alien species, and climate change) (Tylianakis *et al.*, 2005; Potts *et al.*, 2010; Goulson *et al.*, 2015), land-use change is well-documented as one of the most important drivers of wild pollinator declines across the globe (Ghazoul, 2005).

As a whole, insect pollinators are thought to respond quickly to habitat change, locating and colonizing remnant or novel habitat patches within a couple of years after disturbance due to their relatively short generation times, high vagility, and ability to track resources in the novel environment (Kuussaari *et al.*, 2009; Krauss *et al.*, 2010). However, previous work suggests that even mobile species are not immune to the effects of land-use history, the evidence of which is

apparent in the work of Sang *et al.* (2010), Bommarco *et al.* (2014), Cusser *et al.* (2015), and Senapathi *et al.* (2015). Because insect pollinators vary widely in aspects of their life-history, including their resource needs, dispersal, and foraging abilities, one could hypothesize that different taxonomic groups within a pollinator community are likely to respond differently to habitat change. While insect pollinators vary widely within insect order, orders generally share important life-history traits, such as life cycle and general feeding strategies (Triplehorn *et al.*, 2005). Thus, grouping pollinators by order may provide insight into how distinct subsets of the community react to land-use change. Indeed, previous research on a variety of pollinator taxa, including hoverflies (Jauker *et al.*, 2009) and hymenopterans (Steffan-Dewenter *et al.*, 2002), offer evidence that orders with different life histories tend to respond to environmental factors in a manner consistent with their order-specific resource needs and physical constraints.

In this study, we examine how time, geographic distance, contemporary environmental factors, as well as historic land-use, influence the pairwise similarity, or beta diversity, of pollinator communities in an agricultural ecosystem. We examine two hypotheses: H1) Given that contemporary environmental factors, such as the abundance and distribution of semi-natural habitat, are likely to alter the abundance, richness, and distribution of important nesting and forage resources for pollinators, we hypothesize that the abundance of semi-natural habitat will drive pollinator community similarity. Specifically, we predict that sites surrounded by similar amounts of contemporary semi-natural habitat will share similar pollinator communities. Further, we expect to see long lasting effects of land-use history and predict that sites with similar land-use histories will share similar pollinator communities. H2) Second, using subsets of the larger data set to analyze individual pollinator orders separately (e.g. Coleoptera, Diptera, Hymenoptera, and Lepidoptera), we hypothesize that much of the variation in order-specific community similarity is due to variation in species life-history traits. Specifically, we hypothesize that taxa with different life histories will tend to produce different distance-decay relationships. To address these two

hypotheses, we investigate the similarity of 36 pollinator communities over a three-year period within conventionally managed cotton fields differing in contemporary and historic land-use across the U.S.A. Gulf Coast.

METHODS

Cotton agricultural development in the last century has been a major driver of land-use change along the US Gulf Coast. Land-use in the region has entailed systematic and high frequency disturbance events and offers an ideal opportunity to study how land-use change can alter community dynamics. Further, even within a single geographic region, contemporary and historic land-use can vary widely within cotton agricultural development, making the isolation of contemporary and historic variables possible within a relatively small geographic area. Cotton (*Gossypium hirsutum* L.) is managed as an annual crop, and like most annual crops is rotated between years. Thus, cotton offers an excellent opportunity to understand community similarity in a realistic agricultural situation that reflects the ecology of other annually rotated crops, such as sorghum, corn, milo, alfalfa, soybean, squash, tomatoes and watermelon, among many others.

Study System

We conducted our research along the Texas Gulf Coast, U.S.A., where cotton is a primary crop and one of the few crops that offers nectar and pollen resources to insect foragers in the area. In addition to cotton, the publically available Cropscape Database (Han *et al.*, 2014) indicate that the area is also cultivated with sorghum, corn, and soybean (total agriculture is 55% of the landscape). The remainder of the landscape is comprised of ranching (36%), a few disparate low-density developed habitats (6%), and semi-natural habitats, which include forest, shrubland, herbaceous grassland, and wetlands (3%).

We conducted research in 36 conventionally managed cotton fields near the towns of Telferner, Austwell, and Woodsboro, Texas, USA (28.847913, -96.892975; 28.346058, -96.884940; 27.885588, -97.471427, respectively). We sampled 12 sites per year, across three years (2013, 2014, 2015), for a total of 36 sites. We chose sites in an effort to include a wide range of contemporary and historic land-use context. Sites were located within cotton fields that were at least 35 hectares in size and 2 kilometers apart from each other within the sample year (Figure 2.1). According to growers, sites were not sprayed with any chemicals five days prior to, nor during, the sampling period.

Explanatory Variables

We measured the time of sampling, geographic location, cotton bloom density, and contemporary and historical land-use context as explanatory variables for each of our sites. Time was measured categorically as the year that a site was sampled (i.e. 2013, 2014, or 2015). As with all annual crops, cotton replanted each year, such that each site was only sampled for one year. Geographic location refers to the GPS location of the 1st transect at each site (described below under ‘Response Variables: Pollinator Community’).

To measure cotton bloom density, sites were sampled three times over the course of the bloom. The three sampling bouts were conducted at least three days apart and covered the peak of cotton bloom (June 11th- July 2nd) in 2013, 2014, and 2015. During each sampling bout we measured cotton bloom density at each of our sites by counting the number of blooms on 20 randomly chosen plants per sample per site. Bloom density per site was calculated as the average number of blooms per 20 plants across the three sampling bouts. In addition, we surveyed fields and field edges for the abundance and richness of flowering non-cotton plants as well as measured abiotic factors including temperature, wind speed, and yearly rainfall. Due to the lack of variation

in non-cotton floral resources and abiotic variables, these variables were not included in the final models.

To measure the contemporary land-use surrounding each site at a regional scale (1 km radius), we analyzed both land-use abundance and richness of the surrounding area using information from the 2013, 2014, and 2015 Cropscape Database (Han *et al.*, 2014). A one-kilometer radius buffer was chosen as it represents the average foraging distance of many pollinator species (Greenleaf *et al.*, 2007; Zurbuchen *et al.*, 2010) and thus supports the independence of sites. To quantify land-use composition, we created one-kilometer radius buffers surrounding each site in qGIS (Quantum GIS Development Team, 2015) and summed the total amount of semi-natural land-use falling within that buffer. ‘Semi Natural’ land-use includes the Cropscape categories: Evergreen and Deciduous forest, Shrubland, Grassland, Pasture/Hay, and Wetlands. Abundance of semi-natural habitat closely (negatively) correlated with both the abundance of agriculture and human development surrounding each site (Pearson correlation = -0.9846488, p -value < 0.001, Pearson correlation = -0.1179738, p -value < 0.001, respectively). In this way, the amount of semi-natural habitat surrounding sites serves as a proxy for agriculture and human development as well. To quantify the land-use richness associated with each of our sites, we first converted raster maps into vectors in qGIS and then calculated the total edge distance, or perimeter, of all land-use types within the one-kilometer buffer (Turner, 1989). Other indices of habitat richness, like other measures of heterogeneity or habitat diversity, do not capture the added edge distance and complexity created by small patches of habitat that may be important to mobile pollinators. Thus, we selected the edge distance metric, as per past studies in the region (Cusser *et al.*, 2016).

Lastly, to quantify land-use history, we measured land-use abundance and richness surrounding sites using National Land Cover Database (NLCD) maps from the years 1992 and 2011 (Homer *et al.*, 2015). The NLCD map from 2011 is the basis of all subsequent Cropscape

maps (used above for contemporary land-use calculations), and thus all non-crop distinctions (i.e. semi-natural, water, etc.) are identical between Cropscape 2013, 2014, 2015 and NLCD 2011. Change in land-use abundance for each site was calculated as the difference in semi-natural habitat abundance within a one-kilometer buffer surrounding each site between 1992 and 2011. Similarly, change in land-use richness was calculated as the difference in meters of edge habitat surrounding sites within a one-kilometer buffer between 1992 and 2011. In 2016, extensive ground truthing was executed to confirm that land-use in 2016 was similar to that of 2011. Points of each land-use type were randomly chosen across the region and visited to confirm land-use type. Of the 92 randomly chosen points visited, all but three were accurately described by the 2011 NLCD dataset, as well as the subsequent Cropscape (Han *et al.*, 2014) land-use distinctions.

Response Variables: Pollinator Community

To quantify the pollinator community at each site, we netted all floral visitors found actively foraging in cotton flowers during three one-hour sampling bouts at each site. Sampling bouts were the same as those used to determine cotton bloom density, described above. During each one-hour bout, a single collector walked four parallel 50 m x 1 m transects checking cotton blooms for visitors. Transects were located along field edges, 12 m apart, representing an area of 2500 m² (Cusser *et al.*, 2016). Because we only collected insects actively foraging within cotton flowers, we consider all specimens as potential pollinators of cotton. Pollinators were placed into jars with ethyl acetate and then transferred into individual vials of ethanol. While the authors know of no managed colonies near our sites, the European honey-bee, *Apis mellifera*, is managed in the general region and thus abundances do not likely reflect aspects of land-use or land-use history. Consequently, while we collected honey-bees during sampling bouts, we did not include

them in the analyses. We combined specimens from the three sampling bouts to determine pollinator community composition at each site.

H1) Drivers of Pollinator Community Similarity

To investigate the correlation between pollinator community composition and contemporary and historic land-use variables, we used multiple regression on distance matrices (MRDM) (Legendre and Legendre, 1998). MRDM is well-suited for quantifying the strength and direction of correlation between community similarity, or beta diversity, and environmental similarity between sites (Aizen *et al.*, 2016; Barnes *et al.*, 2016). In particular, MRDM can provide inferences about the importance of individual environmental factors and spatial context in explaining community composition. MRDM, while a direct extension of partial Mantel analysis, offers distinct advantages over the more traditional analysis.

First, MRDM uses nonparametric regression methods (Yee and Mitchell, 1991). In this way, once the distance matrices have been converted into vectors, the calculations for fitting an MRDM model are no different than those for multiple regression with raw data. Thus, unlike partial Mantel analysis, which has traditionally investigated linear correlations, MRDM provides a convenient means for modeling non-linear species responses (Tuomisto *et al.*, 2006). The only computational difference lies in significance testing, which is performed by permuting the response distance matrix. Second, expanding the number of explanatory matrices allows each environmental variable to be represented by its own distance matrix. This provides an improved community-environment correlation (because the effects of important variables are not diluted by unimportant ones), and a convenient way to determine the statistical significance and relative importance of each environmental factor (Legendre *et al.*, 1994; Lichstein, 2006).

Concerns about the statistical power and biases related to the application of the partial Mantel test and its derived forms (MRDM) have been raised (Legendre and Fortin, 2010; Guillot

and Rousset, 2013). Nonetheless, in landscape ecology the problem often lies in choosing among several statistically supported models, in which case MRDM may be especially helpful (Wagner and Fortin, 2013; Rioux Paquette *et al.*, 2014). Simulation studies suggest modelling with MRDM is a reliable method to distinguish among competing hypotheses (Balkenhol *et al.*, 2009; Rioux Paquette and Lapointe, 2009; Cushman and Landguth, 2010). Here, we used MRDM to provide an empirical evaluation of the effect of various landscape elements on community similarity.

For this approach, we created a similarity matrix comparing pollinator community composition between each of our sites using the Chao Similarity Index (Chao *et al.*, 2005), in the R statistical package “ecodist” (Goslee and Urban, 2007). Chao Similarity calculates the level of pairwise similarity between communities accounting for the potential of unsampled species and ranges between zero and one. We were especially interested in accounting for unsampled species given that under-sampling may increase the risk of spurious results. We also calculated Sorenson’s and Morisita-Horn similarity indices, which followed very similar patterns, and responded nearly identically in the analyses. Next, we created separate pairwise matrices for each community driver: time when sites were sampled, geographic location, contemporary environmental factors, and historic land-use. These matrices are also referred to as distance, or dissimilarity, matrices and record the difference between each community driver for all site pairs. As such, these pairwise matrices record the difference in the number of years between sampling at each pair of sites, geographic distance between each pair of sites, difference in contemporary environmental factors between each pair of sites, and finally, the difference in land-use history between each pair of sites. All explanatory matrices were tested for collinearity using variance inflation factors (VIFs) in the R statistical package “usdm” (Naimi, 2013). We chose a conservative theta value of $VIF < 4$ to eliminate collinearity from our models (Myers, 1990) and found that none were collinear (Table 2.3).

Finally, we used the package “ecodist” in R (Goslee and Urban, 2007) to investigate the correlation between the response matrix (Chao Similarity) and all explanatory matrices (year, geographic distance, contemporary environmental factors, and land-use history) in the MRDM models. We used permutation and a pseudo-t test to assess significance of variables rather than using the regression coefficients directly. The number of permutations used in ‘ecodist’ was set to 10,000 to ensure stable p -value. A backward selection procedure was then applied (p -value < 0.01) to progressively eliminate nonsignificant variables from the models. While the suitability of stepwise methods (including backward elimination) to select regression variables has been debated (see Whittingham *et al.*, 2006; but Murtaugh, 2009 for a counterargument), we mainly chose this approach because common alternatives (e.g. comparing models on the basis of Akaike information criterion [AIC] values) cannot be applied in a dissimilarity framework in which independence of observations is not respected, a common issue in landscape ecology (Goldberg and Waits, 2010). Lastly, to visualize significant relationships, regression models were plotted.

H2) Drivers of Pollinator Community Similarity by Order

To investigate our second hypothesis, that much of the variation in similarity is due to variation in pollinator species life-history traits, we executed identical statistical methods as described above, but rather than using the full community, we used subsets of the data organized by insect order: Coleoptera, Diptera, Hymenoptera, and Lepidoptera. Because our analysis involves multiple comparisons of the same data set (e.g. one for the full community, and then subsequent comparisons for each of the four orders), we chose to use the conservative alpha value of 0.01 to reduce the risk of finding spurious results, or type one error, for all statistical tests.

RESULTS

Explanatory Variables

Cotton floral resources ranged from 15 blooms per 20 plants to 114 blooms between sites (SE: 0.779 blooms). Semi-natural habitat included Cropscape and NLCD land-use categories of Evergreen and Deciduous forest, Shrubland, Grassland, Pasture/Hay, and Wetlands and ranged from 53,272.5 m² to 5,242,756.1 m² surrounding sites (SE: 145,632.37 m²). Meters edge habitat ranged from 12,916.30 m to 156,348.78 m (SE: 893.511 m). Change in semi-natural habitat over a 19-year period ranged from the loss of 1606373.50 m² to the gain of 160326.30 m² (SE: 9885.657 m²). Lastly, change in meters edge habitat over that same period ranged from the loss of 105821.69 m to a gain of 22768.4 m (SE: 748.57 m). A histogram of explanatory variables, and correlation matrix of variables are shown in Figures 2.2 and 2.3, respectively.

Response Variables: Pollinator Community

We captured a total of 1,793 pollinator specimens of 97 species. The specimens were of four orders: Hymenoptera (801 individuals, 56 species), Diptera (683 individuals, 16 species), Lepidoptera (255 individuals, 18 species), and Coleoptera (54 individuals, 7 species) (Table 2.6). The solitary bee species, *Melissodes tepaneca*, made up 12% of the specimens. The syrphid flies, *Allograpta exotica* and *Toxomerus politus*, comprised 10% and 16% of the specimens, respectively. Lastly, *Lasioglossum* specimens of at least 14 species made up 8% of the specimens. Of the remaining pollinators, 22 were singleton species, and 12 were doubletons. Nine species (9.3%) were present in all three years. Of those nine, none were beetles, two were flies (*A. exotica* and *T. politus*), three were hymenopterans (*Polistes* sp., *Xylocopa virginica*, and *Halictus ligatus/poeyi*), and four were butterflies (*Strymon melinus*, *Eurema lisa*, *Lerodea eufala*, and *Libytheana carinenta*).

H1) Drivers of Pollinator Community Similarity

Addressing our first hypothesis, that similar abundances of semi-natural habitat and similar land-use histories drive pollinator community similarity, our MRDM and backward selection procedure revealed that difference in time (0, 1, or 2 years) between samples has a significant

negative relationship with pairwise similarity in community composition (p -value = 0.001, Table 2.1, Figure 2.5). In other words, an increase in the number of years between samples correlates with a decrease in community similarity. Historic land-use, measured as the change in semi-natural habitat abundance between 1992 and 2011, also has a significant negative relationship with community similarity (p -value = 0.003, Table 2.1, Figure 2.4). Specifically, sites with less similar land-use histories had less similar community compositions. Geographic distance between sites, differences in cotton bloom density, contemporary measures of land-use (semi-natural abundance and meters of edge habitat) and change in meters of edge habitat between 1992 and 2011 were not significantly correlated with community similarity. A histogram of Chao Similarities is shown in Figure 2.5.

H2) Drivers of Pollinator Community Similarity by Order

MRDM further revealed that the community similarity of different insect orders responds to different explanatory matrices. Coleoptera responds to differences in geographic distance, such that as sites become increasingly separated in space, communities become significantly less similar in composition. Further, beetle similarity has a significant negative relationship with both the loss of semi-natural habitat and edge habitat between 1992 and 2011, where sites with dissimilar changes in semi-natural habitat and meters of edge habitat are less similar in community composition (Table 2.2, Figure 2.6a). Diptera similarity has a significant negative relationship with the loss of semi-natural habitat, such that sites with less similar land-use histories have less similar fly community compositions (Table 2.2, Figure 2.6b). Hymenoptera respond only to difference in time (sampling year) (Table 2.2, Figure 2.6c), showing a negative relationship between the number of years between samples and hymenopteran community similarity. Lepidoptera follow a similar pattern, responding only to difference in time (Table 2.2, Figure 2.6d). Put another way, when bee and butterfly communities are further apart in time, they are less similar in community

composition. Overall, models were significant at the $\alpha = 0.01$ level (Table 2.2, Figure 2.6), except for the final Coleoptera model, which was only marginally significant at the $\alpha = 0.01$ level (p -value = 0.043). Non-significant MRDM relationships are shown in Figure 2.7.

DISCUSSION

In this study, we show that similarity in pollinator community composition decreases with time and is driven, in part, by historical land-use. Interestingly, neither geographic distance nor contemporary environmental factors, such as cotton floral resources or contemporary semi-natural habitat abundance, influence pollinator community similarity. Additionally, we show that pollinating insect orders respond differently to time, space, and environmental factors. Overall, our research indicates that predicting community similarity using only contemporary environmental factors would have failed to capture the long-lasting legacy effects of land-use history that we clearly document in our study system.

H1) Drivers of Pollinator Community Similarity

We found a negative relationship between pollinator community similarity and time, with communities becoming less similar as the period of time between sampling increased. There exists a long history of research on the temporal dynamics of both animal and plant communities that show a higher degree of turnover between longer time periods (Ives and Carpenter, 2007). Research on these changes in plant and animal diversity have been instrumental in helping ecologists recognize disturbance and successional dynamics (Tilman, 1986; Lockwood *et al.*, 1997; Chase, 2003), identify important relationships between community stability and biodiversity (Cottingham *et al.*, 2001), and predict how communities may respond to disturbances, including long-term global changes (Fraterrigo and Rusak, 2008; Magurran *et al.*, 2010). Pollinator communities in particular, have been documented to be quite dynamic over time (Herrera, 1988;

Cane *et al.*, 2005; Price *et al.*, 2005). For example, examining natural areas across Europe, Dupont *et al.* (2009) reported drastic changes in the identity of the species visiting a particular plant, with less than 25% of pollinator species present in one year being observed in the next. Between the three years of our study, only 9.3% (9 species) were present in all three years. These nine species are likely generalist pollinators, with large populations that tend to inhabit low quality, highly modified, or fringe agricultural landscapes (Scott, 1992; Branquart and Hemptinne, 2000; Michener, 2007). The remaining ~90% of species, present in only one or two years of our study, may represent insects that only use agricultural habitats occasionally, or those with small, ephemeral populations.

H2) Drivers of Pollinator Community Similarity by Order

Addressing our second hypothesis, which investigated the response of specific orders, we found that taxa with different life histories did indeed tend to produce different species-environment relationships. Specifically, we found that coleopteran community similarity responded negatively to increased geographic distance as well as the loss of both semi-natural habitat and meters edge habitat over the 19-year period. Diptera only became less similar with increasing difference in the loss of semi-natural habitat, and both Hymenoptera and Lepidoptera communities became less similar as the period of time between sampling increased. We hypothesize that several factors may account for these interesting findings.

Our results suggest that coleopteran communities may be the most dispersal-limited order in our study. In general, species with poorer dispersal abilities are likely less able to cross large geographic distances or traverse potential dispersal barriers than those with greater dispersal abilities (Weddell, 1991). This evidence of possible dispersal limitation for coleopterans also fits with our finding that the loss of meters of edge habitat over the past 19 years drives coleopteran community similarity. It is possible that the loss of edge habitat may be reducing the ability of

beetles to disperse, resulting in changes to the composition of coleopteran communities over geographic space. However, it must be noted that coleopterans were the smallest group of pollinators in terms of both abundance and richness and their final model was only marginally significant.

Dipteran pollinators, on the other hand, were very abundant. Small generalist syrphids, like *A. exotica* and *T. politus*, made up a large proportion of the dipteran communities and were present in all three years of the study. These small hoverflies may be especially suited to exploiting resources in open, highly modified agricultural habitats. While syrphids as a group have been associated with land-use intensification in other studies (Biesmeijer *et al.*, 2006; Jauker *et al.*, 2009), syrphids with generalized resource needs like *A. exotica* and *T. politus*, may be especially well suited to agricultural landscapes. Most other dipteran species in our communities, however, are likely to have specialized larval and adult resource needs, including those with larvae that require fresh water (saprophagous), specific tree roots (saproxylic), or specialize on non-crop related insects and plants (Branquart and Hemptinne, 2000; Schweiger *et al.*, 2005). Specific resources such as these may be more easily lost over time with the conversion of natural landscapes, which would explain our finding that sites with different histories of semi-natural habitat loss are characterized by different fly communities.

Lastly, our hymenopteran and lepidopteran communities showed a strong negative relationship between time (number of years between samples) and community similarity. Three Hymenoptera species were present in all three years of our study: *Polistes* sp 1., *Xylocopa virginica*, and *Halictus ligatus/poeyi*. These species are consistent likely for a variety of reasons: larger population sizes, superior dispersal abilities, and/or greater likelihood to exploit cotton floral resources. As the *Polistes* sp. 1 is social, it is possible that it exists at relatively large population sizes and thus may be more likely to have been consistently collected in our three years of sampling. The large carpenter bee, *X. virginica*, while solitary, is very abundant along the Gulf

Coast. In fact, in this region, they are sometimes considered pests because of their tendency to bore nesting cavities into houses, barns, and other wood structures. *X. virginica* are also excellent flyers, with foraging ranges estimated to be nearly six kilometers (Greenleaf *et al.*, 2007), and thus are not likely to be dispersal-limited. So, while the cotton landscape may not offer appropriate nesting resources to *X. virginica*, cotton flowers may still be regularly exploited during long foraging bouts. Lastly, the social *H. ligatus/poeyi* is very common in the region and throughout the state (Michener, 2007). *Halictus ligatus/poeyi* has been found to have a highly generalized diet, visiting a wide range of plant genera, and thus may be more likely to visit cotton for either nectar or pollen (Michener, 2007). It is the remaining 53 bee and wasp species, present in only one or two years, that are likely driving the pattern we found of high turnover between years. Removing species present in all three years from our analysis makes our observed results stronger (Table 2.4). These individuals were largely solitary bees and may be more specialized in their nesting and forage resource needs, less motivated to visit cotton floral resources, from smaller populations, or any combination of those factors. If an insect has a low preference for cotton, we may not find that insect visiting cotton in years when other floral resources are available.

The analogous pattern of turnover between years that we saw in butterfly communities may be the result of similar processes. Four butterfly species (*Strymon melinus*, *Eurema lisa*, *Libytheana carinenta*, and *Lerodea eufala*) were present in all three years of our study. All four butterflies have large ranges, are associated with open, non-forested habitats, use a wide variety of caterpillar host plants, and are known to be common in disturbed weedy areas (Scott, 1992). Therefore, like the common bees and wasps, these four butterflies are not likely to respond strongly to changes in land-use across either space or time. However, the remaining 14 species of butterfly, including *Copaeodes minima* and *Nathalis iole*, which are significantly smaller butterflies, may be more dispersal limited, have smaller population sizes, or have much more specialized larval and adult diets (Scott, 1992). Similar to Hymenoptera, removing species present in all three years from

the analysis makes statistical patterns stronger (Table 2.4). Thus, it is likely that these 14 relatively less common butterflies are responsible for the strong negative relationship we found between the differences in sampling time and community similarity. It should be noted that while we did observe patterns specific to each order, that within-order variation in dispersal ability and life history traits was also great, and future research would benefit from an investigation of genus or species-specific responses.

CONCLUSIONS

Geographic distance and contemporary environmental factors have often been proposed as central drivers of community similarity. In this study, we show that historic land-use is even more important in explaining community similarity in our system. Thus, our research offers evidence of a legacy effect on the composition of communities in a rapidly changing agricultural system. We posit that historic processes may be critical to consider from a conservation perspective as their exclusion may lead to overly optimistic assessments of biodiversity in recently disturbed habitats. The conversion of natural landscapes into agriculture is one of the leading causes of habitat loss world-wide; understanding how to develop strategies that protect and regenerate the diversity of ecosystem providers in these landscapes is essential both for the conservation of biodiversity and the promotion of agro-ecological function.

Table 2.1: Results of multiple regression on distance matrices (MRDM) for the full pollinator community. p -values are estimated from 10,000 permutations. Asterisks show significant p -value at $\alpha = 0.01$. A backward selection procedure was applied (p -value < 0.01) to progressively eliminate nonsignificant variables from the full model to determine the final model.

Full Community: Full Model	Regression	P value
F value: 73.87, R ² : 0.45, P Value: 0.001	Coefficients	
Intercept	-0.38	1
Time	-0.23	0.001 *
Geographic Distance (m)	-0.23	0.04
Cotton Bloom Density	-0.23	0.78
Semi-natural Habitat (m ²)	-0.23	0.28
Meters of Edge Habitat (m)	-0.23	0.91
Change in Semi-natural Habitat (m ²)	-0.23	0.001 *
Change in Meters of Edge Habitat (m)	-0.23	0.28
Full Community: Final Model	Regression	P value
F value: 251.48, R ² : 0.45, P Value: 0.001	Coefficients	
Intercept	-0.42	1
Time	-0.26	0.001 *
Change in Semi-natural Habitat (m ²)	-0.26	0.003 *

Table 2.2: Results of multiple regression on distance matrices (MRDM) for individual orders. p -value are estimated from 10,000 permutations. Asterisks show significant p -value at $\alpha = 0.01$. A backward selection procedure was applied (p -value < 0.01) to progressively eliminate nonsignificant variables from the full model to determine final models.

Coleoptera: Full Model F-value: 4.59, R^2 : 0.22, p -value: 0.015		
	Regression Coefficients	P value
Intercept	0.54	0.77
Time	0.34	0.04
Geographic Distance (m)	0.34	0.006*
Cotton Bloom Density	0.34	0.52
Semi-Natural Habitat (m ²)	0.34	0.87
Meters of Edge Habitat (m)	0.34	0.80
Change in Semi-Natural Habitat (m ²)	0.34	0.02
Change in Meters of Edge Habitat (m)	0.34	0.001*
Coleoptera: Final Model F value: 4.03, R^2 : 0.16, p -value: 0.043		
Intercept	0.53	0.88
Geographic Distance (m)	0.53	0.01
Change in Semi-Natural Habitat (m sq)	0.53	0.006*
Change in Meters of Edge Habitat (m)	0.53	0.007*
Diptera: Full Model F value: 7.57, R^2 : 0.32, p -value: 0.007		
	Regression Coefficients	P value
Intercept	0.079	1
Time	0.06	0.77
Geographic Distance (m ²)	0.06	0.92
Cotton Bloom Density	0.82	0.15
Semi-Natural Abundance (m ²)	0.06	0.16
Meters of Edge Habitat (m)	0.06	0.72
Change in Semi-Natural Habitat (m ²)	0.06	0.002*
Change in Meters of Edge Habitat (m)	0.06	0.76
Diptera: Final Model F value: 169.99, R^2 : 0.24, p -value: 0.001		
Intercept	0.24	1
Change in Semi-Natural Habitat (m ²)	0.24	0.001*

Table 2.2 Continued

Hymenoptera: Full Model		
<i>F</i> value: 0.07, <i>R</i> ² : 0.073, <i>p</i> -value: 0.07		
Intercept	0.58	0.39
Time	0.70	0.008*
Geographic Distance (m ²)	0.70	0.072
Cotton Bloom Density	0.70	0.43
Semi-natural Habitat (m ²)	0.70	1
Meters of Edge Habitat (m)	0.70	0.44
Change in Semi-Natural Habitat (m ²)	0.70	0.401
Change in Meters of Edge Habitat (m)	0.70	0.491
Hymenoptera: Final Model	Regression Coefficients	<i>P</i> value
<i>F</i> value: 32.9, <i>R</i> ² : 0.050, <i>p</i> -value: 0.002		
Intercept	0.50	1
Time	0.09	0.002*
Lepidoptera: Full Model		
<i>F</i> value: 10.70, <i>R</i> ² : 0.11, <i>p</i> -value: 0.001		
Intercept	0.54	0.98
Time	0.38	0.001*
Geographic Distance (m ²)	0.38	0.045
Cotton Bloom Density	0.38	0.30
Semi-natural Habitat (m ²)	0.38	0.11
Meters of Edge Habitat (m)	0.38	0.12
Change in Semi-Natural Habitat (m ²)	0.38	0.91
Change in Meters of Edge Habitat (m)	0.38	0.60
Lepidoptera: Final Model	Regression Coefficients	<i>P</i> value
<i>F</i> value: 54.70, <i>R</i> ² : 0.084, <i>p</i> -value: 0.001		
Intercept	0.55	1
Time	0.11	0.001*

Table 2.3: Variation Inflation Factors (VIFs) for explanatory variables

Explanatory Variable	VIF
Cotton Bloom Density	1.27
Semi-natural Habitat (m ²)	3.20
Meters Edge Habitat (m)	2.99
Change in Semi-Natural Habitat (m ²)	3.51
Change in Meters Edge Habitat (m)	3.96

Table 2.4: Results of multiple regression on distance matrices (MRDM) for Hymenoptera and Lepidoptera orders excluding species that appeared every year. Asterisks show significant *p*-values at alpha = 0.01.

Hymenoptera <i>F</i> value: 18.86217, <i>p</i> -value: 0.00200	Regression Coefficients	<i>P</i> value
Intercept	-0.6404	0.969
Time	-0.7856	0.001 *
Geographic Distance (m)	-0.7856	0.444
Cotton Bloom Density	-0.7855	0.641
Semi-Natural Habitat (m ²)	-0.7855	0.968
Meters of Edge Habitat (km)	-0.7855	0.912
Change in Semi-Natural Habitat (m ²)	-0.7855	0.606
Change in Meters of Edge Habitat (m)	-0.7855	0.444
Lepidoptera <i>F</i> value: 11.998739, <i>p</i> -vValue: 0.001000		
Intercept	-0.4398	0.977
Time	-0.5998	0.001*
Geographic Distance (m)	-0.4407	0.062
Cotton Bloom Density	-0.4386	0.315
Semi-Natural Habitat (m ²)	-0.4397	0.115
Meters of Edge Habitat (m)	-0.4396	0.109
Change in Semi-Natural Habitat (m ²)	-0.4395	0.888
Change in Meters of Edge Habitat (km)	-0.4395	0.590

Table 2.5: List of sites including site specific variables: year sampled, location (longitude and latitude), cotton bloom density, semi-natural habitat, meters edge habitat (m), change in semi-natural habitat, and change in meters edge habitat.

Site	Year	Longitude	Latitude	Cotton Bloom Density	Semi- Natural Habitat (m ²)	Meters Edge Habitat (m)	Change in Semi- Natural Habitat (m ²)	Change in Meters Edge Habitat (m)
Adamek	2013	28.75120804	-96.82521448	82	2919831.81	116592.35	-1606373.46	-105821.69
Austwell 774	2015	28.36750319	-96.84671763	23	484708.50	40909.54	-410790.10	-21399.62
Bayer	2015	28.70756854	-96.92657137	27	2983172.67	88859.15	-238343.98	-23107.04
Bayside 136	2015	28.09218413	-97.22850508	15	2339416.03	95762.50	99429.38	15863.30
Chris	2014	28.78231923	-96.8034964	68	1408627.92	100107.96	-810914.73	-41967.85
Gin House Block	2015	28.11157328	-97.24869031	25	136934.89	12916.30	-33542.70	-3757.12
	2014	28.30436455	-97.41532738	56	1339742.38	73205.83	-434905.27	-29682.72
JJ	2013	28.35317725	-96.86735982	89	535195.17	59414.36	-263714.64	-11123.12
JJ 35	2014	28.40800229	-96.90214143	47	1936891.02	80191.93	-29523.84	-18184.59
JJ Aransas	2014	28.36358956	-96.8181205	54	2342090.92	104940.28	-780932.09	-63784.24
JJ Home	2014	28.3646632	-96.86211152	54	948598.36	53919.81	-602259.66	-42543.64
Johnson	2015	28.35034438	-96.86371382	31	688574.33	62810.58	-389415.52	-10897.13
JR Corner	2014	28.32352668	-96.93442543	48	3144717.67	97900.30	-603164.78	-50210.11
Ken 2013	2013	28.78951045	-96.83845611	114	3731304.36	135425.81	-611286.31	-46205.12
Ken 2014	2014	28.78678028	-96.84345309	55	2464341.84	138694.29	-360986.14	-52211.45
Ken 2015	2015	28.78781127	-96.84179241	22	2920894.27	134839.82	-423403.60	-52274.92
Lee	2013	28.29729993	-97.37670667	110	2886379.10	84184.22	-1190896.17	-50031.64
Lenhardt 1	2013	28.388193	-96.910056	86	3324546.31	74967.34	-231208.84	-20466.94
Lenhardt 2	2013	28.40092691	-96.86069488	88	1268099.12	68666.54	-487504.52	-35238.91
Lenhardt 3	2013	28.33595892	-96.87478485	104	911865.02	39540.71	-782626.56	-30980.67
Mayo	2013	28.77478482	-96.88013482	70	1003307.77	66397.39	-419527.13	-12795.30
Microwave New Ground	2015	28.11721404	-97.22851225	18	340919.56	55377.01	-244021.77	-3552.98
	2014	28.30212708	-97.38016764	63	2863207.09	94619.75	-870484.76	-39816.04
Roy	2013	28.75479579	-96.9053053	64	2597600.12	91069.52	-633942.26	-51137.67

Table 2.5
Continued

Shaw	2015	28.37414886	-						
South			96.88716481	26	90241.87	43097.49	-90241.87	5601.38	
Clarkson	2014	28.28455026	-						
South			97.38604168	43	1220411.60	72859.37	-382482.60	-23159.80	
Floerke	2014	28.31805642	-						
Telferner			97.38834565	52	5242765.31	156348.78	-309372.34	-23790.82	
1686	2015	28.77214303	-						
Telferner			96.82850414	37	1689200.76	85825.11	160326.30	-6910.55	
Highway	2015	28.75731435	-						
			96.94900632	39	446955.58	44969.80	-236767.84	-15291.60	
Tom 1	2014	28.73981648	-						
			96.86173259	47	3049979.16	126263.39	-425981.88	-36235.90	
Tom 2	2014	28.71140291	-						
			96.92031951	60	3216619.70	88606.71	-173460.39	-46233.62	
Vega	2015	28.106277	-						
			-97.213204	24	810940.63	96518.89	-207173.34	22768.40	
Walt 1	2013	28.18978109	-						
			97.25482499	83	894885.66	79180.32	-199193.53	10925.10	
Walt 2	2013	28.17468611	-						
			97.25045063	97	53727.78	42773.78	-35865.87	17192.95	
Walt 3	2013	28.15744052	-						
			97.23597581	80	62840.05	50919.72	-26585.21	14156.49	
Zarsky	2015	28.40881653	-						
			96.88117285	24	3019938.72	69554.26	-286413.02	-2648.06	

Table 2.6: Pollinator species list organized by order.

Coleoptera

Acalymma vittatum
Coccinellidae morphospecies sp. 1
Coccinellidae morphospecies sp. 2
Coccinellidae morphospecies sp. 3
Coccinellidae morphospecies sp. 4
Diabrotica balteata
Diabrotica undecimpunctata

Diptera

Allograpta exotica
Allograpta obliqua
Asilidae morphospecies sp.1
Bombyliidae morphospecies sp.1
Dioprosopa clavata
Dolichopodidae morphospecies sp. 1
Eristalis arbustorum
Eristalis stipator
Eristalis tenax
Hermetia illucens
Muscidae morphospecies sp. 1
Palpada furcata
Palpada mexicana
Sarcophagidae morphospecies sp. 1
Toxomerus marginatus
Toxomerus politus

Table 2.6 Continued

Hymenoptera

Agapostemon splendens

Agapostemon texanus

Ancyloscelis apiformis

Augochlorella bracteata

Augochloropsis metallica

Bombus pennsylvanicus

Ceratina shinneri

Chalcididae morphospecies sp. 1

Chrysididae morphospecies sp. 1

Coelioxys slosoni

Colletes mandibularis

Diadasia diminuta

Dianthidium curvatum

Dianthidium subparvum

Dieunomia heteropoda

Eumeninae morphospecies sp. 1

Halictus ligatus/poeyi

Lasioglossum disparile

Lasioglossum coactum

Lasioglossum connexum

Lasioglossum (Dialictus) morphospecies sp. 1

Lasioglossum (Dialictus) morphospecies sp. 2

Table 2.6 Continued

Lasioglossum (Dialictus) morphospecies sp. 3

Lasioglossum (Dialictus) morphospecies sp. 4

Lasioglossum (Dialictus) morphospecies sp. 5

Lasioglossum (Dialictus) morphospecies sp. 6

Lasioglossum (Dialictus) TX sp. 16

Lasioglossum (Hemihalictus) fedorense

Lasioglossum hunter

Lasioglossum semicaeruleum

Lasioglossum tegulare

Megachile albitarsis

Megachile brevis

Megachile coquillett

Megachile deflexa

Megachile gentilis

Megachile montivaga

Megachile parallela

Megachile polycaris

Melissodes tepaneca

Melitoma marginella

Mutillidae morphospecies sp. 1

Polistes morphospecies sp. 1

Polistes morphospecies sp. 2

Ptilothrix bombiformis

Table 2.6 Continued

Scoliidae morphospecies sp. 1
Sphecidae morphospecies sp. 1
Svastra atripes
Svastra grandissima
Svastra oblique
Svastra petulca
Triepeolus concavus
Triepeolus rufoclypeus
Xylocopa micans
Xylocopa virginica

Lepidoptera

Atlides halesus
Brephidium exile
Echinargus isola
Eurema lisa
Hylephila phyleus
Large Moth morphospecies 1
Lerema accius
Lerodea eufala
Libytheana carinenta
Ostrinia nubilalis
Medium Moth morphospecies 1
Melanchroia chephise
Nathalis iole

Table 2.6 Continued

Phoebis agarithe

Phyciodes tharos

Pontia protodice

Pyrgus communis/albescens

Small Moth morphospecies 1

Strymon melinus

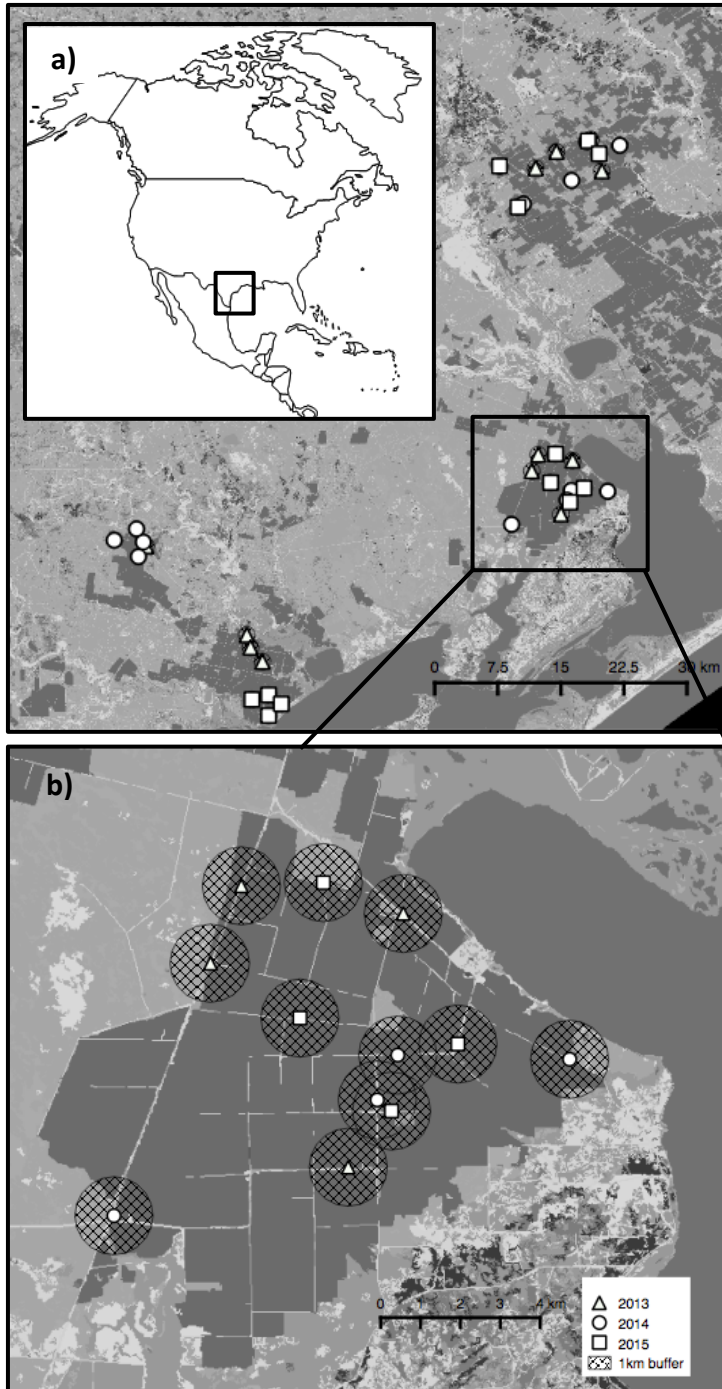


Figure 2.1: Map of study sites. a) Inset: North America. Map of 36 conventional cotton fields used as study sites (shown as white symbols) along the South Texas Gulf Coast, U.S.A. b) An enlargement showing 12 cotton field sites sampled over the three-year period. White symbols represent sampling sites, and hatched buffers represent a 1km radius of habitat surrounding sites. White triangles were sampled in 2013, white circles in 2014, and white squares in 2015. Sites were primarily surrounded by natural areas (light grey) and agriculture (medium grey).

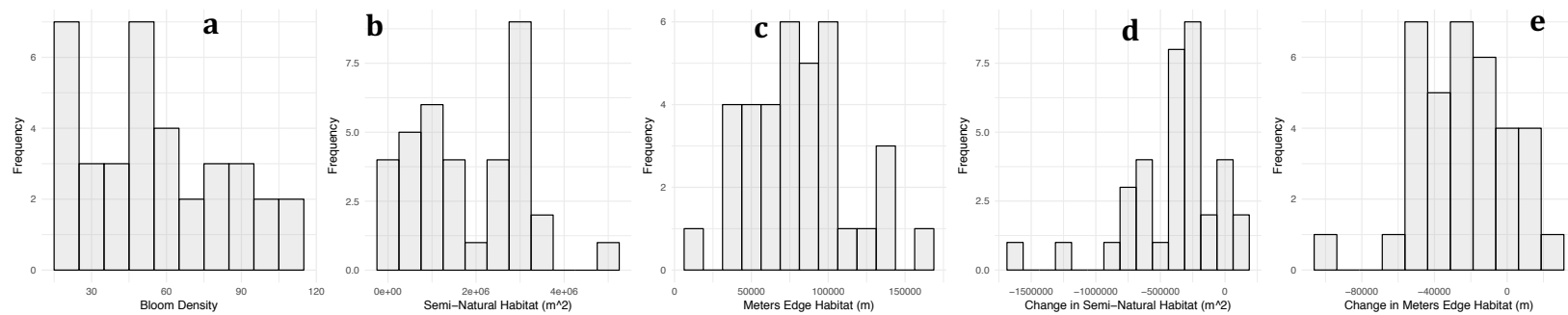


Figure 2.2: Histograms showing the distribution of explanatory variables across sites a) Histogram showing the distribution of bloom density, b) semi-natural habitat (m²), c) meters edge habitat (m), d) change in change in semi-natural habitat (m²), e) change in meters edge habitat (m) across sites.

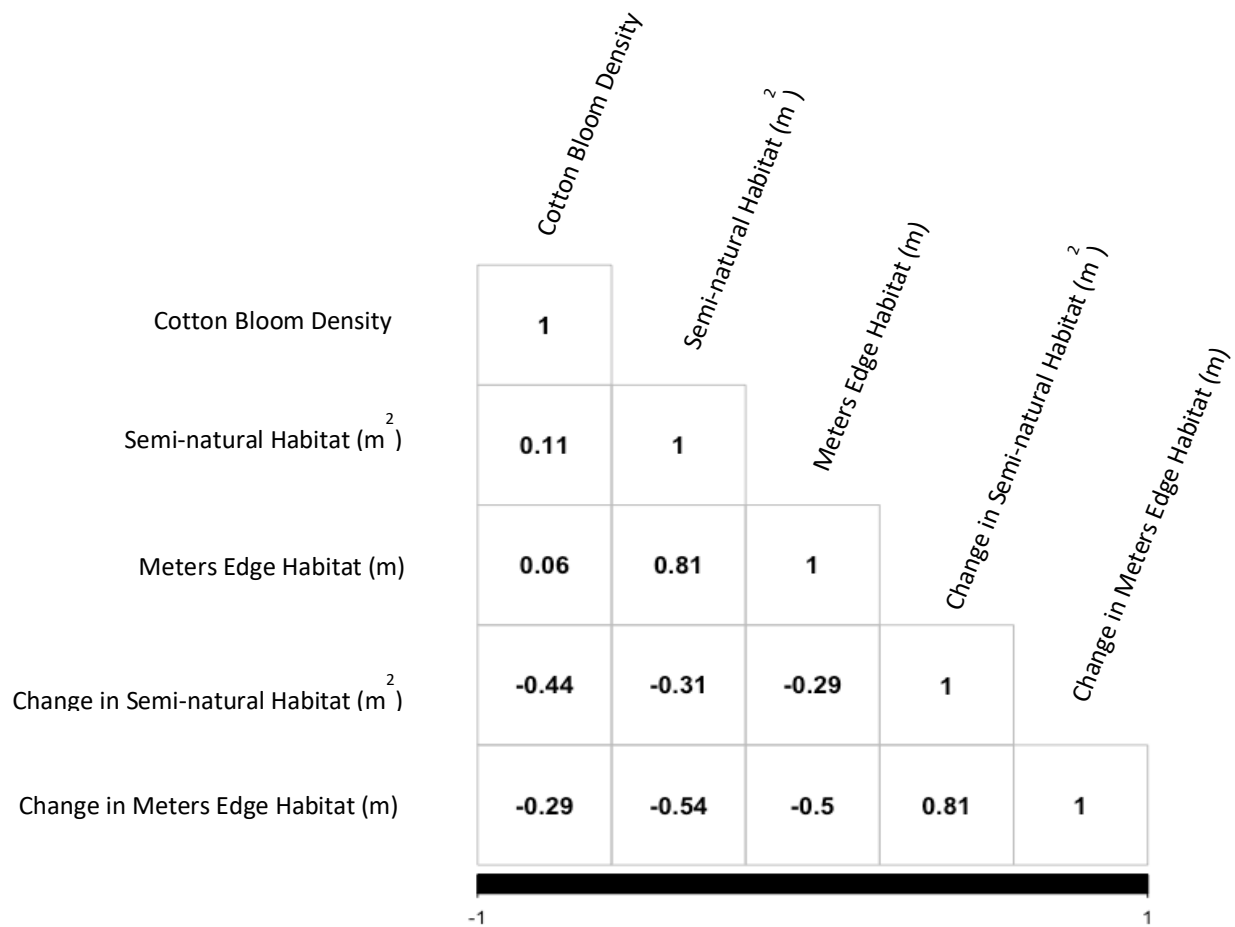


Figure 2.3: Correlation matrix showing the direction and correlation of explanatory variables used in MRDM models

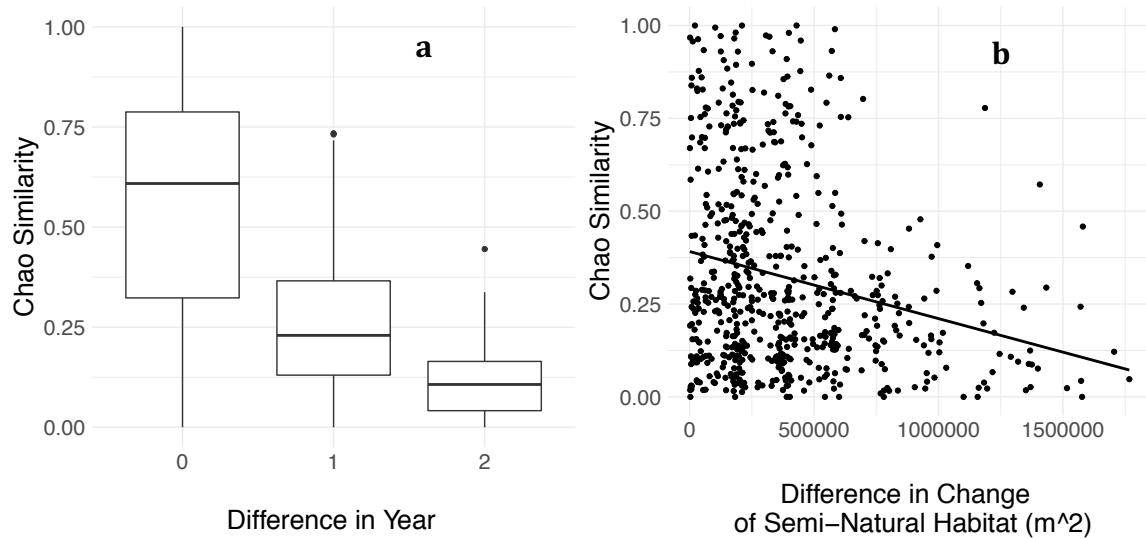


Figure 2.4: Plots showing the relationship between significant explanatory variables of the MRDM and Chao Similarity. a) Plot showing the significant negative relationship between difference in time (Difference in Year between sites) and Chao Similarity (Chao), b) Plot showing the significant negative relationship between difference in land-use history (Difference in Change of Semi-Natural Habitat (m^2) between 1992 and 2011) and Chao Similarity.

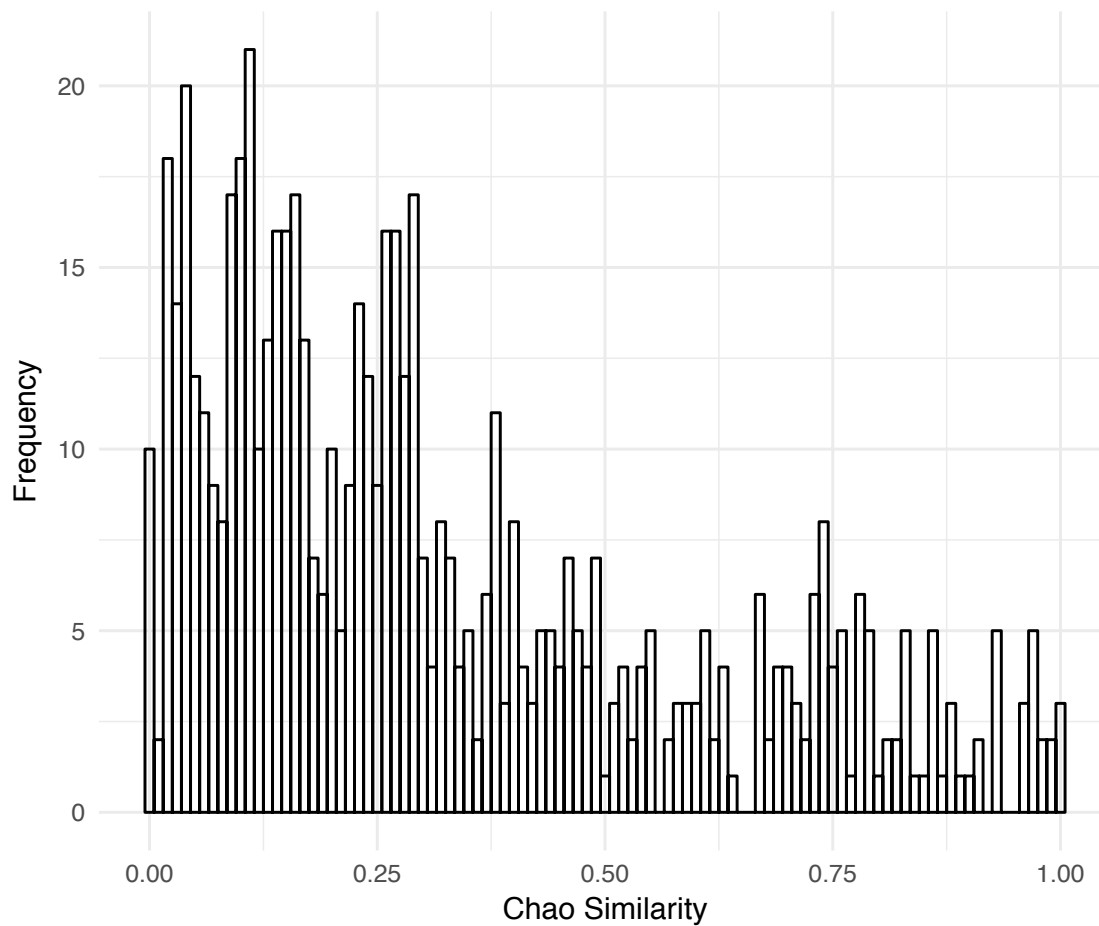


Figure 2.5: Histogram showing the distribution of Chao Similarity between sites. A value of 0 shows no similarity, while a value of 1 shows complete similarity between two sites.

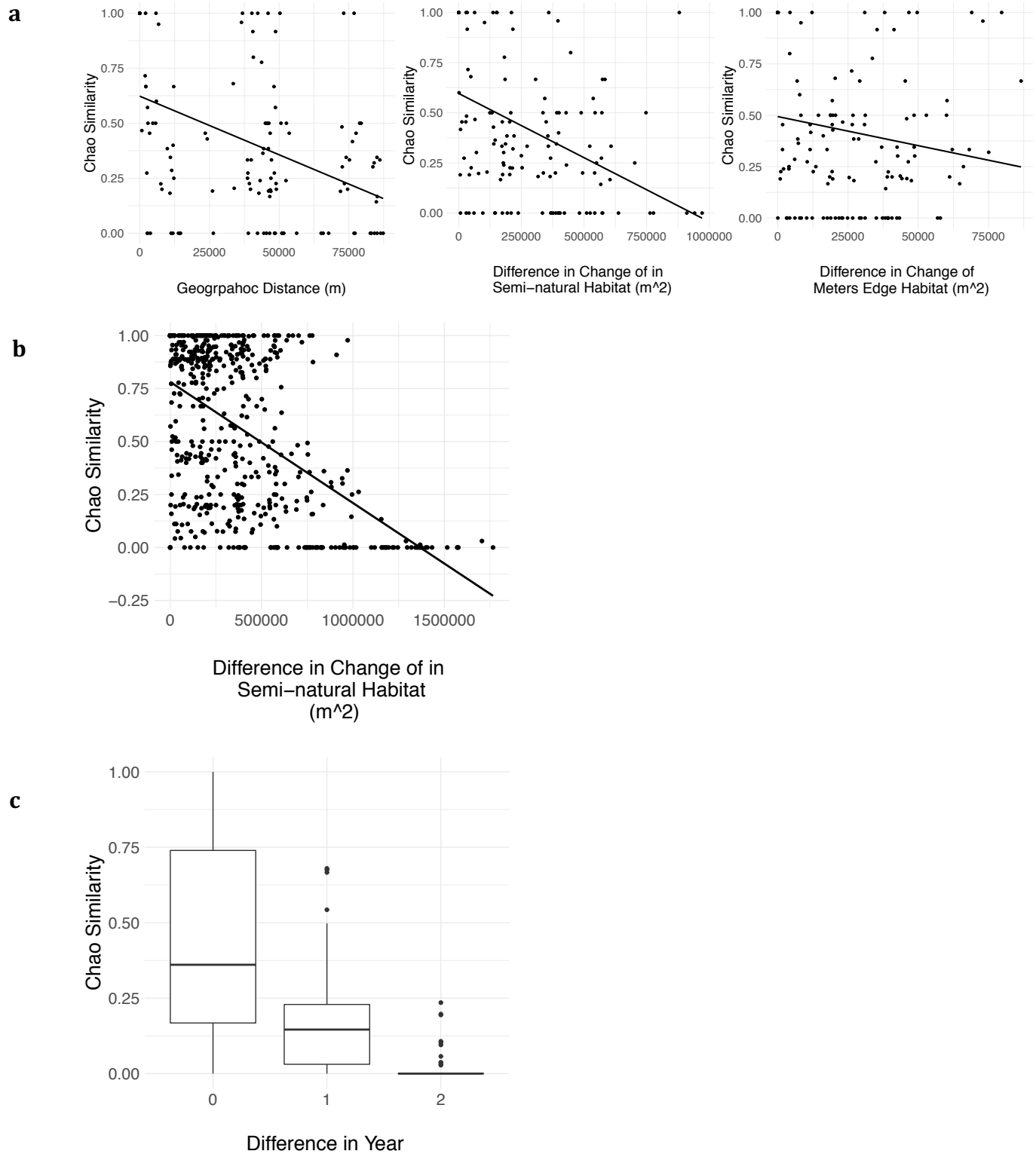


Figure 2.6

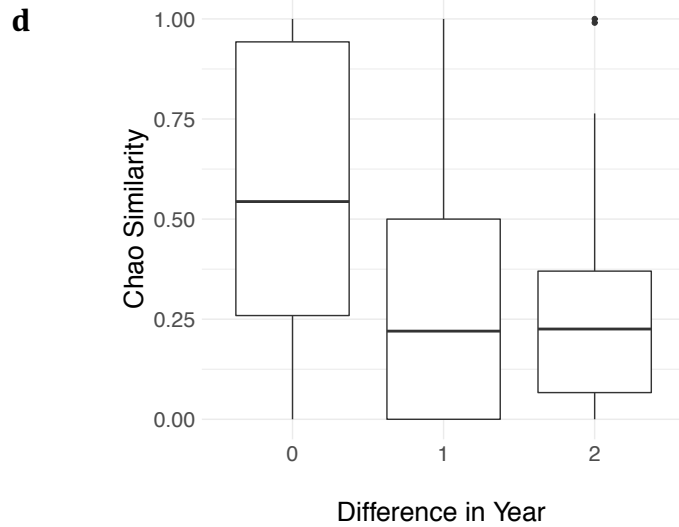


Figure 2.6 Continued: Plots showing the relationship between significant explanatory variables of the MRDM and Chao Similarity for each order. a) Plot showing the significant negative relationships between difference in geographic distance, land-use history (Difference in Change of Semi-Natural Habitat (m^2) and Change in Meters Edge habitat (m) between 1992 and 2011) and Coleoptera Chao Similarity, b) Plot showing the significant negative relationship between difference in land-use history (Difference in Change of Semi-Natural Habitat (m^2) between 1992 and 2011) and Diptera Chao Similarity. c) Plot showing the significant negative relationship between difference in time (Difference in Year between sites) and Hymenoptera Chao Similarity, d) Plot showing the significant negative relationship between difference in time (Difference in Year between sites) and Lepidoptera Chao Similarity.

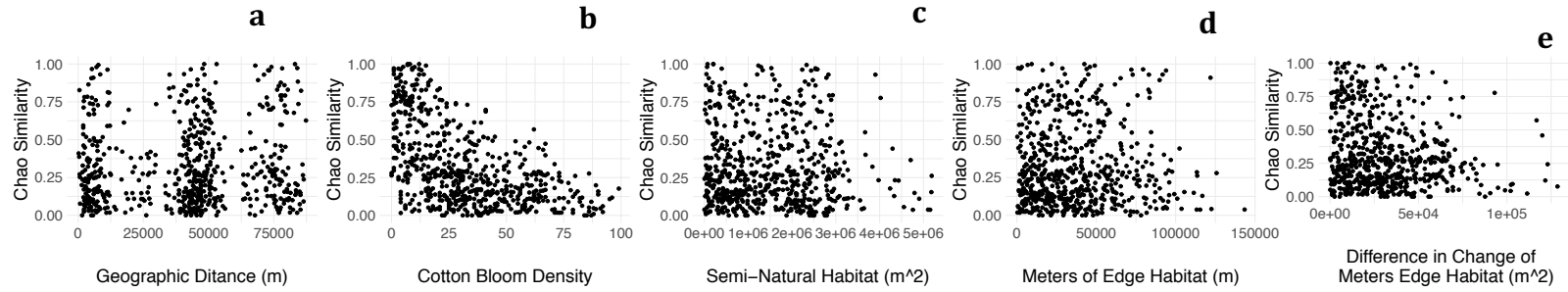


Figure 2.7: Plots showing the relationship between non-significant explanatory variables of the MRDM and full community Chao Similarity. a) Plot showing the non- significant negative relationship between difference in geographic distance (m) and Chao Similarity b) difference in cotton bloom density and Chao Similarity, c) difference in semi-natural habitat (m^2) and Chao Similarity, d) difference in meters edge habitat (m) and Chao Similarity, e) difference in the change of meters edge habitat (m) and Chao Similarity.

Chapter 3: Small but critical: semi-natural habitat fragments promote bee abundance in cotton agroecosystems across both Brazil and the United States

ABSTRACT

Bees are the most important pollinators of agricultural crops worldwide. For bees in agroecosystems, patches of semi-natural habitat within or adjacent to agriculturally-dominated landscapes can potentially provide important nesting and food resources. Despite this, across the globe, land cover change is rapidly reducing the abundance of semi-natural habitat within agricultural landscapes, with unknown consequences to bee communities. Identifying how the availability of semi-natural habitat drives bee community composition in agroecosystems across biogeographic regions may reveal important commonalities and key governing principles that transcend a single region or target taxon. Here, we analyze and compare the composition and drivers of bee communities in cotton fields within Brazil and the U.S.A. to reveal how land cover and land cover change impacts bee community composition and dynamics across these two regions. We show that across sociality, nesting guilds, and taxonomic relatedness, the most critical factors impacting bee communities in cotton agroecosystems are the same in Brazil and the U.S.A.: the contemporary abundance of semi-natural habitat, the density of the cotton bloom, and the loss of semi-natural habitat over a five-year period. Given the importance of bee abundance for the provision of pollination in cotton plants, our findings highlight the significance of semi-natural habitat in supporting key ecosystem service providers for both tropical and temperate cotton agroecological systems. We underscore the important role that land managers play in biodiversity conservation, and the potential contribution they can make to pollination provision by supporting agricultural landscapes that conserve fragments of semi-natural habitat.

INTRODUCTION

Resource availability drives species diversity and abundance across diverse taxa and within both natural and human-managed ecological systems (Tylianakis *et al.*, 2008). By altering the distribution and density of key resources, human-induced land cover change poses a major threat to the persistence of many species in newly altered habitats. In particular, agricultural and pastoral development in the last century has been one of the most important drivers of land cover change (Scialabba and Williamson, 2004). The conversion of forest, grassland, and pasture, to agriculture also offers an ideal opportunity to study how large-scale land cover change and habitat loss alter population and community dynamics on a global scale. Because similar crops are grown across multiple biogeographic regions, researchers can use a comparative approach to investigate the fundamental dynamics of how environmental drivers, such as contemporary land cover or recent change in land cover, affects biological communities. By identifying common drivers of community response to land cover change across multiple biogeographic regions, there is great potential to reveal key principles governing community composition that transcend a single biogeographic region or population.

Pollination by animals is critical for more than 80% of all plant species (Ollerton and Winfree, 2011) including more than 60% of global crop species (Klein *et al.*, 2007) and bees are the most important pollinators of agricultural crops worldwide (McGregor, 1976; Nabhan and Buchmann, 1997). The decline of bee abundance and diversity has been shown to lead to parallel declines in plant species, as well as crop yield (Biesmeijer *et al.*, 2006, Kearns *et al.*, 1998; Ashman *et al.*, 2004). While, the decline of wild bee populations has been attributed to a variety of causes (e.g. agrochemicals, pathogens, alien species, and climate change, Tylianakis *et al.*, 2005; Potts *et al.*, 2010; Goulson *et al.*, 2015), land cover change and subsequent habitat loss is well-documented as one of the most powerful threats to bee populations across the globe (Ghazoul, 2005).

For wild bees to persist in a landscape, they need two major resources: food in the form of pollen and nectar, provided by flowers, and nesting habitat, provided by access to soil, woody vegetation/debris, and existing cavities. Of these two resource groups, bee pollinators are thought to closely track floral resources within a landscape (Waser, 1983; Ghazoul, 2006). Assumed to act as optimal foragers, bees prefer to visit forage patches with high floral density, a phenomenon called the ‘concentration effect’ (Hegland and Boeke, 2006). Conversely, bee foragers have also been documented to visit proportionally fewer flowers as patch size increases, known as the ‘dilution effect’ (Goulson, 2000; Kunin, 1993; Hegland *et al.*, 2009; Veddeler *et al.*, 2006; Root, 1973). Beyond the availability of floral resources, the diversity and abundance of bee communities is also thought to be dependent on landscape characteristics that specifically mediate the distribution of important nesting resources. While difficult to directly measure, the amount of semi-natural habitat surrounding the sampling area is commonly used as a proxy for nesting habitat and has been found to be a significant predictor of bee abundance and diversity in many landscapes (Kearns and Oliveras, 2009; Xie *et al.*, 2008; Kruess and Tscharntke, 2002).

As a whole, bees are thought to respond rapidly to habitat loss, reaching a new equilibrium in remnant habitat patches within a few years of disturbance due to their relatively short generation times, high mobility, and ability to track resources in a new environment (Krauss *et al.*, 2010). Nevertheless, within this larger group of bee pollinators, distinct species are likely to respond differently to habitat loss depending on factors unique to that species: (i) their foraging range and (ii) their degree of nest resource specialization. First, bees are central place foragers and require suitable floral resources within their flight range, which may be limited. Some of the smallest bees in our region forage only a few hundred meters from their nesting habitat (Greenleaf *et al.*, 2007). Thus, limited foraging abilities may prevent smaller bees from quickly emigrating out of unsuitable habitat. Second, given that some bees exhibit very specific nesting preferences (e.g. Michener, 2007) it is possible that nest resource availability drives response to land-use change

for bee communities. If nesting substrates are removed as semi-natural habitat is converted to agriculture, bees may exhibit declines or may go locally extinct (Potts *et al.*, 2005).

In this study, we first quantify and compare bee community composition across cotton agroecosystems in Mato Grosso, Brazil and in a portion of southern Texas, U.S.A. Second, we investigate how floral density, land cover, and land cover history influence bee abundance and diversity across these two biogeographic regions. Given differences in natural habitat and bee fauna sociality, nesting preference, and composition between the two regions (Free, 1993; Roubik, 1995), we hypothesize that different environmental factors, such as the abundance of semi-natural habitat, might differentially drive bee abundance and diversity in Brazil and the U.S.A. However, in both bioregions, we expected to find evidence of delayed reaction to land cover change, and thus we predict we will find a negative effect of the recent semi-natural habitat loss on contemporary bee communities in both Brazil and the U.S.A.

METHODS

Study System

We conducted our research in cotton agroecosystems across two distinct biogeographic regions. Within eastern Brazil, we sampled within the state of Mato Grosso and within the southern U.S.A., we sampled a cotton growing region in the southern part of the state of Texas. In Brazil, the eastern state of Mato Grosso is the largest cotton producing area of the country, responsible for about 60% of Brazilian cotton (1.7 million hectares) (Mato Grosso Institute of Agricultural Economics, 2014). Mato Grosso grows primarily *Gossypium hirsutum* L. (Malvaceae). Aside from cotton, the state also stands out as a major producer of soybean and corn, and agricultural land cover makes up the majority of the region (58%). The remainder of the region is covered by semi-natural habitat (22%), including forest, shrub, and grassland, that are a mixture of two dominant vegetation types: Cerrado and Amazon Forest. The Cerrado is a savanna-like biome with drylands

that range from open grassland fields with a few shrubs to closed forests with canopy height of 12-15 m. These include periodically flooded wetlands called “Gallery Forests” (Ratter *et al.*, 1997). The Amazon Forest is a biome in which drylands are composed primarily of closed canopy rainforests with canopy height with more than 20 m, as well as wetlands (Myser, 2016).

In the U.S.A., the state of Texas grows more than 25% of the country’s cotton crop, and cotton covers roughly 1.9 million hectares of farmland in the state (Alvarez and Plocheck, 2014). Texas also grows primarily Upland cotton varieties (*Gossypium hirsutum* L. (Malvaceae)). Agriculture makes up the majority of land cover (55% in the southern Texas region we studied), and, similar to Mato Grosso, consists primary of cotton, sorghum, corn, and soybean cropland. The remainder of the region is comprised of low density developed areas (e.g. low density suburban development) (6%), and semi-natural habitat (38%), including pastureland, shrub, mixed woodland, and marsh areas along the Gulf Coast. Semi-natural habitat along the coast includes barrier islands, salt grass marshes surrounding bays and estuaries, remnant tallgrass prairies, oak parklands and oak mottes, and tall woodlands in the river bottomlands (Conner *et al.*, 1989).

We conducted research in 17 sites in Mato Grosso located in three geographic regions between Primavera do Leste (-12.35138, -55.5235) and Campo Novo do Parecis, Mato Grosso (-13.33132, -57.50479) in 2016. Specifically, the three geographic regions were separated on average by 228 km, and were near the towns of Primavera do Leste, Sorriso, and Campo Novo do Parecis. Sites were located within cotton fields that were at least 40 ha in size and two km apart from each other. Similarly, in Texas, we conducted research in 12 sites located in three geographic regions between Telferner (28.847913, -96.892975) and Woodsboro, Texas (28.303701, -97.381612) in 2014. The three geographic regions of study were separated on average by 52 km and were located near the towns of Woodsboro, Austwell/Tivoli, and Telferner, Texas (Cusser *et al.*, 2016). Sites were located within cotton fields that were at least 35 ha in size and two km apart from each other (Figure 3.1).

Bee Community Sampling

To quantify the bee community at each site we netted bees found actively foraging within cotton flowers during one-hour sampling bouts at each site. During each one-hour bout, collectors walked four parallel 50 m $\times$ 1 m transects, checking blooms for visitors and collecting them by net. Transects were located at the edge of fields and were $\sim$ 12 m apart, representing an area of 2500 m². In Mato Grosso, bee collections were performed during peak bloom, from late March to early May in 2016. Due to weather, the number of sampling bouts per site ranged from one to four. In Texas, each site was sampled three times and sampling bouts were performed from June to July in 2014 (Cusser *et al.*, 2016). In Mato Grosso, bees were identified using entomological identification keys, as well as the Entomological Collection of Professor J. M. F. Camargo (RPSP), in Department of Biology at Faculty of Philosophy, Sciences and Literature of Ribeirão Preto, University of São Paulo (FFCLRP/USP). In Texas, bees were identified by J. Neff at the Central Texas Melittological Institute.

Landscape Composition

Floral Resources

In both Mato Grosso and Texas, we collected data on the quantity of available floral resources that were attributable to the cotton crop during each of our bee sampling bouts. To calculate cotton bloom density, we counted the number of blooms on each of five randomly chosen cotton plants within each of the four transects, for a total of 20 cotton plants per sample per site. Bloom density was then calculated as the average number of blooms per plant across samples per site in both Mato Grosso and Texas.

Regional and Historic Land Cover

In both Mato Grosso and Texas, we calculated the percentage of different land cover types surrounding each sampling site at a regional scale (1.5 km radius). This radius was chosen as it is small enough to ensure that the regional scale did not overlap between sites, but also large enough to reflect the foraging distance of many bees (Greenleaf *et al.*, 2007). In Mato Grosso we used information from the 2009 GlobCover Database (Arino *et al.*, 2012). To quantify land cover abundance, we summed the total amount of semi-natural habitat cover falling within the 1.5 km buffer in qGIS (Quantum GIS Development Team, 2015). ‘Semi-Natural’ land habitat includes the GlobCover categories: evergreen and deciduous forest, as well as all types of shrub and grassland. Semi-natural abundance was negatively correlated with the abundance of agriculture land surrounding each site (Pearson’s correlation = -0.55, p -value = 0.021). Thus, semi-natural habitat in Mato Grosso serves as a description of land cover surrounding sites in general.

Similarly, in Texas, we measured the abundance of different types of land cover surrounding each site at a regional scale (1.5 km radius), using information from the 2011 Cropscape Database (Han *et al.*, 2014). As described above, we quantified land cover abundance as the total amount of semi-natural habitat falling within the 1.5 km buffer around each site. ‘Semi-Natural’ habitat cover in Texas includes the Cropscape categories: Evergreen and Deciduous forest, Shrubland, Grassland, Pasture/Hay, and Wetlands. As in Mato Grosso, semi-natural abundance was negatively correlated with both the abundance of agriculture and human development surrounding each site, but the magnitude of the correlation was much stronger (Pearson’s correlation = -0.9976, p -value < 0.001). Thus, semi-natural habitat in Texas serves as description of the land cover abundance surrounding sites in general.

We used the difference in the abundance of semi-natural habitat from the years 2004 and 2009 to determine how semi-natural habitat cover has recently changed in Mato Grosso. The difference in the semi-natural habitat cover between these years was calculated for each site within a 1.5 km buffer, using the GlobCover Database maps (Arino *et al.*, 2012). Similarly, to quantify

land cover change in Texas, we measured the change in semi-natural habitat cover surrounding sites within a 1.5 km buffer using National Land Cover Database maps from the years 2006 and 2011 (Han *et al.*, 2014). These data sets were the most similar in years and time between years publicly available for both biogeographic regions.

Statistical analysis

First, to summarize the differences in bee community composition between Mato Grosso and Texas, we used the extant entomological literature to group bees by their taxonomic tribe (which generally share important life-history characteristics), their sociality (Solitary, Social, or Kleptoparasitic), and nesting preference where ‘Ground’ refers to ground-nesting bees, ‘Wood/Stem’ refers to wood-nesting bees, and ‘Large Cavity’ refers to the nesting habit of bees that occupy large (>5 cm) tree hollows or rodent burrows, including honey, stingless, and bumble bees. To examine statistical differences in groupings between Mato Grosso and Texas, we used non-metric multidimensional scaling (NMDS, Anderson, 2001). Using the vegan package in the R statistical computing language (Oksanen *et al.*, 2009), we calculated Bray–Curtis dissimilarity using the abundance of pollinators of each tribe, sociality, and nesting group. Permutational MANOVA (function ‘adonis’ in the vegan package) was used to determine if differences between the states were statistically significant (Anderson, 2001), after verifying the assumption of homogeneity of group dispersion.

To determine the relationship between landscape variables and bee abundance (pooled across species), we used Poisson generalized linear mixed models (GLMMs). We fit separate models for data from Mato Grosso and Texas. We first screened land cover variables for multicollinearity by calculating variance inflation factors (VIFs) using the “vifstep” function in the R package ‘usdm’ (Naimi, 2013). Because land cover variables are inherently related to one another, we chose a conservative threshold of $VIF < 4$ as an indicator of substantial collinearity (Myers,

1990). Of the three variables checked for collinearity in Mato Grosso and Texas (bloom density, semi-natural abundance, and change in semi-natural abundance over a five-year period), none were found to be collinear. To determine the specific aspects of land cover that drive bee diversity, we pooled specimens from the multiple sampling bouts within each site, and calculated species diversity using the Chao diversity metric (Chao, 2005). Chao diversity accounts for the potential role of unsampled species in the estimate of diversity. We used Gaussian generalized linear mixed models (GLMMs) and fit separate models for data from Mato Grosso and Texas.

For both bee abundance and diversity, we included a random intercept per geographic region (within Mato Grosso: Primavera do Leste, Sorriso, Campo Novo do Parecis; within Texas: Woodsboro, Austwell/Tivoli, and Telferner), and included bloom density, semi-natural abundance, and change in semi-natural abundance as fixed effects. To account for differences in sampling effort among sites in Mato Grosso, we included the number of sampling rounds as an offset in this model. We tested for overdispersion for the Poisson GLMMs and found no evidence of over dispersion. We fit the GLMMs with the ‘glmer’ function in the R package lme4 (Bates *et al.*, 2014); and we used the second-order Akaike Information Criterion (AICc, Burnham and Anderson, 2003) to select among all possible combinations of the fixed effects, using the ‘dredge’ function in the R package MuMIn (Barton, 2016).

RESULTS

Bee community sampling

In Mato Grosso, we captured a total of 1,476 bee specimens of 29 species, represented by 12 tribes. The European honey bee, *Apis mellifera*, and the solitary bee, *Melissodes nigroaenea* made up 59% and 27% of total specimens, respectively. Of the remaining bee species, 7 were singletons (only found once), and 4 were doubletons. 69% of specimens from Mato Grosso were social, including honey and stingless bees, 31% were solitary and none of the collected bees were

kleptoparasitic. In terms of nesting, 59% of specimens in Mato Grosso prefer large cavities (> 5 cm), 30% nest in the ground, 9% nest in trees, and less than 1% nest in pithy stems or small cavities (< 5 cm).

In Texas, we captured a total of 601 bee specimens comprised of 45 species, represented by 9 tribes. The European honey bee, *Apis mellifera* and the solitary bee, *Melissodes tepaneca*, made up 27% and 30% of total specimens collected, respectively. *Lasioglossum* specimens of at least 18 morpho-species made up 21% of the Texas specimens. Of the remaining bee species, 20 were singletons, and 7 were doubletons. 31% of specimens from Texas were social, including honey and bumble bees, 69% were solitary, and the remaining bees were kleptoparasitic (0.04%). In terms of nesting, 28% of specimens in Texas prefer large cavities (>5 cm), 67% nest in the ground, 0% nest in trees, and 4% nest in pithy stems or small cavities (<5 cm).

Regional and Historic Land Cover

Mato Grosso sites averaged 1.07 cotton blooms / plant (SE: 0.0219). Regional land cover surrounding sites averaged 24.7 % (SE: 1.2%) semi-natural habitat in the 1.5 km radius buffer. Mato Grosso sites lost on average ~ 4% of semi-natural habitat (SE: 0.5%) between the years 2004 and 2009. Texas sites averaged 2.69 cotton blooms / plant (SE: 0.030). Regional land cover surrounding sites averaged 32.8% (SE: 1.6%) semi-natural habitat in the 1.5 km radius buffer. Texas lost on average ~ 2% of semi-natural habitat (SE: 0.04%) between the years 2006 and 2011.

Statistics

Visualization via NMDS, and the PERMANOVA confirm that the composition of bee tribes, sociality, and nesting preference were statistically different between Mato Grosso and Texas ($df=1$, $r sq=0.14922$, $p\text{-value}=0.001$, $df=1$, $r sq=0.11875$ $p\text{-value}=0.005$ $df=1$, $r sq=0.092$, $p\text{-value}=0.016$, respectively, Figure 3.2 a, b, c).

Exploring our primary question, we found that Mato Grosso bee abundance was positively correlated with bloom density and the abundance of semi-natural habitat within a 1.5 km radius of the site and was negatively correlated with the loss of semi-natural habitat between 2004 and 2009. Texas bee abundance followed a similar pattern, responding positively to bloom density and negatively to the loss of semi-natural habitat between the years 2006 and 2011 (Table 3.3, Figure 3.3).

DISCUSSION

We found significant differences in the composition of tribes, sociality, and nesting preferences of bees visiting cotton flowers in Mato Grosso, Brazil and Texas, USA. Interestingly, despite these differences, bee community abundance in both states responded positively to the same landscape variables. Specifically, we show that bee abundance increased with increasing bloom density in both states. In Mato Grosso and Texas, bee abundance also increased with the abundance of semi-natural habitat within 1.5 kilometers of the sampling point, though this pattern was only statistically significant in Mato Grosso. Further, we show that historic land-use also influenced the abundance of bees in both systems. We found the abundance of bees to decrease with greater losses of semi-natural habitat over a five-year period. In other words, those sites that lost the most semi-natural habitat were among those with the fewest bees. Lastly, none of the landscape variables significantly influenced bee diversity (Chao) in either state. Overall, our results provide evidence that both current and historic aspects of land cover impact the abundance of bee pollinators in the cotton agroecosystems across two distinct biogeographic regions.

Our exploratory analyses of bee communities in each state highlight some of the key differences of the bee community between Mato Grosso and Texas. Mato Grosso had more Augochlorini and Meliponini, than Texas, which had significantly more Emphorini and Halictini. Meliponines, which were abundant in Mato Grosso, are eusocial, stingless bees that can be found

in most tropical and subtropical regions of the world. Brazil is home to several species of stingless bees, with more than 300 species described and probably more yet to be discovered (Pedro, 2014). Meliponines are, on average, small bees, but they vary greatly in shape, size, and habit (Roubik, 1992), and mainly nest in tree cavities, attaining their highest species richness in pristine tropical wet forest (Roubik 2000). Small *Lasioglossum* of the subgenus *Dialictus* likely drove much of the Halictini abundance we observed in Texas. *Dialictus* are well known for their abundance and diversity in temperate regions. Most species are believed to be eusocial or semi-social (Batra 1966; Eickwort, 1986), with colony sizes ranging from small (< 2 workers, Packer, 1992) to large (>100 workers, Michener, 2007) although a few are known to be solitary (Packer, 1994) or communal (Eickwort, 1986).

In comparing nesting preferences, we found that Mato Grosso had more large cavity nesting bee species, while Texas had more wood/stem nesters. This again, is likely related to the high abundance of social meliponine bees in Mato Grosso which prefer to nest in large tree cavities common to parts of the Cerrado and Amazon Forest. In Texas, most small cavity-nesting bees require above-ground, pre-excavated holes in which they provision their young. Above-ground nesting resources are likely to be ample in semi-natural grassland habitats which were more abundant in Texas. Both states had similar abundances of ground-nesting bees, which require exposed soil in which they excavate tunnels (Potts *et al.*, 2005). As a result of tilling and other land management practices, agricultural landscapes in both Texas and Mato Grosso likely have an abundance of exposed soil, catering to ground nesting bees. However, tilling practices may negatively affect ground nesting bees. Specifically, plowing may kill bees, either by destroying nests near the soil surface or by collapsing tunnels above the cells (Shuler *et al.*, 2015; Williams *et al.*, 2010). Reduced tilling practices may allow for the persistence of ground-nesting bees in both of these cotton agroecosystems.

Mato Grosso had more social bees than Texas. While both states had a large proportion of social honey bees, the meliponines of Mato Grosso are all eusocial bees, whereas most non-*Apis* bees in Texas were solitary. Studies of other recently fragmented agricultural landscapes have found that large expanses of tropical cropland tend to be dominated by social bees. These studies suggest that the success of social bees in these novel tropical environments lies in their recruitment-based foraging strategy and versatile nesting preferences (Roubik, 1980; Brosi *et al.*, 2008). It has been hypothesized that these advanced foraging strategies may have evolved for the exploitation of mass-flowering patches, common in tropical forests, and consequently predispose social bees to take advantage of the flowering events that characterize the modern agro-industrial landscape (Raine *et al.*, 2006). Solitary bees on the other hand, may lack this ability to exploit pulses and mass flowering events as they are constrained by their lack of communication. Thus, it may be that the abundance of agricultural cover in the Mato Grosso region predisposes the landscape towards social bees, like Meliponines.

Despite substantial differences in community composition and natural history, we found that bee community abundance responded to bloom cover, land cover, and land cover change in similar ways in Mato Grosso and Texas. Bees in both states responded positively to the density of cotton bloom, becoming more abundant at higher densities of bloom. This finding is an example of the concentration effect as found in other pollination studies (Hegland and Boeke, 2006). As such, flowers that are located in high density patches experience increased visitation. Bee abundance in both states also responded positively to the abundance of semi-natural habitats surrounding sites, the trend being significant in Mato Grosso. Semi-natural habitat has been shown to provide an abundance of nesting resources important to bees, including trees, large and small cavities, and pithy stems (Potts *et al.*, 2005). Several reviews have highlighted that bee abundance responds to the abundance of semi-natural habitat (Ricketts *et al.*, 2008; Garibaldi *et al.*, 2011; Klein *et al.*, 2012). Because bees are central place foragers, the proximity of nesting habitat within

flight range of target crops is essential if land managers hope to take advantage of wild bee pollination services. Lastly, we saw that in both states, bee abundance responded negatively to the loss of semi-natural habitat over a five-year period. Historically, bee pollinators have been thought to respond quickly to habitat loss, reaching a new equilibrium in remnant habitat patches within a couple of years after disturbance. Despite this, along with the results we present here, studies have shown that even mobile species are not immune to the effects of extinction debt (Sang *et al.*, 2010; Bommarco *et al.*, 2014; Cusser *et al.*, 2015).

Interestingly we found that none of our explanatory variables predicted differences in bee diversity in either state. We hypothesize that this has to do with the short temporal survey period (seven weeks) in both systems, as pollinators emerging at different time periods may respond to different land cover cues (Hegland *et al.*, 2009). As such, given our focus on the short cotton-bloom period, our investigation of changes in diversity with changes in land cover may have missed longer-term shifts in bee community composition.

While our study may not have detected changes in bee diversity with land cover, changes in bee abundance have been shown to have important and far reaching effects on service provision in both natural and agricultural systems. When species contribute to community function in proportion to their abundance, as proposed by the mass ratio hypothesis (Grime, 1998), then a small number of dominant species can make a disproportionate contribution to ecosystem function. Indeed, previous research of pollination service provision has shown that the abundance of a few, common, bee species in a community can contribute disproportionately to overall community function (Kleijn *et al.*, 2015; Vázquez *et al.*, 2005). Thus, while it is the many rare species that may drive changes in regional diversity, changes in diversity may have little effect on overall pollination service provision. As such, our finding that bee abundance responds to semi-natural land cover across biogeographical regions has important implications for managing landscapes to promote the provision of important ecosystem services, like pollination.

Overall, despite very different bee communities in the two regions, we found that the same overall factors drive bee community abundance in cotton agroecosystems across the two hemispheres. The density of cotton floral resources, along with the abundance of current and historic semi-natural land cover, are key factors regulating bee abundance in these agriculturally modified landscapes.

CONCLUSIONS

Declines in semi-natural habitat have been shown to be critically linked to biodiversity loss within agricultural landscapes (Benton *et al.*, 2003). Here, we found evidence in support of that claim across two distinct biogeographic regions. Our results indicate that cotton growers may not need to rely solely on regional scale semi-natural reserves to provide pollinator resources. Instead, farmers can contribute to biodiversity conservation by preserving small patches of semi-natural habitat on their own farms (Jha and Vandermeer, 2009) by creating heterogeneous and resource-rich agricultural matrices (Perfecto and Vandermeer, 2008). Specifically, cotton growers can promote bee abundance within their own farms by diversifying their landscapes, creating a mosaic of flowering patches and nesting resources that attract and support foraging bees. Given the benefit of bee pollination to cotton yields (Cusser *et al.*, 2016; Pires *et al.*, 2014), there is great incentive for growers to improve agro-ecosystem management, dually garnering ecosystem services and supporting biodiversity conservation.

Table 3.1: Model summaries for top models about bee abundance in Mato Grosso and Texas

Mato Grosso Bee Abundance	Estimate	Std. Error	Z Value	Pr(> z)
Intercept	-2.52127	1.50727	-1.67	-0.0944
Bloom Density	1.54709	0.08941	17.30	<0.001*
Semi Natural Habitat	1.05701	0.03281	132.22	<0.001*
Change in Semi Natural Habitat	-1.84545	0.03719	-49.62	<0.001*
Texas Bee Abundance	Estimate	Std. Error	Z Value	Pr(> z)
Intercept	3.60043	0.46394	7.761	<0.001*
Bloom Density	0.28344	0.04836	5.861	<0.001*
Semi Natural Habitat	0.05601	0.05124	1.093	0.274
Change in Semi Natural Habitat	-0.25743	0.06499	-3.961	<0.001*

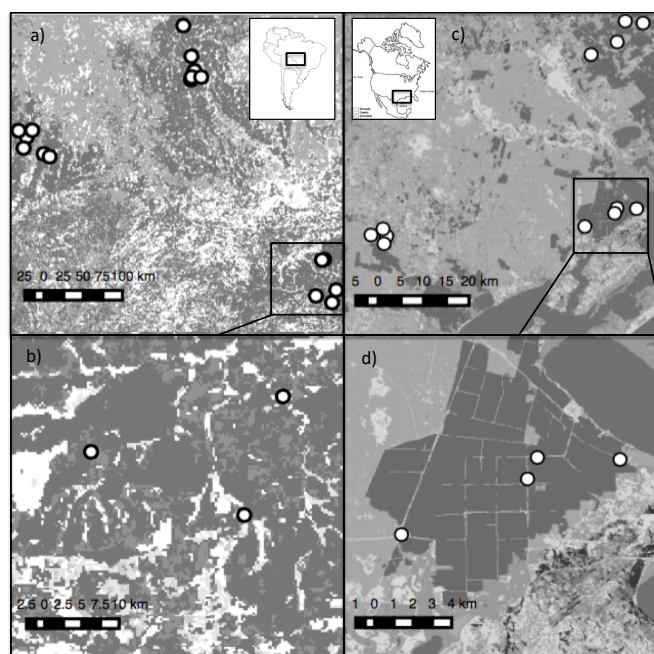


Figure 3.1: Map of study sites. Top Left Inset) South America. a) Map of 17 cotton fields used as study sites in Mato Grosso, Brazil. Sites were located in three geographic areas near the towns of Sorriso, Primavera do Leste, and Campo Novo, Mato Grosso, Brazil. Sites are shown as white dots. b) An enlargement of the Primavera do Leste region showing three cotton field sites. Top Right Inset: North America. c): Map of 12 cotton fields used as study sites. Sites were located in three geographic areas near the towns of Woodsboro, Austwell/Tivoli, and Telferner, Texas, U.S.A. d) An enlargement of the Austwell/Tivoli region showing four cotton field sites.

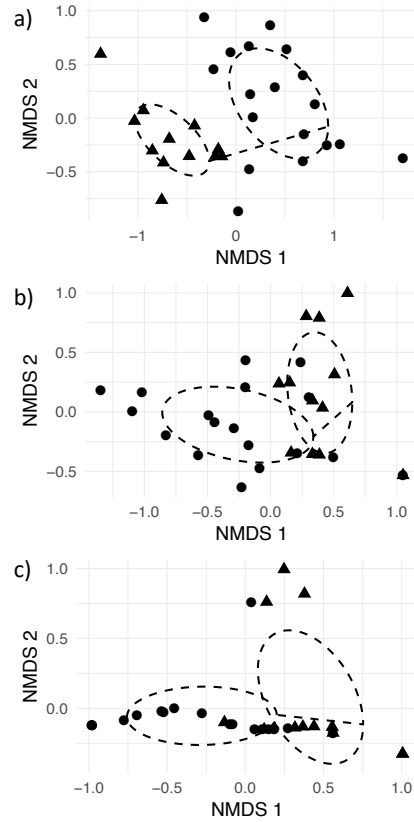


Figure 3.2: Non-metric multidimensional scaling (NMDS) comparing the composition of a) tribes b) sociality c) nesting preferences between Mato Grosso, Brazil and Texas, U.S.A. We calculated Bray–Curtis dissimilarity using the abundance of pollinators of each tribe. Permutational MANOVA was used to determine that differences between states were statistically significant, after verifying the assumption of homogeneity of group dispersion. Sites in Mato Grosso are shown as circles, and Texas as triangles.

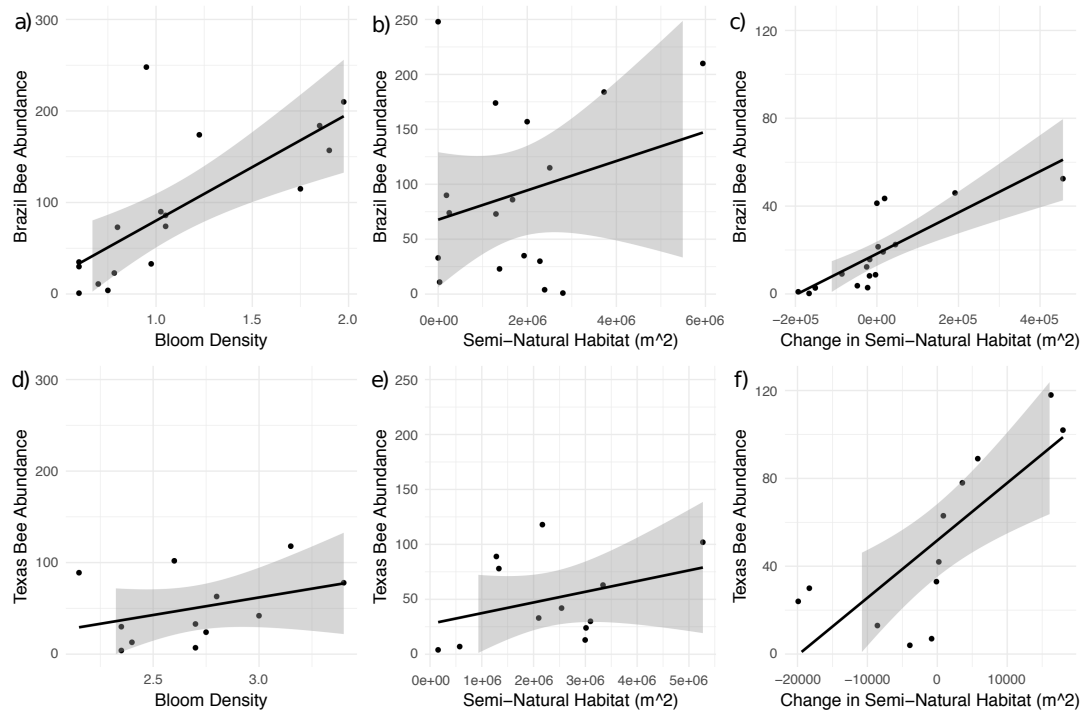


Figure 3.3: Regression plots of Mato Grosso and Texas Bee Abundance and a, d) Bloom density, b,e) Semi-Natural Habitat Abundance, c,f) Changes in Semi-Natural Habitat Abundance. Asterisks denote a significant relationship. NS denotes the lack of a significant relationship.

Chapter 4: Dually improving biodiversity and pollination services for enhanced cotton yields and sustainability

ABSTRACT

Cotton is the most economically and culturally important fiber crop worldwide. Like other self-compatible plants, cotton may potentially benefit from animal mediated pollination, but it is unknown if the species is indeed pollen limited across agro-ecological landscapes. Furthermore, most cotton growers do not manage domesticated pollinators nor employ land management practices that promote the long-term stability of wild pollinator communities in the cotton agro-ecosystem. Our study had three objectives: 1) determine the extent of pollen limitation across a heterogeneous set of cotton-growing landscapes, 2) investigate the relationship between cotton pollen limitation and pollinator community composition and 3) identify the land-use attributes that impact wild pollinator abundance and diversity. To address these objectives, we used a combination of pollen limitation experiments, pollinator community surveys and GIS analysis across 12 cotton landscapes in South Texas. Overall, we found that cotton in this region is pollen-limited, as significantly larger bolls are produced with the addition of outcross pollen. Further, we reveal that pollen limitation was negatively correlated with pollinator abundance and richness. Pollinator community composition was closely related to land-use heterogeneity, a measure of land-use diversity and edge effects. Specifically, land-use heterogeneity at local scales had a significant positive influence on pollinator richness and abundance. Our results reveal great potential for increased crop yields via wild pollinator-mediated fruit set, equivalent to more than 108 \$/acre with a regional gain of over \$1.1 million USD.

Cusser, S., Neff, J. L., & Jha, S. (2016). Natural land cover drives pollinator abundance and richness, leading to reductions in pollen limitation in cotton agroecosystems. *Agriculture, Ecosystems & Environment*, 226, 33-42.
S. Cusser was responsible for designing research, performing research, analyzing data, writing the dissertation and writing the manuscript.

Further, our research provides insight into the specific land management practices that support pollinator communities within cotton agro-ecosystems; landscapes that maintain habitat heterogeneity, including rangeland, fallow hedgerows, and/or riparian strips, in addition to cotton fields, promote wild pollinator abundance and diversity, and subsequently experience reduced pollination limitation and increased local crop yields.

INTRODUCTION

Given the rapidly expanding human population, it is estimated that by the year 2050 we will be challenged to provide fiber, food, and fuel for ~9.6 billion world inhabitants (United Nations News Centre, 2013). One proposed solution to this problem is to increase the intensity and homogenization of agricultural and forestry landscapes (Robinson and Sutherland, 2002; Benton *et al.*, 2003). Although landscape homogenization has the potential to increase crop yield and efficiency (Green *et al.*, 2005), increased agricultural intensity is also irrefutably one of the main causes of biodiversity loss (Adger *et al.*, 2002). As a result, within intensely managed, homogeneous agricultural landscapes, yields often increase at the expense of biodiversity. Some insects comprise an economically important group of biodiversity in agricultural systems as they provide ecosystem services critical to human survival; these organisms may act as natural enemies to crop pests or provide pollination services that benefit yield (Daily, 1997; Losey and Vaughn, 2006). One particularly important group of beneficial insects are the pollinators, such as bees, butterflies and flies, which move pollen between plants, and increase yield and quality in many crops (Kevan *et al.*, 1990). More than 60% of world crops benefit from animal pollination service (Klein *et al.*, 2007), worth an estimated \$300 million annually (Gallai *et al.*, 2009). As land-use intensification increases, however, and beneficial insect populations decline (Benton *et al.*, 2003), services provided by these beneficial insects may be lost, negatively affecting yield (Elmqvist and

Maltby, 2010), and potentially undermining the ecosystem processes on which these agro-ecosystems rely (Tscharntke *et al.*, 2012).

Thus, although agriculture and biodiversity conservation may have traditionally been viewed as incompatible (Mittermeier *et al.*, 2003), the two should be mutually considered in order to maximize long-term yields and promote the preservation of ecosystem services. Past research investigating this subject has taken place largely in low intensity agriculture and polycultural systems such as shade coffee and cacao (Giller *et al.*, 1997; Tscharntke *et al.*, 2005; Schroth and Harvey, 2007; Firbank *et al.*, 2008). These studies have shown that diversified agricultural practices can promote the establishment and long-term stability of biodiversity to enhance ecosystem services and aid in biodiversity conservation (Estrada and Coates, 2002; Daily *et al.*, 2003; Mayfield and Daily, 2005) while also contributing to increased crop production and rural income (Pretty *et al.*, 2003). However, critics to this approach claim that it is largely relevant only in polycultural settings rather than in highly intensified agro-ecosystems (Green *et al.*, 2005). Thus, at present it is not known if the dual optimization of biodiversity and crop yields is achievable in highly intensified agricultural landscapes.

Worldwide, cotton is one of the most intensely managed and economically important agro-ecosystems (*Gossypium spp.* (Malvaceae)). The industry generates more than \$1 billion USD per annum and employs over 200,000 people in the US alone (USDA ERS, 2013). Worldwide, although cotton covers only 2.5% of cultivated land, cotton growers use 16% of the world's pesticides, more than any other single crop (Environmental Justice Foundation, 2007), making it one of the most intensely managed crops in the world. Despite their intensive management, cotton agro-ecosystems can host a wide range of beneficial insects, including lacewings, ladybird beetles, and spiders (Eyhorn *et al.*, 2005). Furthermore, the large flowers of cotton, which produce copious amounts of pollen and nectar (Free, 1970), can serve as a food resource for a diverse group of pollinating insects (Moffett *et al.*, 1976; Berger *et al.*, 1988; Pires *et al.*, 2014). Although cotton is

known to be self-compatible, previous studies suggest that it benefits from pollination service (Free, 1993; Rhodes, 2002) because cotton pollen is too heavy to move between flowers without an insect vector (Free, 1993). Despite the likely importance of pollinators in cotton, cotton growers do not currently utilize managed pollinators (e.g., honey bees or bumble bees) nor do they use agricultural practices that promote the visitation of wild pollinator communities in the southern US (Delaplane *et al.*, 2010).

The composition of wild pollinator communities may be particularly critical within agro-ecosystems because pollination service stability is often associated with pollinator diversity and abundance (Garibaldi *et al.*, 2013). Pollinator diversity, in particular, appears to enhance the resilience and security of pollination ecosystem services, especially in the face of regional land-use change (Peterson *et al.*, 1998). Because of natural fluctuations in pollinator populations, the diversity of wild pollinator communities is important in providing stable crop pollination service between years (Williams *et al.*, 2001). Specifically, pollinator diversity can buffer pollination services against asynchronous fluctuations in single pollinator species over time (Williams *et al.*, 2001). Mechanistically, greater fruit set observed in more diverse pollinator communities is attributable to greater pollination functional diversity across both space and time (e.g., Hoehn *et al.*, 2008).

Finally, pollinator community composition and pollination service assessment should be considered at multiple spatial scales because many insects are highly mobile, and often respond to land-use change in areas that consist of multiple habitat types (Turner, 1989; Dunning *et al.*, 1992). Land-use at the regional landscape scale can be characterized by changes in the diversity of habitats, and the size and spatial arrangement, or complexity, of those habitats across the region (Gustafson, 1998). Recent studies have also demonstrated a strong relationship between landscape complexity and the abundance and diversity of insect pollinators (Steffan-Dewenter *et al.*, 2002). This is probably due to the fact that many pollinator species have different requirements with

respect to foraging and nesting resources, which are likely to occur in spatially separated habitats within a specific foraging range (Westrich, 1996). Therefore, in addition to the area of suitable local and regional habitat, the diversity and arrangement of habitat types is an important factor in determining pollinator abundance and diversity in human modified landscapes (Wiens *et al.*, 1985; McCoy *et al.*, 1986; Turner and Bratton, 1987; Steffan-Dewenter *et al.*, 2002). Further, past work has revealed that changes in the abundance of particular land-use types can have important impacts on pollinator communities. For example, increasing isolation from natural habitats has been found to be strongly associated with decline in crop pollination (reviewed in Ricketts *et al.*, 2008).

To determine how land management practices effect pollinator community composition and the pollination service provided across cotton agro-ecosystems, we examine three fundamental hypotheses: first, cotton yield is limited by the pollination service it receives. Given the prevalence of monoculture-style land management across cotton-growing regions, we expect to find evidence of pollen limitation and predict yield increases with manual cross-pollination. Second, we hypothesize that pollen limitation is lower in the presence of abundant and diverse pollinator communities. If cotton is indeed pollen limited, we predict that the degree of limitation is negatively associated with the local abundance and richness of pollinators. And lastly, we hypothesize that greater natural land cover and land-use heterogeneity will lead to a greater richness and abundance of wild pollinator communities, and thus predict that pollinator species richness and abundance would respond positively to natural habitat cover and landscape heterogeneity at local and regional scales.

METHODS

Study System

Texas grows more than 25% of the total US cotton crop, and cotton covers roughly 6 million acres of farmland (4%) in the state. In Texas, cotton is grown in four major regions: South

Texas, the Blacklands and North Texas, El Paso, and West Texas. We conducted our research in the South Texas region, where cotton is a primary crop and one of the only crops that offers nectar and pollen resources to potential insect foragers in the area (National Cotton Council of America, 2014). The South Texas region is responsible for about 15% of the annual Texas cotton crop and grows primarily Upland cotton varieties (*Gossypium hirsutum* L. (Malvaceae)). The region is characterized by shrink-and-swell clay soils and its agriculture is predominantly rain fed. In addition to cotton, sorghum, corn, and soybean are also grown in the region (total agriculture regionally is 55%). Cattle ranching (36%), a few dispersed low-density developed areas (6%) and natural areas, including shrub, mixed woodland, and marsh areas along the gulf coast (2%), make up the remainder of the region.

We conducted research in 12 sites located in three regions between Telferner (28.847913, -96.892975) and Woodsboro, Texas (28.303701, -97.381612). We chose sites in an effort to include a wide range of landscape-level habitat heterogeneity, while controlling for geographic region. Specifically, the three geographic regions of study were near the towns of Woodsboro, Austwell/Tivoli, and Telferner, Texas. Within each region, four cotton landscapes were chosen that were primarily comprised of one of four habitat types: Natural Areas, Rangeland, Developed, and Agricultural areas. Within each landscape, sites were located within cotton fields that were at least 35 ha in size and 2 km apart from each other (Figure 4.1). Sites were not sprayed with any chemical five days prior to, nor during, the 16-day sampling period.

Pollen Limitation Experiment

First, we conducted a pollen limitation experiment at each of the 12 sites. Early in the flowering season (June 13th-15th, 2014), 10 virgin flowers were exposed to one of 5 treatments: closed (C), closed self-cross (CS), closed outcross (CO), open (O), and open outcross (OO), for a total of 50 experimental flowers at each site. Throughout the flowering period, closed (C) flowers

remained bagged with lightweight white organza bags that excluded pollinators. Closed self-cross flowers (CS) were bagged but supplemented with self-cross pollen from the same flower, and closed outcross (CO) flowers were bagged but received outcross pollen from three to five donors within a 10 m radius of the focal plant. These three treatments evaluate the importance of outcross pollen to seed/fruit production as conducted in other systems (Parker, 1997; Yang *et al.*, 2005; Benjamin *et al.*, 2014). The fourth and fifth treatments were left unbagged throughout the flowering period to receive ambient pollination service: open flowers (O) measured the amount of ambient pollination service and open outcross flowers (OO), remained open but were also supplemented with an abundance of additional outcross pollen from three to five donors within a 10 m radius of the focal plant, saturating the stigma, and thus representing maximum pollination service. From the fourth and fifth treatments, we evaluated potential limitation in boll set resulting from inadequate pollination service. All pollination treatments happened between the hours 0800 and 1300, when cotton flowers are most receptive (Free, 1993). Bolls were left to mature for six weeks in the field, were hand-collected, and were then assessed for seed-cotton weight and seed number per boll. Seed-cotton weight is the total weight of both the lint and seeds of a single cotton boll, and was used as a metric of yield. Pollen limitation was measured as the difference in seed-cotton weight, or number of seeds per boll, between open outcross (OO) treatment flowers and open (O) flowers that received ambient pollination service (as per Klein *et al.*, 2003; Groeneveld *et al.*, 2010; Holzschuh *et al.*, 2012; Benjamin *et al.*, 2014). To test for differences between treatments, we used a Shapiro test to check for a normal distribution of seed-cotton weight and seeds per boll, then ANOVA and Tukey HSD tests to determine significant differences in seed-cotton weight and seeds per boll between treatments.

Pollinator Community Composition

We also performed insect sampling to quantify the pollinator community at each site. Here, we netted insects found actively foraging within cotton flowers during three one-hour sampling bouts at each site. During each one-hour bout, a single collector walked four parallel 100 m x 1 m transects checking blooms for visitors. Transects were located 25 m apart, representing an area of 7500 m². The three sampling sessions were at least five days apart and covered the peak of cotton bloom in 2014 (June 16th- July 2nd). Because we only collected insects actively foraging within flowers, we consider all specimens as potential pollinators. Time spent moving specimens from the net into the killing jar was not included in the sampling time to ensure an even sampling effort between sites. Pollinators were placed into jars with ethyl acetate until the end of the sampling period and then transferred into individual vials of ethanol. From these samples, we determined pollinator richness and abundance at each site. We used linear regression to determine the relationship between pollinator abundance and richness and the extent of pollen limitation at each of our sites (see section ‘Pollen Limitation Experiment’, above).

Land-use Analysis

Finally, we analyzed land-use surrounding sites at local (250 m radius) and regional (1 km radius) scales using information from the 2011 National Land Cover Database (Jin *et al.*, 2013). Land-use was analyzed in two ways. First, we calculated the percentage of land-use types (26 types total) surrounding each site grouped into four broad categories: Agricultural, Developed, Natural, and Rangeland at local and regional scales (Agriculture: row crops including corn, sorghum, and soy as well as cotton itself. Developed: any man-made surface. Rangeland: pasture, hay, and grassland. Natural: riparian forest, deciduous and evergreen forest, and scrubland). Next, we analyzed land-use heterogeneity surrounding each of our sites. As per Turner (1989), heterogeneity was calculated as the total edge distance, or perimeter, between the different 26 land-use types. Other indices of heterogeneity, like habitat richness or diversity, do not capture the added edge

distance and complexity created by small patch sizes. Thus, in this study, a landscape with a few land-use types and minimal edge between types would have low heterogeneity, while a landscape with a large number of different land-use types and a maximum edge between types would have high heterogeneity.

To determine the relationship between land-use and pollinator abundance and richness, we used linear mixed models using geographic region (Woodsboro, Austwell/Tivoli, and Telferner) as a random effect, and land-use variables at local and regional scales (percent Agriculture, Developed, Rangeland, Natural, and Heterogeneity) as fixed effects. Local and regional land-use variables were not correlated and were explored in a single model. Values were shifted to have a mean of zero to account for differences in magnitude between fixed effects.

RESULTS

Pollen Limitation Experiment

We found that flowers receiving outcross pollen had heavier bolls with more seeds than either flowers receiving no pollen or self-cross pollen (Figure 4.2 and 4.3: C, CO, CS). Also, our results revealed that flowers supplemented with additional outcross pollen produced bolls that were significantly heavier with more seeds than those flowers receiving ambient pollination service (Figure 4.2 and 4.3: O, OO) (Seed-cotton weight: f -value = 47.86, p -value < 0.001; Seeds per boll: f -value = 24.11, p -value < 0.001). Outcross pollen increased seed-cotton weight by 18%, and the number of seeds per boll by 17% in this comparison. The closed outcross (CO) treatment was statistically indistinguishable from the open outcross (OO) treatment, revealing that hand-pollination resulted in adequately and effectively pollinated flowers with no negative effect of the closed bagged treatments on fruit set (Figure 4.2 and 4.3). We also found a great deal of variation in the degree of limitation between sites (Figure 4.4). Although most sites were pollen limited, producing bolls between 0.3 grams to nearly 4 grams heavier in seed-cotton weight with the

addition of outcross pollen, two sites produced smaller bolls on average and revealed no pollen limitation.

Pollinator Community Composition:

We captured a total of 841 pollinator specimens of 52 species. The specimens were of four orders: Hymenoptera (37 species), Diptera (5 species), Lepidoptera (7 species), and Coleoptera (3 species). The solitary bee species, *Melissodes tepaneca*, made up 21% of the specimens, and the European honey bee, *Apis mellifera*, made up 19%. The syrphid fly, *Allograpta exotica*, comprised another 16% of the specimens. Lastly, *Lasioglossum* specimens of at least nine species made up 15% of the specimens. Of the remaining pollinators, 24 were singleton species, and 10 were doubletons (Table 4.1).

Statistically, there was a significant negative relationship between pollinator abundance and the degree of pollen limitation and a marginally significant negative relationship between richness and the degree of pollen limitation (Abundance: t -value = -2.551, p -value = 0.03; Richness: t -value = -2.006, p -value = 0.06). Sites suffering least from pollen limitation were among those with the most abundant and rich pollinator communities (Figure 4.5).

Land-use Analysis

We found no strong association between any single type of land-use, either locally or regionally, and aspects of the pollinator community, either abundance or richness. There was however, a strong significant positive relationship between local habitat heterogeneity and both pollinator abundance and richness (Figure 4.6) (Abundance: t -value = 4.087, p -value < 0.001; Richness: t -value = 4.236, p -value < 0.001). Those sites located in locally heterogeneous landscapes hosted the greatest richness and abundance of pollinators.

DISCUSSION

In this study we document substantial pollen limitation across cotton agro-ecological landscapes. Additionally, we reveal a direct negative relationship between the extent of pollen limitation and the abundance and richness of the local pollinator community, providing strong support for two of our three initial hypotheses. We found partial support for our third hypothesis by documenting a positive relationship between local land-use heterogeneity and pollinator abundance and richness, but no relationship between the percent of local or regional natural habitat and the pollinator community.

First, we provide clear evidence that outcross pollen improves boll set and show that cotton yield in our study system is indeed limited by the pollination service it receives. Thus, our pollen limitation findings offer evidence that crop yields in South Texas cotton agro-ecosystems can be increased through the improved management of native pollinators. Although only a few of the world's crops are completely dependent on animal pollination (Richards, 2001), most have been found to exhibit yield benefits from pollination service (reviewed in Klein *et al.*, 2007). Specifically, a meta-analysis shows that 39 of the leading 57 world crops exhibit increased yield with services provided by pollinating animals (Klein *et al.*, 2007); though it is the most valued non-food crop on the planet, cotton was not on this list. Our results reveal cotton growers could gain as much as an 18% increase in seed-cotton weight and a 17% increase in seeds per boll with increased pollination service. Given the average production of 826lbs of cotton per acre in the U.S. and the current price of cotton, this increased production could be estimated to be valued at as much as 108 \$/acre with a regional gain of over \$1.1 million USD (National Agriculture Statistics Service, 2013). Our findings, and those of Pirens *et al.* (2014), are the first to quantify pollinator service contribution to cotton.

Second, we show that pollen limitation is closely linked to local pollinator abundance and richness, a pattern also detected in native plant communities (reviewed in Ashman *et al.*, 2004) as

well as crop systems (reviewed in Garibaldi *et al.*, 2013). Indeed, diverse pollinator assemblages have been shown to maximize crop yields, compared to the abundance of a single pollinator species (e.g., the European honey bee, *Apis mellifera*) (e.g., Klein *et al.*, 2003; Hoehn *et al.*, 2008; Winfree and Kremen, 2009; reviewed in Garibaldi *et al.*, 2013), and help to ensure consistent pollination service over multiple years, even in the face of natural fluctuations in the abundances of some species (Kaiser-Bunbury *et al.*, 2010).

Looking more closely at the composition of our pollinator community, we found that the majority of our pollinators were of three species: *Melissodes tepaneca* (Hymenoptera), *Apis mellifera* (Hymenoptera), and *Allograpta exotica* (Diptera). Interestingly, these three species vary widely in their life history, which has important implications for how they respond to agricultural land-use change. The most abundant pollinator, *Melissodes tepaneca*, is a native ground nesting solitary bee, whose females singly excavate cavities in bare soil, lay eggs and provision their young (Michener, 2007). In contrast, the social European honey bee, *Apis mellifera*, lives exclusively in large and complex social nests with overlapping generations and a division of labor. Both bees are central place foragers, and forage within some specific distance from their nest site, and thus are only able to exploit floral resources within that spatial range. The native hover fly species, *Allograpta exotica*, however, is not a central place forager nor does it directly provision its young with nectar and pollen. Rather, *Allograpta* adults move through landscapes, visiting flowers to drink nectar, and lay eggs on larval host plants where the larvae develop and feed on aphids and other crop pests (Bugg, 2008). Given that these three pollinators have vastly different life history strategies, land management practices are likely to differentially affect their population dynamics. For instance, deep tillage, a practice popular in cotton, could have devastating effects on ground nesting bee populations (Shuler *et al.*, 2005), such as those of *Melissodes*, (nest depths of *M. tepaneca* are unknown, however all known *Melissodes* nests are relatively shallow (3-18 cm, n=7, Cane and Neff, 2011) but would have no effect on either *Apis* or *Allograpta* species. On the

other hand, pesticides sprayed onto crop plants during the larval development of *Allograpta*, may negatively affect fly populations (Moens *et al.*, 2011). Given that pollinator diversity is important to crop yield, we argue that the development of land management practices, like low till and low pesticide application, must be kept in mind to preserve ecosystem service provision.

Addressing our final hypothesis, we show that land-use heterogeneity is an important driver of pollinator community composition, and consequently impacts the services provided within the cotton agro-ecosystem. Agricultural intensification in modern landscapes, where fields are amalgamated and enlarged to enhance farming efficiency, results in homogeneous landscapes with little to no non-crop area. The homogenization of agricultural habitats may be negatively affecting pollinators in two major ways. First, pollinators may exhibit decline due to a reduction in overall resource diversity. It is well supported that landscape diversity creates a wider array of foraging niches for different functional groups of pollinators (Fenster *et al.*, 2004) and environmental changes that alter the spatial and temporal distribution of important nesting and food resources will influence pollinator community composition (reviewed by Kremen *et al.*, 2002; Williams *et al.*, 2010). Second, the homogenization of habitats may alter the ability of pollinators to move across landscapes. Because many invertebrate pollinators must forage across multiple land-use types to exploit resources important for their livelihood (e.g. food or nesting resources), homogenized landscapes may actually prohibit the persistence of some pollinators unable to travel long distances between resources in homogenous landscapes that function as only “partial landscapes” (Westrich, 1996). In addition to foraging movement, homogenization may negatively affect pollinator populations by reducing the likelihood of dispersal and recolonization events. Because pollinator populations naturally fluctuate over time, pollinator assemblages are characterized by rare local extinction events. Thus, a patchwork of high quality habitats, even as small remnants or restored hedgerows, could provide refuge and sources of recolonization for pollinators into newly disturbed sites, thus reducing the negative influence of local extinction

events over time (Perfecto and Vandermeer, 2010; Tschardtke and Brandl, 2004; Steffan-Dewenter *et al.*, 2007).

Understanding the importance of heterogeneity in supporting biodiverse communities is especially relevant given the current trajectory of global farming towards homogeneous landscapes (Robinson and Sutherland, 2002). As the demands on agricultural lands to produce fiber, food, and fuel continue to expand, effective strategies are needed to balance biodiversity conservation and agricultural production. Two approaches, with opposing strategies but similar goals, have been proposed to approach this problem: land sharing and land-sparing. The land sparing approach promotes smaller, more intensely farmed, homogeneous areas to maximize yields, while separate reserves target biodiversity conservation. The argument for land-sparing is rooted in the increased efficiency and productiveness it supposedly affords in intensely farmed landscapes, while preserving remaining wild areas exclusively for biodiversity (Balmford *et al.*, 2005; Green *et al.*, 2005). Land sharing, in contrast, integrates conservation and agriculture within more heterogeneous landscapes, relying on farming practices that benefit wildlife and, presumably, biodiversity in general. Typical characteristics of land-sharing landscapes include patches of native remnant or restored vegetation scattered throughout the productive landscapes, agricultural areas that are structurally similar to native vegetation, and overall spatial heterogeneity (Luck and Daily, 2003; Fischer *et al.*, 2006; Perfecto and Vandermeer, 2008). In this way, heterogeneity is maintained by planting a diversity of crops in a range of small fields, retaining habitat features within fields (e.g. scattered trees), or conserving habitat features along the margins of fields (e.g. hedgerows: Benton *et al.*, 2003; Harvey *et al.*, 2006; Manning *et al.*, 2006).

In this study, we offer evidence that landscapes with higher levels of local heterogeneity positively affect total yield via the pollination services they receive from beneficial insects. We argue that management objectives that promote land-use heterogeneity can be developed and widely applied across a variety of agricultural systems (Benton *et al.*, 2003). Interestingly, we

found that local (250m radius) heterogeneity was more important than regional (1km radius) heterogeneity in driving pollinator richness and abundance. This result has been documented in other agro-ecosystems (e.g., Jha and Vandermeer, 2010) and underscores the importance of local, small-scale restoration efforts for the conservation of diverse pollinator populations. These findings are encouraging, given that even small changes to local land-use may have far reaching beneficial effects for the pollinator community, even within highly homogeneous regional landscapes.

CONCLUSIONS

This research is one of the first to document the entire set of interactions between pollination service provision, pollinator community composition, and landscape drivers of biodiversity within a single study, and the first to do so within the cotton agro-ecological landscape. Further, these results provide compelling evidence that the cotton agro-ecosystem can serve as a novel habitat in which to dually improve biodiversity while also enhancing pollination services and yield. Our work has on-the-ground implications for crop management and, more generally, offers insights into conservation policy. Given the immense scale of the cotton agro-ecosystem, the benefits it receives from animal-mediated pollination services, and its potential to provide important forage for a wide range of insects, we propose that cotton is an ideal system in which to promote the conservation of pollinator diversity and simultaneously increase crop yields. Through these combined efforts, it may be possible to provide cotton for our growing global population, while increasing the ecological sustainability of the fiber that touches all our lives.

SUPPLEMENTAL MATERIAL

Table 4.1: Pollinator species list organized by order

Hymenoptera		
	<i>Agapostemon</i>	<i>splendens</i>
	<i>Agapostemon</i>	<i>texana</i>
	<i>Ancyloscelis</i>	<i>apiformis</i>
	<i>Apis</i>	<i>mellifera</i>
	<i>Augochlorella</i>	<i>bracteata</i>
	<i>Augochloropsis</i>	<i>metallica</i>
	<i>Bombus</i>	<i>pensylvanicus</i>
	<i>Ceratina</i>	<i>shinnersi</i>
	<i>Coelioxys</i>	<i>slossoni</i>
	<i>Colletes</i>	<i>mandibularis</i>
	<i>Diadasia</i>	<i>enavata</i>
	<i>Dianthidium</i>	<i>subrufulum</i>
	<i>Dieunomia</i>	<i>heteropoda</i>
	<i>Halictus</i>	<i>ligatus/poeyi</i>
	<i>Lasioglossum</i>	<i>coactum</i>
	<i>Lasioglossum</i>	<i>connexum</i>
	<i>Lasioglossum</i>	<i>sp. 1</i>
	<i>Lasioglossum</i>	<i>disparile</i>
	<i>Lasioglossum</i>	<i>fedorense</i>
	<i>Lasioglossum</i>	<i>hunteri</i>
	<i>Lasioglossum</i>	<i>tegulare</i>
	<i>Lasioglossum</i>	<i>TX 16</i>
	<i>Megachile</i>	<i>albitarsis</i>

Table 4.1 Continued

	<i>Megachile</i>	<i>brevis</i>
	<i>Megachile</i>	<i>deflexa</i>
	<i>Megachile</i>	<i>montivaga</i>
	<i>Megachile</i>	<i>parallela</i>
	<i>Melissodes</i>	<i>tepaneca</i>
	<i>Melitoma</i>	<i>marginella</i>
	<i>Ptilothrix</i>	<i>bombiformis</i>
	<i>Svastra</i>	<i>atripes</i>
	<i>Svastra</i>	<i>obliqua</i>
	<i>Svastra</i>	<i>petulca</i>
	<i>Triepeolus</i>	<i>concavus</i>
	<i>Xylocopa</i>	<i>micans</i>
	<i>Xylocopa</i>	<i>virginica</i>
	<i>Polistes</i>	<i>sp. 1</i>
Diptera		
	<i>Allograpta</i>	<i>exotica</i>
	<i>Hermetia</i>	<i>illucens</i>
	<i>Dioprosopa</i>	<i>clavata</i>
	<i>Palpada</i>	<i>furcata</i>
	<i>Palpada</i>	<i>mexicana</i>
Coleoptera		
	<i>Diabrotica</i>	<i>undecimpunctata</i>
	<i>Coccinellidae</i>	<i>sp. 1</i>
	<i>Coccinellidae</i>	<i>sp. 2</i>

Table 4.1 Continued

Lepidoptera	<i>Brephidium</i>	<i>exile</i>
	<i>Eurema</i>	<i>lisa</i>
	<i>Libytheana</i>	<i>carinenta</i>
	<i>Lerodea</i>	<i>eufala</i>
	<i>Phyciodes</i>	<i>tharos</i>
	<i>Pyrgus</i>	<i>communis/albescens</i>
	<i>Strymon</i>	<i>melinus</i>

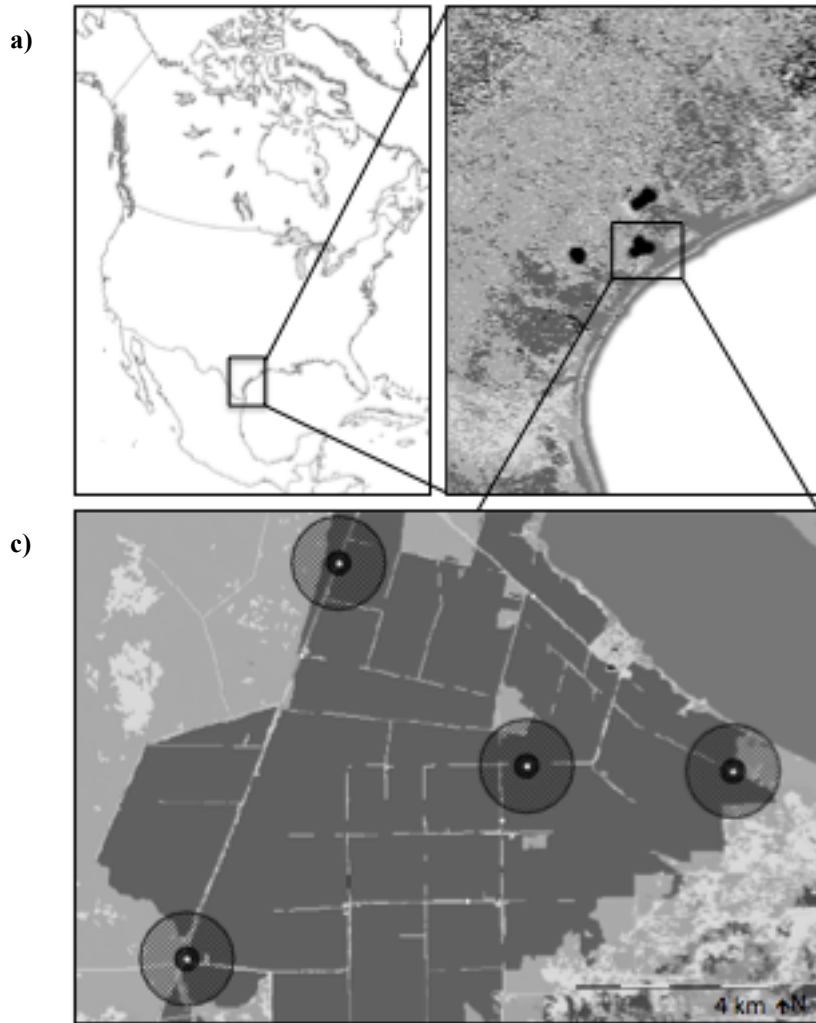


Figure 4.1: Map of study sites. a) North America. b) Map of 12 conventional cotton fields used as study sites along the South Texas Gulf Coast. Sites were located in three geographic areas near the towns of Woodsboro, Austwell/Tivoli, and Telferner, Texas, USA. c) Within each region, four cotton field sites were chosen that were primarily surrounded by one of four habitat types: Developed (white), Natural Areas (light grey), Rangeland (medium grey), and Agriculture (dark grey). Sites were at least 35ha in size and 2km apart from each other. White dots represent sampling sites, dark buffers represent the 250m radius local habitat, and hatched buffers represent 1km radius regional habitat.

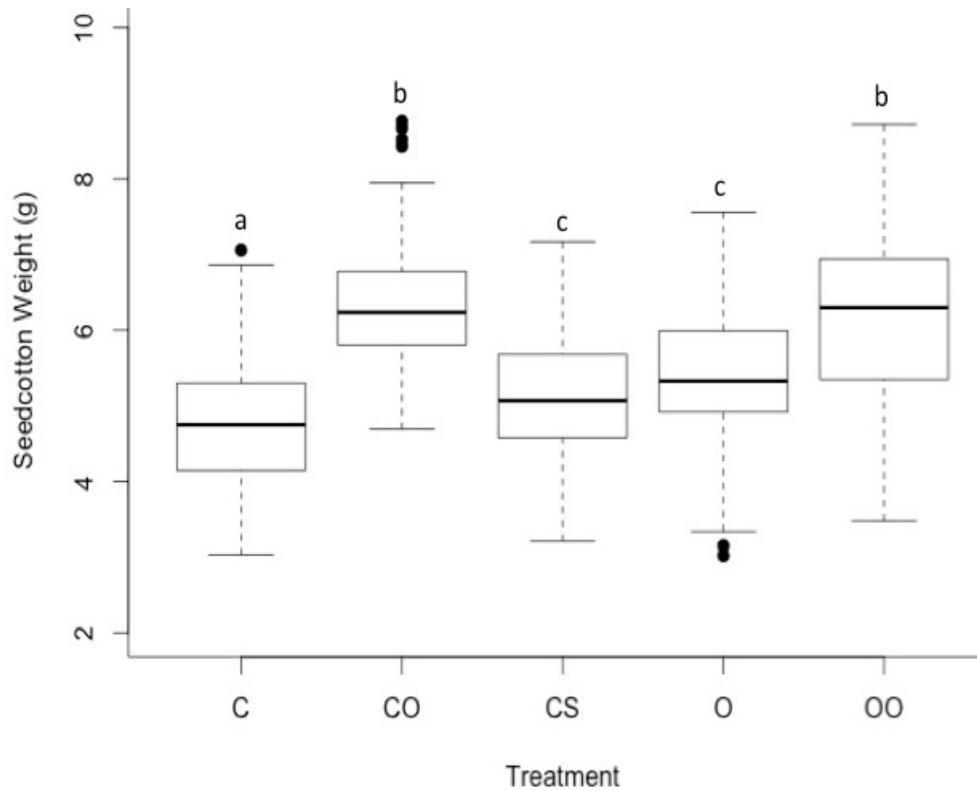


Figure 4.2: Boxplot comparing seed-cotton weight between the five pollen limitation treatments: closed (C), closed outcross (CO), closed self-cross (CS), open (O), and open outcross (OO). Closed (C) flowers remained bagged with lightweight white organza bags throughout the flowering period, closed out (CO) flowers were bagged but received outcross pollen from 3-5 donor flowers in the vicinity, closed self-cross flowers (CS) were bagged but supplemented with self-cross pollen, open flowers (O) remained unbagged to measure the amount of ambient pollination service, and open out cross flowers (OO) remained open but were also supplemented with an abundance of additional outcross pollen. The central bar gives treatment mean, boxes give the 1st and 3rd quartiles, and closed circles show outliers. ANOVA and Tukey HSD statistics were used to compare seed-cotton weight between treatments. Significance is shown with letters.

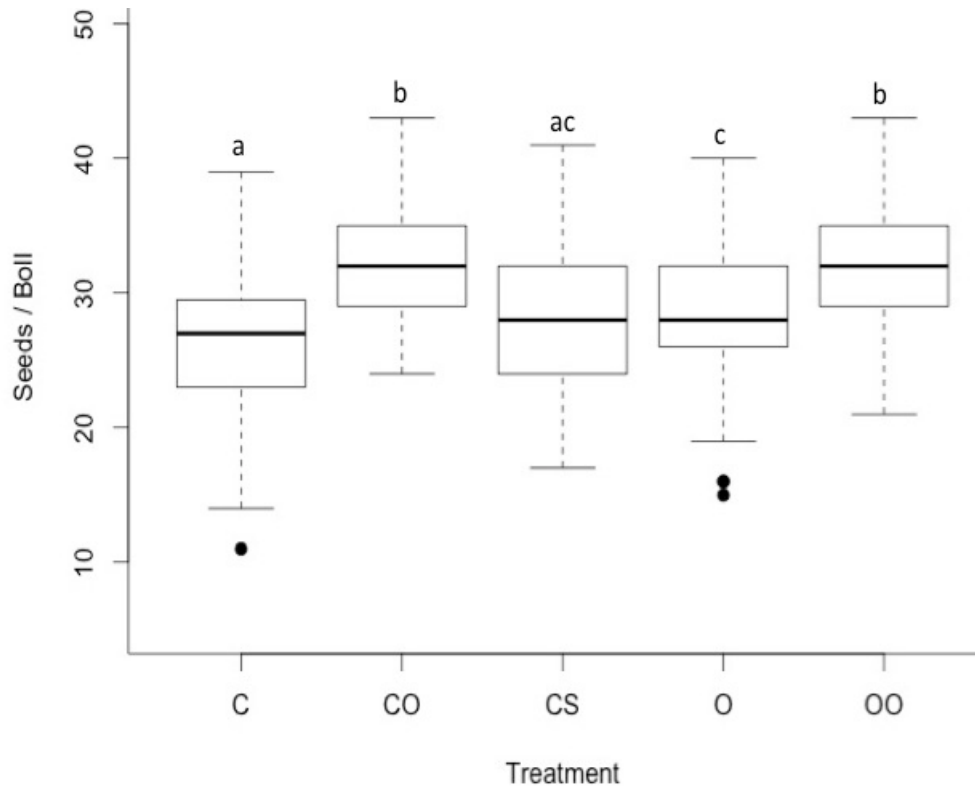


Figure 4.3: Boxplot comparing the number of seeds per boll between the five pollen limitation treatments: closed (C), closed outcross (CO), closed self-cross (CS), open (O), and open outcross (OO). The central bar gives treatment mean, boxes give the 1st and 3rd quartiles, and open circles show outliers. ANOVA and Tukey HSD statistics were used to compare seeds per boll between treatments. Significance is shown with letters.

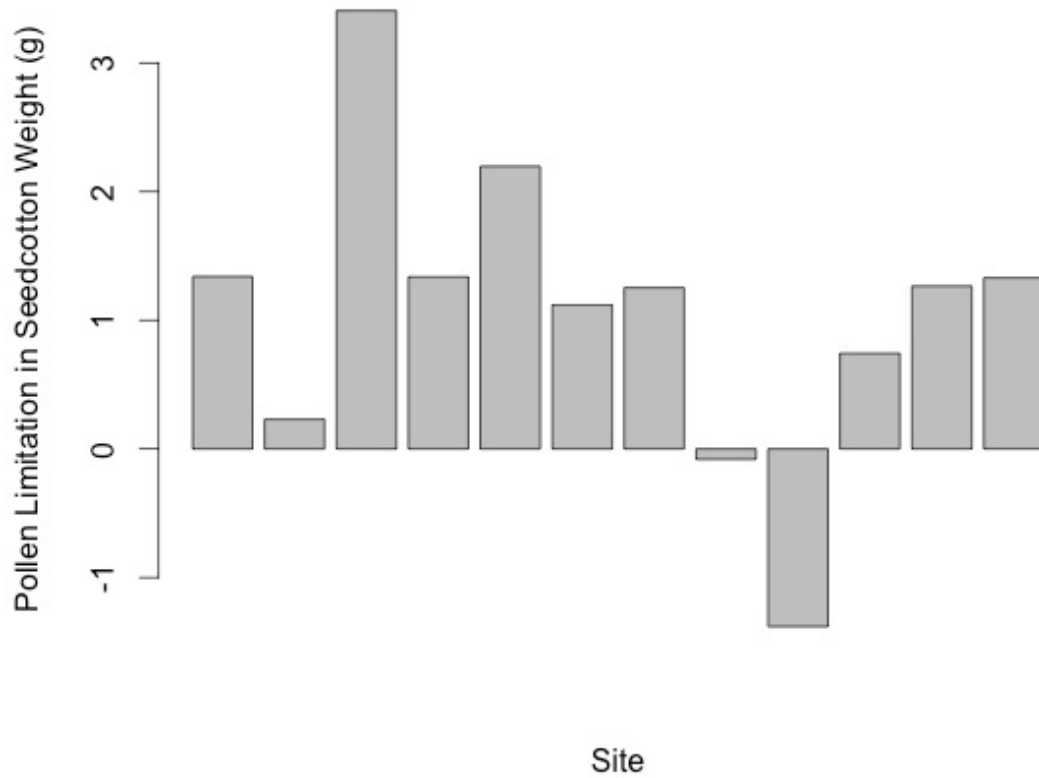


Figure 4.4: Barplot showing the variability of pollen limitation between sites. Pollen limitation is determined as the difference in seed-cotton weight between the open outcross (OO) pollen treatment and the open (O) pollen treatment.

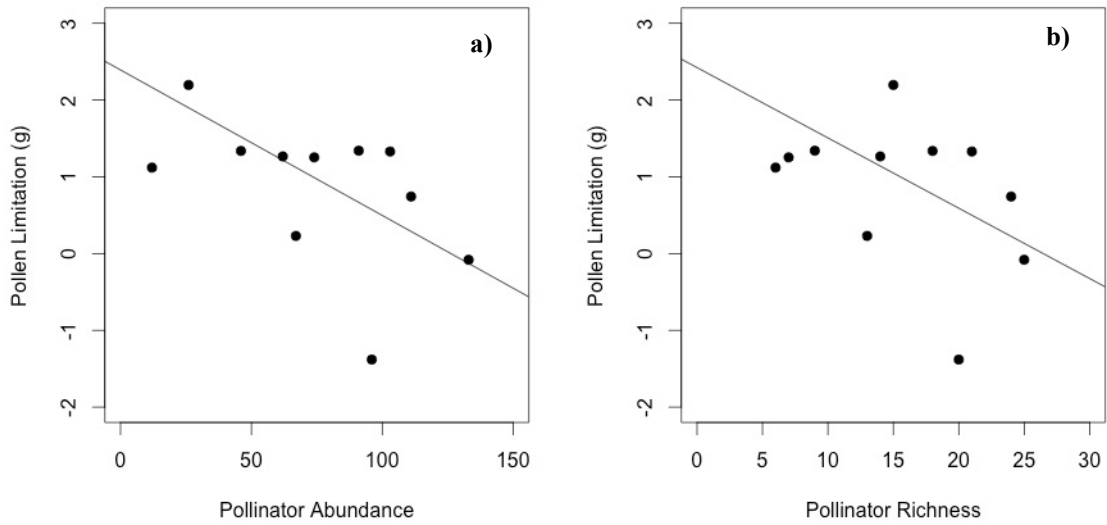


Figure 4.5: (a) Linear regression showing the negative relationship between pollinator abundance and pollen limitation in seed-cotton weight. (b) Linear regression showing the negative relationship between pollinator richness and pollen limitation in seed-cotton weight. Pollen limitation is determined as the difference in seed-cotton weight between the open outcross (OO) pollen treatment and the open (O) pollen treatment.

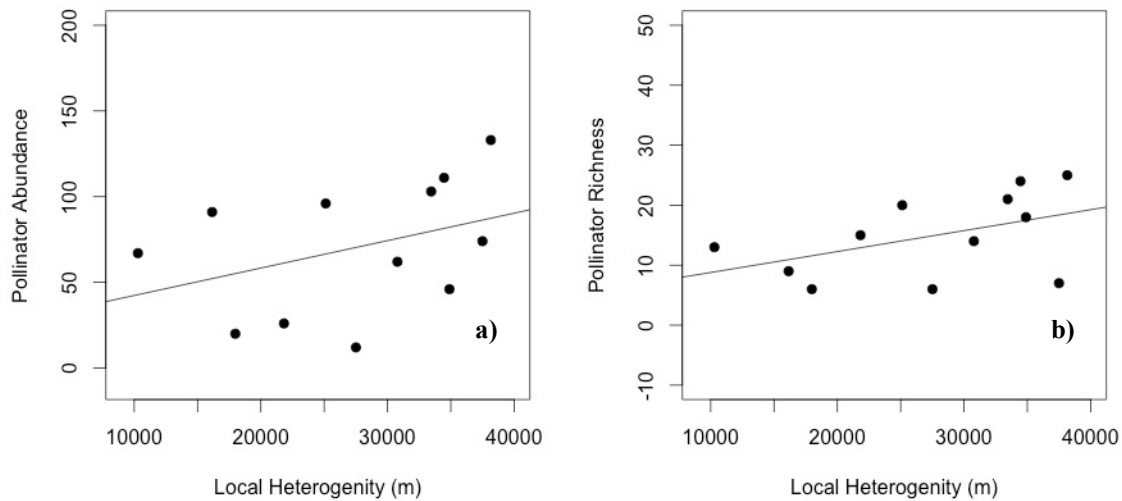


Figure 4.6: (a) Linear regression showing the positive relationship between the residuals of pollinator abundance from a mixed linear model where geographic area is a random effect and local heterogeneity is a fixed effect. (b) Linear regression showing the positive relationship between the residuals of pollinator richness from a mixed linear model where geographic area is a random effect and local heterogeneity is a fixed effect.

Chapter 5 Landscape composition differentially drives diet breadth for key pollinator species

ABSTRACT

An animal's diet breadth is critical to its survival and reproductive success, and yet it can vary considerably between species and across landscapes. Such variation can have far reaching effects on the stability and function of the community-level resource-consumer network, yet much remains unknown about different drivers of animal diet breadth. This study is to uses a network approach to better understand how pollinator diet breadth, or generalization in plant usage, changes at local and regional landscape scales and if these patterns are consistent between species. To answer these questions, we conducted rigorous field surveys to build 36 quantitative plant-pollinator networks across the gulf coast cotton growing region of Texas, U.S.A. Networks were built using both field-recorded pollinator-plant visitation data as well as light microscopy-recorded pollen-derived pollinator-plant visitation data. Specifically, we focused on the two most common cotton pollinator species in the region: the social European honey bee, *Apis mellifera*, and the solitary native long-horned bee, *Melissodes tepaneca*. First, we show that despite being collected directly from cotton blooms, both bee species were found to be carrying substantial amounts of non-cotton pollen, indicating that pollinators use and often prefer non-crop pollen. Second, we showed that the extent of pollinator generalization is highly landscape- and species-dependent. Specifically, local landscape factors better explained proportional generalization than regional factors, and *A. mellifera* and *M. tepaneca* respond to local landscape features with opposing patterns. *A. mellifera* became significantly more generalized in local areas with higher local floral richness, while *M. tepaneca* became less generalized in response to the same factor. Further, *M. tepaneca* became significantly more generalized at sites with high floral abundance, while *A. mellifera*, showed the opposite pattern. These results suggest that *A. mellifera* tracks local floral

resources more closely than *M. tepaneca*, possibly due to greater foraging efficiency and/or social communication. An understanding of the drivers of generalization among and between pollinator species can provide important insights into the functioning of plant-pollinator communities. These findings may have important ramifications on pollination service provided to wild and cultivated plants in agro-ecosystems, especially as increases in generalization are likely to enhance the likelihood of hetero-specific pollen transfer between plant species, negatively affecting plant reproduction.

INTRODUCTION

Interactions between consumers and resources are often generalized (Waser *et al.*, 1996; Chase *et al.*; 2002). Generalist species form the backbone of, and offer stability to, a range of ecological food webs and interaction networks. Indeed, generalized interactions are thought to slow the extinction of more specialized species within networks when interactions are disrupted or become degraded (Memmott *et al.*, 2004, 2007; Fortuna and Bascompte, 2006; Cusser *et al.*, 2013). On a basic level, generalism can be described by the number of resources that a species exploits or consumes, where a generalist would be a species that exploits a large number of resources available within a landscape. Within this framework, a resource-consumer network approach can explicitly describe diet generalization across different local and landscape contexts (e.g., Kremen *et al.*, 2007; Frund *et al.*, 2010). Characterizing how generalization varies across species and landscape contexts could provide insight into the function and stability of entire resource-consumer communities, especially in the face of land cover and climate change. It is particularly important to understand interaction networks for species that provide important ecosystem services, such as pollinators, as changes in network structure will mediate plant reproduction and gene flow, food acquisition for pollinators, and the abundance of plants in need of pollination services (Memmott *et al.*, 2004).

Historically, pollinator generalization has been described as an inherent attribute of a given pollinator species and assumed to be static for that species across time and space. In this way, pollinator species were classically described as either specialists, restricting their foraging to few, closely, related plant species belonging to the same family (Westrich, 1989; Wcislo and Cane, 1996; Cane and Sipes, 2006), or, generalists, having a broad host range that encompasses two or more plant families (Westrich, 1989; Muller, 1996). The breadth of a pollinators diet is thought to be the result of a variety of morphological, physiological, and evolutionary constraints. Morphologically, certain plants are believed to be adapted to attract a narrow range of pollinators (Faegri and van der Pijl, 1979; Harborne, 1982; Kevan and Baker, 1983; Bertin, 1988). For example, floral corolla tube length and width, and the presence or absence of a keel can be highly variable between plant species and can limit which pollinators are able to successfully access rewards (Inouye *et al.*, 1980; Waser and Price, 1981). Beyond the shape of the flower, the viscosity of the nectar reward may morphologically limit access of particular pollinators. As such, pollinators with long feeding apparatuses will be limited to more dilute nectars likely because of adhesive forces or capillary action. For example, honey bees (a long-tongued species) prefer nectar concentrations of 30–50%, whereas short-tongued bees can utilize higher concentration, more viscous, nectars of 45–60% (Roubik and Buchmann, 1984). Further, flower species with physically large, or especially prickly, pollen grains may deter smaller pollinators unable to handle and/or transport such provisions (Castellanos *et al.*, 2004). Physiologically, pollinators are also confined to particular diets by the nutritional composition of the nectar and pollen on which they feed. Studies which have traced larval development of specialized bee species and found that larvae failed to develop when reared on non-preferred pollen, suggesting that certain plant species possess protective properties that hamper pollen digestion by non-specialized species (Praz *et al.*, 2008; Sedivy *et al.*, 2011; Alder, 2000; Arnold *et al.*, 2014; Eckhardt *et al.*, 2014; Muller and Kuhlmann, 2008). As a result, it's likely that pollinators selectively collect or avoid floral resources based on

their phytochemical and nutritional needs. Lastly, from an evolutionary stand point, pollinators are thought to have consistent diets as a result of a tight co-evolutionary history (Gilbert and Raven, 1975) that acts to reduce hetero-specific pollen transfer, which benefits the plant, and acts to reduce foraging and handling time, which benefits the pollinator. Thus, combined, morphological, physiological, and evolutionary constraints, for both plants and pollinators, can serve as the basis for pollinator diet consistency across space and time (Grant, 1993, 1994; Zung *et al.*, 2015).

On the other hand, rather than being limited to a small, fixed subset of floral resources, it may be adaptive for pollinators to be able to change their diets to include a broad range of flowers as they become available in a landscape. In this case, pollinator response to resource availability may be a function of both species-specific as well as landscape characteristics, depending on species sociality, flight range, and flight period. For example, pollinator responses to resource availability may be dependent on aspects of pollinator sociality. Research on foraging eusocial bumble bees (*Bombus impatiens*), which are known to be highly plastic in their foraging, has shown that individual foragers utilize information acquired from sisters (e.g. flower color previously associated with reward) and social information (e.g. flowers chosen by other bees) in foraging decisions over time (Dunlap *et al.*, 2016). Using this information, social bee foragers can alter their behavior to optimally forage within a landscape; this information is not likely to inform the behavior of solitary pollinator species, which forage independently and do not communicate. Further, as the flight range and length of flight period increase, pollinator generalization may increase in plasticity. In other words, as a pollinators ability to traverse long distances over landscapes and pollinator lifespan becomes longer relative to the flowering of individual plant species, pollinators may be forced to expand their diets as plants wink in and out of bloom over time and space (Waser *et al.*, 1996).

The specific drivers of pollinator diet breadth are also likely to vary across spatial scales. At the local scale, pollinator generalization is expected to change largely as a result of floral traits.

First, as floral morphology and rewards become more similar between plant species, and floral handling time decreases, pollinators may become more likely to switch between floral species (Chittka *et al.*, 1999). Second, as the constraints of pollinator behavior and feeding morphology become less significant, pollinators may become more inclined to seek out and exploit multiple floral species (Thomson and Wilson, 2008). At a broader landscape scale, pollinator diet breadth is expected to respond more to floral resource density and distribution. In other words, pollinators may become more plastic in their foraging behavior as individual floral species become less abundant and more patchily distributed across landscapes (Goulson, 2000). If pollinators are foraging optimally, they should be risk averse, optimizing their diet and becoming less choosy as resources become more heterogeneous (Willmer and Stone, 2004; Westphal *et al.*, 2006). Further, if either travel across landscape or between patches, becomes more difficult, either because the landscape is hazardous or because the pollinator is unable to travel long distances, then one would expect the pollinator to expand their diet to include reliable floral resources available within their forage range.

In this study, we use plant-pollinator networks to examine drivers of diet breadth across species and in response to landscape composition. In particular, we compared diet preference and proportional diet generality of two key pollinator species in Central Texas, the social European honey bee, *Apis mellifera*, and the native solitary long horned bee, *Melissodes tepaneca*. The two species are abundant across the region, are the primary pollinators of the major agricultural crop, cotton, and while being similar in size, represent different life history strategies (i.e. social and solitary). We had two hypotheses: (H1) pollinators have species-specific preferences for floral resources and (H2) pollinators have species-specific responses to landscape-context. Specifically, we predicted that the two key pollinators will utilize non-crop floral resources in their diets, exploiting both locally and regionally available floral resources, and showing a preference for these non-crop species relative to their abundance in the landscape. Further, we predicted that both

pollinators will respond to the abundance and diversity of floral resources at both local and regional scales, becoming more specialized as individual floral species become more abundant. In terms of landscape patchiness or heterogeneity, we predicted that at a local scale, increased floral diversity would promote generalization in pollinator diet, while at the regional scale, heterogeneity, or the patchiness of resources, would discourage generalization. Lastly, we expected to find differences between the two species in how they track floral resources, specifically predicting that *A. mellifera*, which is social and can communicate between foragers, would track floral resources more closely than *M. tepaneca*. To address these hypotheses and predictions, we surveyed 36 plant-pollinator communities across a 3-year period in order to construct and analyze quantitative visitation-based and pollen-based plant-pollinator networks for cotton agro-ecosystems across the gulf coast of Texas.

METHODS

Study System

We conducted our research in cotton fields (*Gossypium hirsutum* L.) along the gulf coast of Texas, U.S.A, where cotton is a primary crop and one of the few to offer nectar and pollen resources to floral foraging insects in the area (Cusser *et al.*, 2016; Cusser *et al.*, in review). In addition to cotton, land-cover maps (Han *et al.*, 2014) show that the region is heterogeneous, and is also cultivated with sorghum, corn, and soybean (total agriculture is 55% of the landscape). The remainder of the landscape is comprised of ranching (36%), a few disparate low-density developed areas (6%), and semi-natural habitats, which include forest, shrubland, herbaceous grassland, and wetlands (3%).

We conducted research in 36 conventionally managed cotton fields located across three geographic regions that were consistently sampled over a three-year period (Cusser *et al.*, 2016). The three geographic regions of study were near the towns of Telferner, Austwell/Tivoli, and

Woodsboro, Texas, U.S.A. (28.847913, -96.892975; 28.346058, -96.884940; 27.885588, -97.471427, respectively). Within each geographic region, four sites were selected each year for a total of 12 cotton sites per region (36 sites total) across three years (2013, 2014, 2015). While controlling for region, we selected sites across a gradient of landscape composition (e.g. ranging from 1.7%-16.6% semi-natural habitat cover). All sites were located within cotton fields that were each at least 35 hectares in size and 2 kilometers apart from each other within the sample year (Figure 5.1). According to growers, sites were not sprayed with any chemicals five days prior to, nor during, the sampling period.

Local and Regional Floral Abundance and Richness

To measure floral abundance and richness at the local scale, we collected field-based data at each site. Sites were sampled three times over the course of cotton bloom. The three sampling bouts were conducted at least three days apart and covered the peak of cotton bloom (June 11th-July 2nd) in 2013, 2014, and 2015. During each sampling session, we recorded local floral abundance and local floral species richness. To measure local floral abundance and richness, we counted the number of blooms within the cotton field in 20 randomly placed 1 m² quadrats. In addition, we counted the number of blooms and species identity of flowers in 12 randomly placed 1 m² quadrats along field edges. Local floral abundance and richness was then calculated as the average abundance and richness in the 32 1 m² quadrats across the three sampling sessions.

At the regional scale (1 km radius), we analyzed both land-cover abundance and land-cover richness in the surrounding area using information from the 2013, 2014, and 2015 Cropscape Database (Han *et al.*, 2014). A 1 km radius buffer was chosen as it represents the average foraging distance of many pollinator species (Greenleaf *et al.*, 2007; Zurbuchen *et al.*, 2010). Specifically, to quantify regional semi-natural land-cover, we created one-kilometer radius buffers surrounding each site in qGIS (Quantum GIS Development Team, 2015) and summed the total amount of semi-

natural land-cover falling within that buffer. ‘Semi-Natural’ land-cover includes the Cropscape categories: Evergreen and Deciduous forest, Shrubland, Grassland, Pasture/Hay, and Wetlands. Because cotton is one of the only crops in the region to offer nectar and pollen resources to foraging pollinators, semi-natural habitat is the only other land-cover type that provides floral resources (Cusser, personal observation). As such, we used the abundance of semi-natural habitat within 1 km as a proxy for floral abundance in the region. Abundance of semi-natural habitat closely (negatively) correlated with both the abundance of agriculture and human development surrounding each site (Pearson correlation = -0.9846488, p -value < 0.001, Pearson correlation = -0.1179738, p -value < 0.001, respectively). In this way, the amount of semi-natural habitat surrounding sites serves as a proxy for agricultural and human development, as well. To quantify the habitat heterogeneity associated with each of our sites, we calculated the total edge distance, or perimeter, of all land-cover types within the one-kilometer buffer (Turner, 1989). Other indices of heterogeneity or habitat patchiness, do not capture the added edge distance and complexity created by small patches of habitat that may be important to mobile pollinators. Thus, we selected the edge distance metric, as per past studies (Cusser *et al.*, 2016; Turner, 1989). During floral surveys, pollen from each flowering plant species in the region was opportunistically collected and preserved in vials filled with ethanol. Later, pollen was dyed with Fuchsin and mounted on slides to serve as a pollen reference collection.

Pollinator Species Collection

To understand pollinator foraging generalization, we netted floral visitors found actively foraging in cotton flowers during three one-hour sampling sessions at each site. Similar to past research on oil seed rape (Stanley and Stout, 2014), we focused sampling on crop pollinators as those insects are likely to impact not only crop yield, but also pollination services delivered to co-flowering wild plants. Pollinator sampling sessions were performed at the same time as those

used to determine local floral abundance and richness, described above. During each one-hour sampling session, a single collector walked four parallel 50 m x 1 m transects checking cotton blooms for all visitors. Transects were located along field edges, 12 m apart, representing an area of 2500 m². Because we only collected insects actively foraging within flowers, we consider all specimens as potential pollinators. Pollinators were placed into jars with ethyl acetate and then transferred directly into individual vials of ethanol. Individual vials were used for each insect to avoid pollen contamination between specimens.

Pollen load examination

To examine our first hypothesis and determine what, if any, non-crop floral resources are used and possibly preferred by pollinators, we examined the pollen loads on each collected specimen. Pollen samples were visualized by vortexing the ethanol in which specimens were stored, extracting 20 µl of suspended pollen, and combining pollen with 60 µl of dilute Fuchsin dye. Then we pipetted 50 µl of the Fuchsin-pollen mixture onto a slide (Kearns and Inouye, 1993; Ritchie *et al.*, 2016). The slide was then examined under 400 × magnification using differential interference contrast microscopy. To avoid contamination of slides, laboratory utensils and work surfaces were cleaned after each specimen. In most cases, pollen grains were identified to species or genera with the aid of a reference collection, as described above. However, in the cases of *Fabaceae morphospecies 1* and *Pinus morphospecies 1*, we recorded pollen to the morphospecies level because those species could not be clearly distinguished by pollen morphology.

Once pollen loads were identified, pollen grain abundance and richness were compared between *A. mellifera* and *M. tepaneca*. To determine if differences were significant between species, we first used a Shapiro-Wilk test to check for a normal distribution of pollen abundance and richness, then ANOVA to determine the statistical significance of differences. To determine if bees were visiting particular plant species significantly more than expected relative to their local landscape availability we used compositional analyses of habitat use (Johnson, 1980; Aebischer *et al.*, 1993; Davis *et al.*, 2012; Jha *et al.*, 2013). To test if pollen species usage is significantly nonrandom relative to availability per site, we used the ‘adehabitatHS’ package in R (Calenge, 2006). Only sites at which pollen found on bee specimens was also found in local floral surveys were able to be analyzed in this way. Preference could not be assessed for those sites at which bees carried non-local pollen, as the matrices are inappropriate for compositional analyses. Matrices were evaluated using a randomization test (500 repetitions) where significant preference for one plant species over each other species was assessed using Wilks lambda (Aebischer *et al.*, 1993).

Network Analysis

To determine how pollinator generalization changes with landscape context, we built quantitative plant-pollinator interaction networks for each of our 36 sites. Networks were constructed in which we combine visitation and pollen load data to build a network of consumers and resources. The network values indicate the number of times that the individual pollinators of species “a” (in our case, *A. mellifera* and *M. tepaneca*) were collected foraging on flowers of plant species “p” (in our case, cotton) plus the number of times that the pollen of plant species “p” was found on pollinator species “a”. By combining visitation and pollen load data in the same network, we avoid the dangers of under-sampling interactions and can be confident in our estimation of

network parameters, even with rare species (Blüthgen *et al.*, 2006). While all pollinator specimens were processed and included in networks, for the two focal species, *A. mellifera* and *M. tepaneca*, we calculated the species-level metric “proportional generality”. Proportional generality is akin to pollinator degree (i.e. the number of observed links of that species within a network) but is preferred as it accounts for context dependence, calculating the number of plant partners for a given pollinator adjusted for the total number of plant resources available in that network. In this way, proportional generalization is normalized by the number or diversity of (resource) species in the network and consequently accounts for variation in local floral richness between sites. The rationale for the importance of normalized network metrics was given by Blüthgen *et al.* (2006): “Hurlbert (1978) emphasized that not only proportional utilization, but also the proportional availability of each niche should be taken into account. A species that uses all niches in the same proportion as their availability in the environment should be considered more opportunistic than a species that uses rare resources disproportionately more.” Further, proportional generality is a quantitative measure, meaning that links are weighted by the value within the network matrix. Thus, common links are given more weight than rare, or less frequent links, consequently accounting for variation in local floral abundance between sites. In summary, a higher value of proportional generality indicates a higher level of species generalization (Blüthgen *et al.*, 2006). To establish that proportional degree was indeed an appropriate metric to measure diet breadth, we also determined other metrics of generality (i.e. degree, normalized degree, proportional similarity, and effective partners). All calculated metrics followed similar patterns.

To analyze our findings, we first screened explanatory land-cover variables for multi-collinearity. To do this, we calculated variance inflation factors (hereafter referred to as VIFs) using the ‘vifstep’ function in the R statistical package usdm (Naimi, 2013). Because land-cover variables are inherently related to one another, we chose a conservative theta value of $VIF < 4$ to eliminate collinearity from our models (Myers, 1990). To determine how pollinator generalization

changed with local and regional floral abundance and richness, we used generalized linear mixed-effects models (GLMM). We used geographic region (Woodsboro, Austwell/Tivoli, and Telferner) and year (2013, 2014, and 2015) as random effects and explanatory land-cover variables at local and regional scales as fixed effects (Local Floral Abundance, Local Floral Richness; Regional Semi-Natural Habitat Abundance (m²), and Regional Meters of Edge Habitat (m)). We used proportional generality as our response variable. Lastly, to determine species-specific differences, we used bee species (*A. mellifera* or *M. tepaneca*) as a categorical interaction variable in our models. Models were investigated using the ‘glmer’ function in the package lme4 (Bates *et al.*, 2014).

RESULTS

Local and Regional Floral Abundance and Richness

Locally, floral abundance averaged 2.82 blooms per m² within cotton fields across sites (SE= 0.019), and field edges were surrounded by on average 0.6 flowering species (SE= 0.012). Regionally, sites were surrounded by 1.8 km² of semi-natural habitat (SE= 0.04 km²), and 81.3 km of edge habitat (SE= 0.89 km).

Pollinator Species Collection

In three years, we collected 645 *A. mellifera* and 285 *M. tepaneca*. For *A. mellifera*, sites ranged from 0 to 117 bee specimens, and averaged 18.1 specimens (SE=0.64) per site. For *M. tepaneca*, sites ranged from 0 to 40 bee specimens, and averaged 7.9 specimens (SE=0.29) per site.

Pollen Load Examination

Bee specimens were recorded carrying 16 species of non-cotton pollen: *Prosopis glandulosa*, *Parthenium hysterophorus*, *Helianthus annuus*, *Ruellia caroliniensis*, *Monarda citriodora*, *Amaranthus retroflexus*, *Lactuca serriola*, *Mimosa stringillosa*, *Amaranthus palmeri*, *Heterotheca subaxillaris*, *Phyla nodiflora*, *Helenium amarum*, *Chamaesyce prostrata*, *Opuntia* sp., *Pinus morphospecies 1*, and *Fabaceae morphospecies 1*. For *A. mellifera*, pollen loads averaged 37.1 pollen grains per specimen (SE= 0.17), ranged from 0 to 5 pollens species, and averaged 0.91 species per specimen (SE=0.002). For *M. tepaneca* pollen loads averaged 52.58 pollen grains per specimens (SE= 0.39), ranged from 0 to 4 pollens species, and averaged 1.22 species per specimen (SE=0.002). Pollen on *M. tepaneca* was significantly more abundant (t -value = 8.381, p -value < 0.001) and richer (t -value = 4.983, p -value < 0.001) than *A. mellifera*.

Preference analysis revealed significant patterns of pollen selection for both *A. mellifera* and *M. tepaneca* (*A. mellifera*: λ = 0.32, p -value = 0.002; *M. tepaneca*: λ = 0.27, p -value = 0.002, Table 5.1). Both species exhibited a preference for non-cotton floral resources given their relative availability in the local landscape. For *A. mellifera*, the most preferred plant species was the non-native *Parthenium hysterophorus*, followed by cotton. *M. tepaneca* preferred the native mint, *Monarda citriodora*, equally to cotton (Table 5.1, Figure 5.2).

Network Analysis

Overall, proportional generalization ranged from 0 to 1.76, and averaged 0.68 (SE=0.006) across species. For *A. mellifera*, proportional generalization ranged from 0 to 1.76 at sites, and averaged 0.97 (SE=0.01). For *M. tepaneca*, proportional generalization ranged from 0 to 1.16 at sites, and averaged 0.43 (SE=0.001). Across sites, *A. mellifera* was significantly more generalized than *M. tepaneca* (t value = 39.64 p -value < 0.001). Figure 5.3 shows three example networks, the first of which highlights a network in which *A. mellifera* has a highly generalized diet, the second

of which, network in which *M. tepaneca* has a highly generalized diet, and in the last network, both focal species have generalized diets.

Investigating our second hypothesis, we found that the two pollinator species reacted differently to local and regional landscape contexts: *A. mellifera* became more generalized with greater local floral richness while *M. tepaneca* became less generalized in response to the same variable (estimate: 0.14, p -value = 0.023, estimate = -0.11, p -value = 0.003, respectively). *M. tepaneca* became more generalized with increased local floral abundance (estimate = 0.18, p -value = 0.022). Neither species responded significantly to regional landscape floral abundance (regional semi-natural habitat abundance (m^2)) or richness (regional meters edge habitat (m)) (estimate = 0.14, p -value = 0.16, estimate = 0.07, p -value = 0.60, respectively, Table 5.2, Figure 5.4).

DISCUSSION

In this study, we first showed that crop pollinating insects not only utilize, but also prefer a number of non-crop floral resources in agro-ecosystems. Second, our results demonstrate that the extent of generalization of a given pollinator is both species- and local landscape-dependent. We showed that local landscape factors contribute more to proportional generalization than regional factors, and that the social honey bee (*A. mellifera*) and the long-horned solitary bee (*M. tepaneca*) respond to local landscape features with opposing patterns. In particular, *A. mellifera* became significantly more generalized in local landscapes with higher floral richness, while *M. tepaneca* responded in the opposite direction to the same factor. This may be due to *A. mellifera*'s increased preference for *Parthenium hysterophorus* as floral resources become more diverse along field edges. Further, *M. tepaneca* became significantly more generalized at sites with high local floral abundance, while *A. mellifera*, though not significant, showed the opposite pattern.

H1) Species-specific preferences for floral resources

We found support for our first hypothesis: that pollinators did utilize and, in some cases, prefer non-crop floral resources relative to their abundance. Of the 16 non-cotton pollen species found on pollinators, only 62% (10/16) were found along field edges, indicating that pollinators were using floral resources at both the local and regional scale. The six floral species not found along crop edges included Prostrate Sandmat (*Euphorbia prostrata*), Camphorweed (*Heterotheca subaxillaris*), Prickly Pear (*Opuntia*) and Pine (*Pinus*) species. These species collectively made up a very small portion of the total number of grains found (1.09%). In addition, we found two morphospecies of pollen of which we did not find an example of in our reference collection. On the other hand, in our reference collection, we collected willow (*Salix sp.*), Red Prickly Poppy (*Argemone sanguinea*), Blanket Flower (*Gaillardia sp.*), and Purple Phacelia (*Phacelia tanacetifolia*) but found no evidence of pollen on insect specimens. It is likely that while present in the landscape, these plants may not be abundant enough or palatable to foraging pollinators and consequently not collected.

Preference analysis revealed that both bee species, despite being collected directly from cotton blooms, preferred non-cotton floral resources. Compared to their abundance, *A. mellifera* preferred weedy *Parthenium hysterophorus* pollen to cotton. A member of the Asteraceae family, *P. hysterophorus* is an invasive plant in North America, invading disturbed lands, including roadsides, field edges, and pastureland. Sometimes called “famine weed”, while *P. hysterophorus* invasion has been associated with the loss of crop yield, in the case of supporting crop pollinators, such as *A. mellifera*, the plant may actually bolster populations by providing pollen when other resources in the landscape are sparse. Preference analysis also found that *M. tepaneca* equally preferred *Monarda citriodora* to cotton pollen. *M. citriodora*, a member of the Lamiaceae family, is native to much of North America. Sometimes called lemon beebalm, *M.*

citriodora is a widespread plant in prairies, roadsides and other sunny habitats. The purple, nectar rich, flowers have been shown to be attractive to a wide range of pollinators including butterflies, flies, and bees. Cotton (*Gossypium hirsutum* L.) is also native to North America. Overall, these preference patterns fit those expected from an evolutionary perspective. The European honey bee, *A. mellifera*, prefers pollen provided by a non-native plant, while the native solitary bee, *M. tepaneca*, prefers two native floral resources. Showy invasive species may draw pollinators away from native species, decreasing visit quantity (Free, 1968; Rathcke, 1983). Such changes in visit quantity may affect plant reproduction by altering the amount of pollen arriving on stigmas, which can affect seed and fruit production (Burd, 1994).

H2) Species-specific responses to landscape-context

We also found support for our second hypothesis, with network evidence indicating that species differ in how they track floral resources within the local landscape. We had predicted that at a local scale, increased floral diversity would promote generalization in pollinator diet, which we found to be true for honey bees, but not for the long-horned bee. Our findings support previous studies and reviews suggesting that categorization of pollinators as static specialists or generalists does not describe foraging behavior throughout a population over time and space (Waser *et al.*, 1996; Ne'eman *et al.*, 2006; Alarcón *et al.*, 2008; Kleijn and Raemakers, 2008; Burkle and Alarcón, 2011; Smith *et al.*, 2012). In particular, we found that *A. mellifera* became significantly more generalized in local landscapes with higher field edge floral richness, while *M. tepaneca* responded in the opposite direction to the same factor. Further, *M. tepaneca* became significantly more generalized at sites with high floral abundance, while *A. mellifera*, though not significant, showed the opposite pattern.

We hypothesize that these differences have to do with aspects of pollinator sociality, as well as the nutritional, and morphological constraints of each species. First, we predicted and found

that the social *A. mellifera*, which can communicate between foragers, would track resources more closely by narrowing their diets with decreases in resource abundance and richness. Past studies have shown that honey bees can recruit sister foragers to high quality resource patches when available (Greenleaf *et al.*, 2007). In contrast, the solitary *M. tepaneca* do not exhibit the same intraspecific recruitment interactions (Vaudo *et al.*, 2015). While the majority of literature investigating the resource needs of bees has focused on social bees, like honey bees, it has been assumed that other species have similar resource demands. However, even for social species, the proportion of macronutrients required by bees may be species-specific, as exemplified in other closely related insect species that differ in floral resource needs (Behmer, 2009; Behmer and Joern, 2008). Indeed, when foraging in the same habitat, among the same plant species, closely related bumble and honey bees collect resources that vary in degree of protein concentration, which may be linked to different foraging strategies (e.g. bumble bees may preferentially forage for pollen quality, while honey bees may forage for quantity to meet the vast demands of their large colonies) (Leonhardt and Blüthgen, 2011). This tradeoff between quantity and quality likely exists in other, less studied, bee species. In our system, it may be the case that *A. mellifera*, foraging on behalf of a vast hive, may choose quantity of pollen over quality. Where quality may be preferred by the solitary *M. tepaneca*, altering the foraging decisions of the two species. Third, differences in the morphology of pollinators, especially of their feeding apparatuses (Inouye, 1980; Gilbert, 1981; Grant and Temeles, 1992), as well as other body parts, including pollen transport structures (Feinsinger and Colwell, 1978; Collins and Paton, 1989) and in overall body size, interact with the morphology and size of flowers to influence the foraging ability of pollinators. While *A. mellifera* and *M. tepaneca* are roughly the same size, the two species have somewhat different morphologies. First, *A. mellifera* has a shorter tongue than *M. tepaneca* (6.6 mm and roughly 7 mm respectively, Balfour *et al.*, 2013; Michener, 2007). Further, the two bees have different pollen collecting structures. *A. mellifera* packs collected pollen into baskets on its hind legs called corbicula, while

M. tepaneca uses the dense scopal hair on its hind legs to carry pollen. These morphological differences may confine species to particular foraging behaviors. It is possible that the corbicula of *A. mellifera* are predisposed to carrying different types of pollen (i.e. *Parthenium hysterophorus*) than the scopa of *M. tepaneca* (i.e. *Monarda citridora*) in our system. In fact, morphological constraints have been proposed to ensure some degree of floral specialization across pollinator species (Grant, 1949, 1994), allowing for mechanical isolation of floral species (Ramirez, 1970).

Conclusions

An understanding of the variation in degree of generalization between pollinator species provides important insights into the functioning of plant-pollinator communities and pollination service provision across landscapes. Thus, this work has critical impacts on network ecology and plant reproductive biology beyond the cotton agroecosystem. Specifically, our findings offer evidence that both non-native social bees and native solitary bees are capable of foraging plasticity. Overall, the solitary bee, *M. tepaneca*, was found to be more specialized on cotton pollen during bloom than the social honey bee, *A. mellifera*. Further, *M. tepaneca* became more specialized on cotton pollen despite increased availability of nearby floral resources. Both bees however, visited a wide range of non-cotton floral resources, actually preferring some floral species to cotton based on their relative local abundance.

Pollination service provision in natural and agricultural environments is an ecologically and economically important ecosystem service, underpinning the reproductive output of both wild and cultivated plants (Klein *et al.*, 2007). For native and cultivated plants, pollinator fidelity has been shown to have far reaching effects on the quality of service provided. From the plants perspective, pollinators that specialize on a single focal plant species during the bloom, but that are able to exploit novel floral resources to support themselves during non-blooming periods are

ideal, reducing the amount of hetero-specific pollen transferred to the focal plant species. Hetero-specific pollen deposition on the stigma may interfere with fertilization by conspecific pollen, leading to reciprocal losses for male and female functions of the plant, with potentially important ecological and evolutionary consequences (Morales and Traveset, 2008).

Table 5.1: Results of pollen preference analysis for *A. mellifera* and *M. tepaneca*. Top five preferred floral species are listed. Rank denotes the level of preference for the particular flower species, with ‘A’ being the most preferred and different letters indicating a significantly (p -value < 0.05) different level of preference. The results of the overall preference selection test (p -value and Lambda) are listed for each plant species (where an asterisk indicates p -value < 0.05).

Species	Floral species	p	λ	Rank
<i>A. mellifera</i>		0.002*	0.32	
	<i>Parthenium hysterophorus</i>			A
	<i>Gossypium hirsutum</i>			B
	<i>Monarda citriodora</i>			C
	<i>Helianthus annuus</i>			C
<i>M. tepaneca</i>	<i>Ruellia caroliniensis</i>			C
		0.002*	0.27	
	<i>Monarda citriodora</i>			A
	<i>Gossypium hirsutum</i>			A
	<i>Prosopis glandulosa</i>			B
	<i>Parthenium hysterophorus</i>			C
	<i>Helianthus annuus</i>			D

Table 5.2: Model summaries for GLMM. We used geographic region and year as random effects and land-cover variables at local and regional scales as fixed effects (Local: Floral Abundance, Local Floral Richness; Regional: Semi-Natural Habitat Abundance, Meters of Edge Habitat). Bee species (*A. mellifera* or *M. tepaneca*) was used as a categorical interaction variable. Bolded estimates and asterisks denotes significance at the $\alpha < 0.05$ level.

<i>A. mellifera</i>						<i>M. tepaneca</i>				
Proportional Generality	Std.		t	p		Std.		t	p	
	Estimate	Error				Estimate	Error			
Intercept	0.97	0.12	2.26	8.33	0.010*	0.40	0.07	58.54	-8.00	0.003*
Local Floral Abundance	-0.01	0.06	4.26	0.05	0.96	0.18	0.08	58.54	2.35	0.022*
Local Floral Richness	0.14	0.06	48.94	2.36	0.023*	-0.11	0.08	58.54	-3.09	0.003*
Regional Semi-Natural Habitat	0.15	0.09	59.44	-1.66	0.10	0.06	0.13	58.54	0.71	0.48
Regional Meters Edge Habitat	0.14	0.10	60.86	1.43	0.16	0.07	0.13	58.54	-0.53	0.60

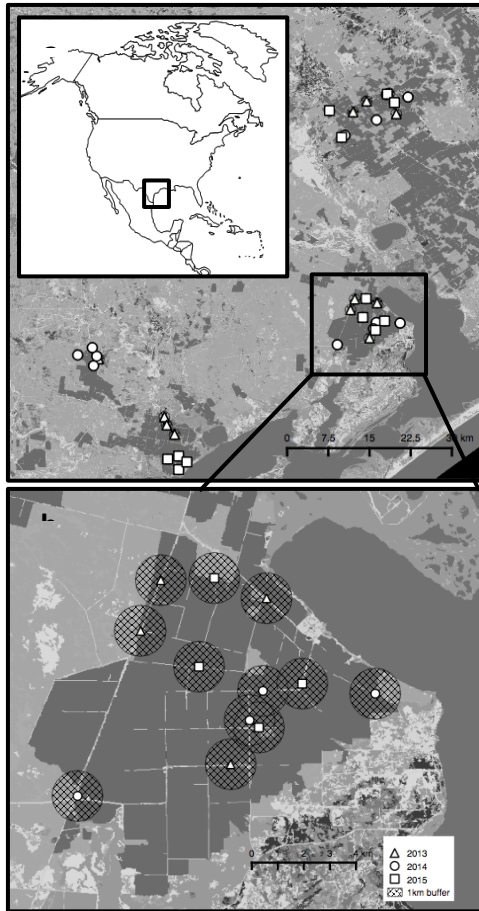


Figure 5.1: Map of study sites. a) Inset: North America. Map of 36 conventional cotton fields used as study sites along the South Texas Gulf Coast, U.S.A. Sites, shown as white symbols, were located in three geographic regions near the towns of Woodsboro, Austwell/Tivoli, and Telferner, Texas. b) An enlargement of the Austwell/Tivoli region showing 12 cotton field sites sampled over the three-year period. White symbols represent sampling sites, and hatched buffers represent a 1km radius of regional habitat surrounding sites. White triangles were sampled in 2013, white circles in 2014, and white squares in 2015.

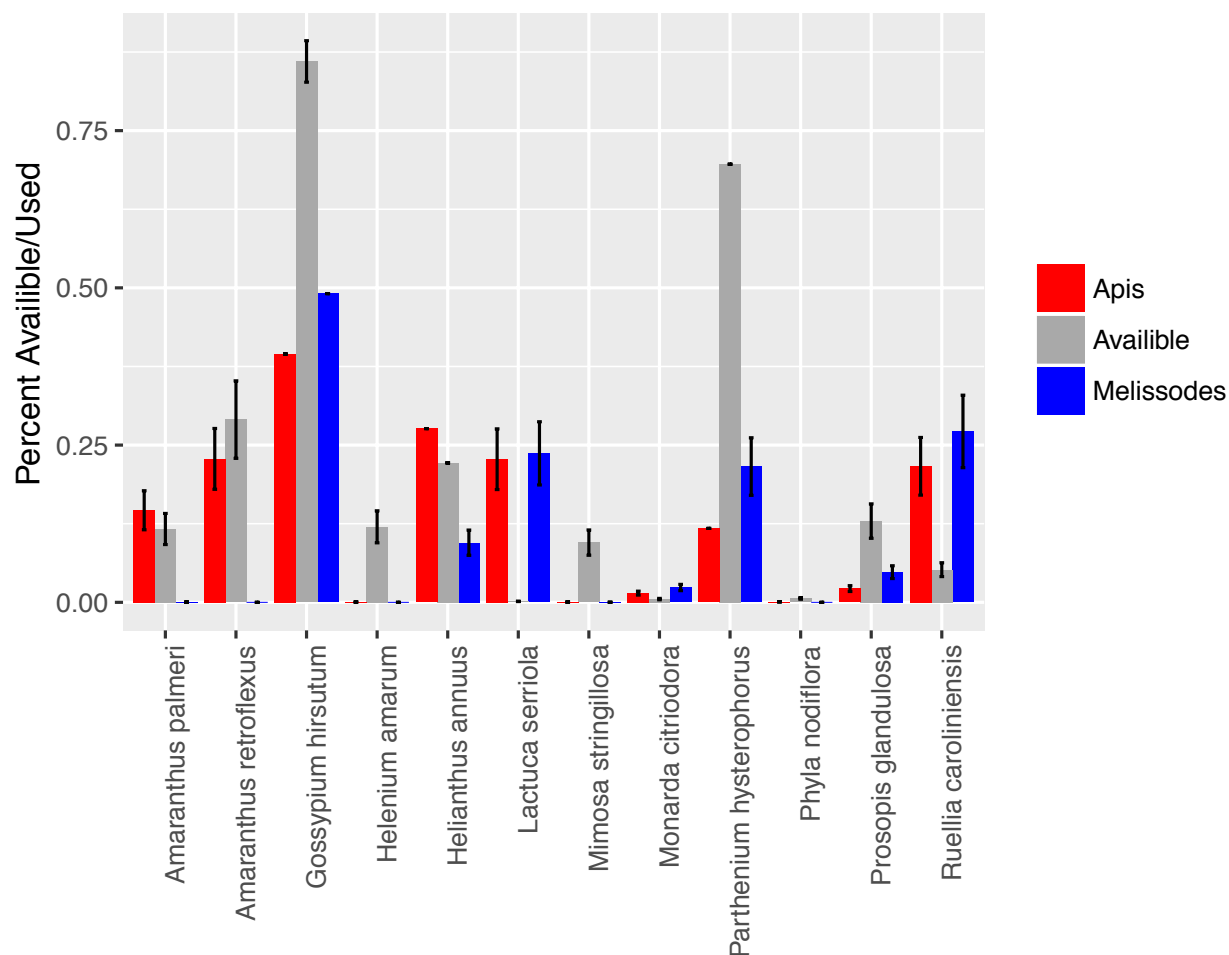


Figure 5.2: Barplot showing the average local availability (Dark Grey), the average percent of pollen load found on *Apis mellifera* (Red), and *Melissodes tepaneca* (Blue) specimens. Of the 16 non-cotton pollen species analyzed, only 11 plants or pollen that occurred in at least two of the three categories (*A. mellifera*, Local Availability, or *M. tepaneca*) are shown. Error bars represent standard error.

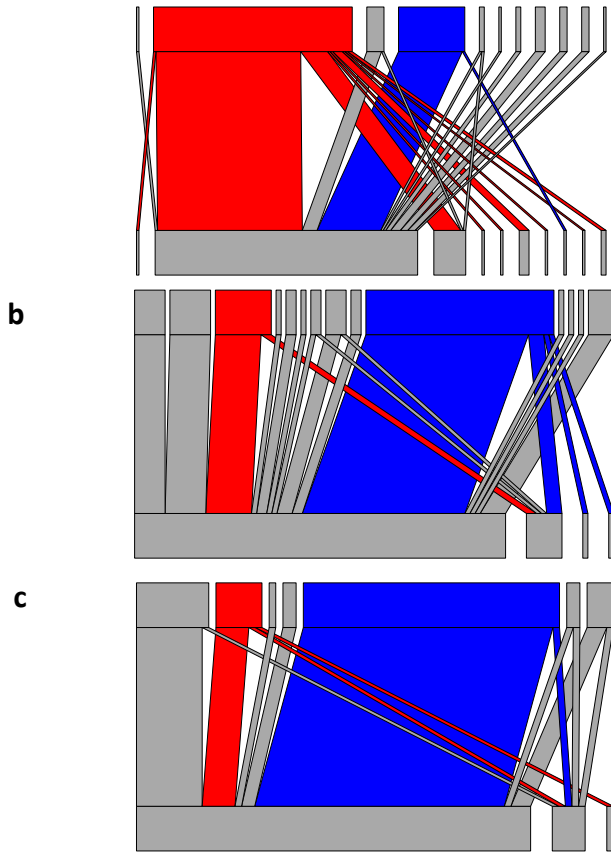


Figure 5.3: Examples of plant and pollinator interactions at three sites. Pollinators are described by the top bar, plants as the bottom bar, and interactions as lines connecting the two. *Apis mellifera* and associated interactions are shown in red, *Melissodes tepaneca* in blue. Pollinators, plants, and interactions that do not include either *A. mellifera* or *M. tepaneca* are shown in dark grey. a) An example of a network in which *A. mellifera* has a high proportional generality (proportional generality = 1.25) and *M. tepaneca* has a relatively low proportional generality (proportional generality = 0.53). b) An example of a network in which *A. mellifera* has a low proportional generality (proportional generality = 0.79) and *M. tepaneca* has relatively high proportional generality (proportional generality = 1.16). c) An example of a network in which both *A. mellifera* and *M. tepaneca* have a relatively high proportional generality (1.05 and 1.10, respectively).

*

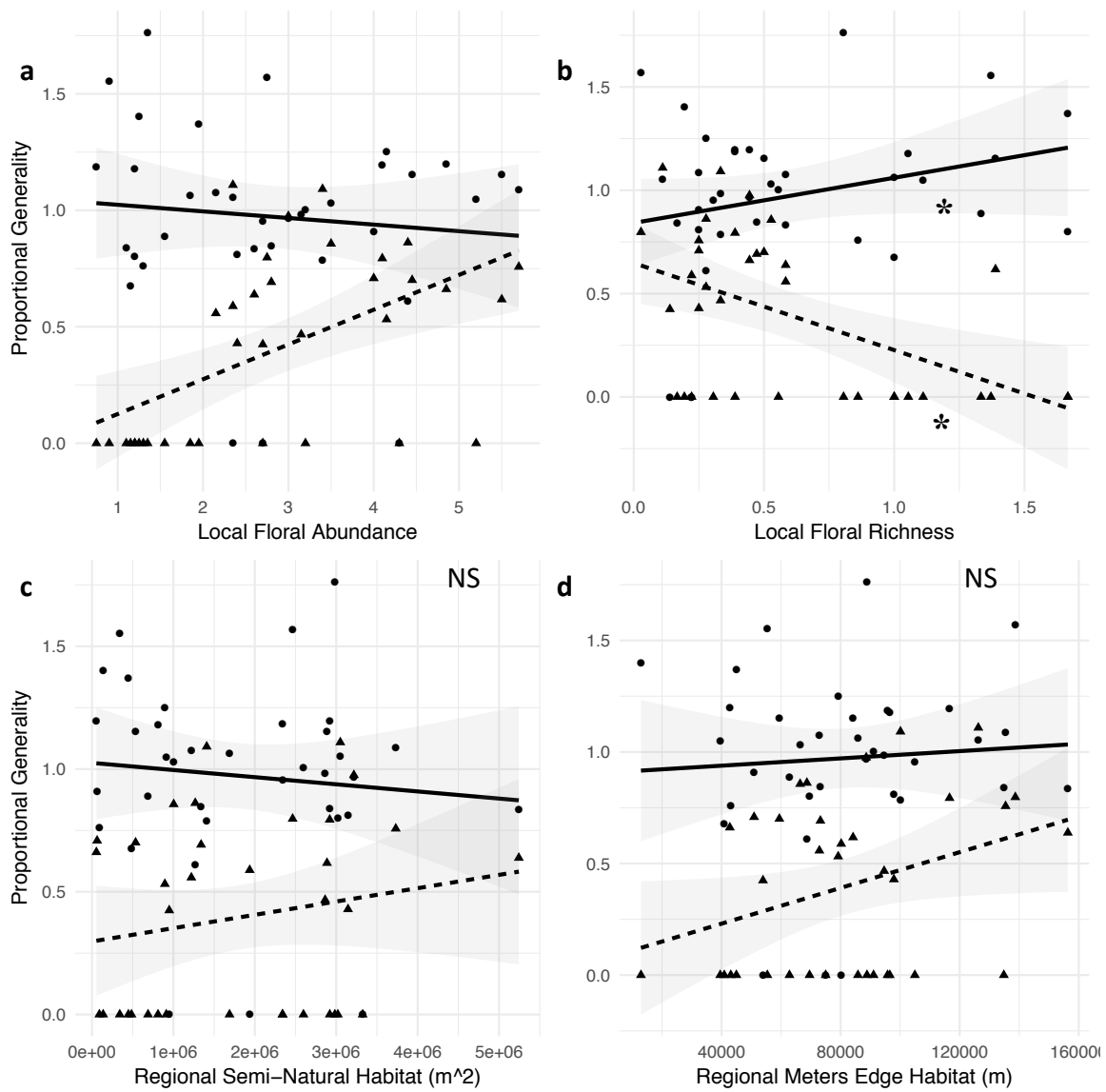


Figure 5.4: Regression plots of proportional generalization and local and regional land-cover. Proportional generalization of *A. mellifera* at each site is shown as filled circles and species-specific regression as a solid line. Proportional generalization of *M. tepaneca* at each site is shown as filled triangles and species-specific regression as a dashed line. Asterisks denote regression significance, while NS denotes the lack of significance at the $\alpha < 0.05$ level.

References

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- Adger, W.N., Kelly, P.M., Winkels, A., Huy, L.Q., and Locke, C. 2002. Migration, remittances, livelihood trajectories, and social resilience. *AMBIO*. 31, 358-366.
- Alvarez, E.C., and Plocheck, R. 2014. Texas Almanac 2014–2015. Texas State Historical Association. <http://www.estremolembo.com/texas-almanac-2012-150-2013-english.pdf> Accessed 29 August 2017
- Anderson, M.J. .2001. A new method for non-parametric multivariate analysis of variance. *Austral Ecology*. 26, 32-46.
- Arino O., Perez J.J.R., Kalogirou V., Bontemps S., Defourny P., and Van Bogaert E. 2012. Global land cover map for 2009. *ESA and UCL*
- Ashman, T.L., Knight, T.M., Steets, J.A., Amarasekare, P., Burd, M., Campbell, D.R., and Wilson, W.G. 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*. 85, 2408-2421.
- Balkenhol N., Waits L.P., and Dezzani R.J. .2009. Statistical approaches in landscape genetics: an evaluation of methods for linking landscape and genetic data. *Ecography*. 32, 818-830.
- Balmford, A., Bennun, L., Ten Brink, B., Cooper, D., Côté, I. M., Crane, P., and Walther, B.A., 2005. The convention on biological diversity's 2010 target. *Science*. 307.
- Barton, K. 2016. multi-model inference. R package version 1.15. 6. 2016.
- Batáry, P., Báldi, A., Kleijn, D., and Tscharrntke, T. .2011. Landscape-moderated biodiversity effects of agri-environmental management: a meta-analysis. *Proceedings of the Royal Society B: Biological Sciences*. 278, 1894-1902.
- Bates D., Mächler M., Bolker B., and Walker S. 2014. Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:1406.5823.

- Batra, S.W.T. 1966. Nests and social behavior of halictine bees of India
Hymenoptera: (Halictidae). *Indian Journal of Entomology* 28, 375-393.
- Bawa, K.S., and Webb, C.J. 1983. Floral variation and sexual differentiation in
Muntingia calabura .Elaeocarpaceae., a species with hermaphrodite
flowers. *Evolution*. 37.6, 1271-1282.
- Beatley, T. 2000. Preserving biodiversity: challenges for planners. *Journal of the
American Planning Association*. 66, 5-20.
- Benjamin, F., R Reilly, J., and Winfree, R., 2014. Pollinator body size mediates the
scale at which land-use drives crop pollination services. *Journal of Applied
Ecology*. 51, 440-449.
- Benton, T.G., Vickery, J.A., and Wilson, J.D. 2003. Farmland biodiversity: is
habitat heterogeneity the key? *Trends in Ecology and Evolution*. 18,
182-188.
- Berger, L.A., Moffettii, J.O., and Rummel, D.R. 1981. The foraging activity of
Agapostemon angelicus Cockerell relative to hybrid cottonseed production in
Texas. *Southwestern Entomology*. 13, 47- 54.
- Berglund, H., and Jonsson, B.G. 2005. Verifying an extinction debt among lichens
and fungi in northern Swedish boreal forests. *Conservation Biology*. 19, 338-
348.
- Bergman, K.O., Askling, J., Ekberg, O., Ignell, H., Wahlman, H., and Milberg, P.
2004. Landscape effects on butterfly assemblages in an agricultural region.
Ecography. 27, 619-628.
- Biesmeijer, J.C., Roberts, S.P., Reemer, M., Ohlemüller, R., Edwards, M., Peeters
T., Schaffers, A.P., Potts ,S.G., Kleukers, R., Thomas, C.D., and Settele, J.
2006. Parallel declines in pollinators and insect-pollinated plants in Britain
and the Netherlands. *Science*. 313, 351-354.
- Blair, R.B. 1996. Land-use and avian species diversity along an urban gradient.
Ecological Applications. 6, 506-519.
- Blüthgen, N., Menzel, F., and Blüthgen, N. 2006. Measuring specialization in
species interaction networks. *BMC Ecology*. 6.1, 9.
- Bommarco, R., Lindborg, R., Marini, L., and Öckinger, E. 2014. Extinction debt for
plants and flower visiting insects in landscapes with contrasting land-use
history. *Diversity and Distributions*. 20, 591-599.

- Bosch, M., and Waser, N. M. 1999. Effects of local density on pollination and reproduction in *Delphinium nuttallianum* and *Aconitum columbianum* .*Ranunculaceae. American Journal of Botany*. 86.6, 871-879.
- Bosch, M., and Waser, N. M. 2001. Experimental manipulation of plant density and its effect on pollination and reproduction of two confamilial montane herbs. *Oecologia*. 126.1, 76-83.
- Branquart E., Hemptinne J.L. .2000. Selectivity in the exploitation of floral resources by hoverflies .*Diptera: Syrphinae. Ecography*. 23, 732-742.
- Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Política Agrícola. Ato Portaria nº13 de 30 de julho de 2015.
<http://www.agricultura.gov.br/assuntos/riscos-seguro/risco-agropecuário/portarias/safra-2015-2016/matogrosso/word/PORTN13ALGODOHERBACEOMT.rtf> Accessed in 12 October 2017
- Brosi B.J., Daily G.C., Shih T.M., Oviedo F., and Durán, G. 2008. The effects of forest fragmentation on bee communities in tropical countryside. *Journal of Applied Ecology*. 45, 773-783.
- Brown, B.J., and Mitchell, R. J. 2001. Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. *Oecologia*. 129.1, 43-49.
- Brown, D.G., Johnson, K.M., Loveland, T.R., and Theobald, D.M. 2005. Rural land-use trends in the conterminous United States, 1950-2000. *Ecological Applications*. 15, 1851-1863.
- Bugg, R.L. 2008. Flower flies *Syrphidae*. and other biological control agents for aphids in vegetable crops. University of California, Division of Agriculture and Natural Resources.
- Burnham, K.P., and Anderson, D.R. 2003. Model selection and multimodel inference: a practical information-theoretic approach. Springer Science and Business Media, Fort Collins, CO.
- Bush, M.B., and Whittaker, R.J. 1991. Krakatau: colonization patterns and hierarchies. *Journal of Biogeography*. 18, 341-356.
- Cane, J.H., Minckley, R., Kervin, L., and Roulston, T.A. 2005. Temporally persistent patterns of incidence and abundance in a pollinator guild at annual

- and decadal scales: the bees of *Larrea tridentate*. *Biological Journal of the Linnean Society*. 85: 319-329.
- Cane, J. H., and Neff, J. L. 2011. Predicted fates of ground-nesting bees in soil heated by wildfire: thermal tolerance of life stages and a survey of nesting depths. *Biological Conservation*. 144, 2631-2636.
- Chao, A., Chazdon, R.L., Colwell, R.K., Shen, T.J. 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. *Ecology Letters*. 8, 148-159.
- Chase, J.M. 2003. Community assembly: when should history matter? *Oecologia*. 136, 489-498.
- Concepcion, E.D., Díaz, M., Kleijn, D., Baldi, A., Batary, P., Clough, Y., and Verhulst, J. .2012. Interactive effects of landscape context constrain the effectiveness of local agri-environmental management. *Journal of Applied Ecology*. 49, 695-705.
- Conner, W.H., Day, J.W., Baumann, R.H., and Randall, J.M. 1989. Influence of hurricanes on coastal ecosystems along the northern Gulf of Mexico. *Wetland Ecology and Management*. 1, 45-56.
- Cottingham, K.L., Brown, B.L., and Lennon, J.T. 2001. Biodiversity may regulate the temporal variability of ecological systems. *Ecology Letters*. 4, 72-85.
- Cousins, S.A., Ohlson, H., and Eriksson, O. 2007. Effects of historical and present fragmentation on plant species diversity in semi-natural grasslands in Swedish rural landscapes. *Landscape Ecology*. 22, 723-730.
- Cowlishaw, G. 1999. Predicting the pattern of decline of African primate diversity: an extinction debt from historical deforestation. *Conservation Biology*. 13, 1183-1193.
- Crone, E.E. 2013. Responses of social and solitary bees to pulsed floral resources. *The American Naturalist*. 182, 465-473.
- Crone, E.E. 2013. Responses of social and solitary bees to pulsed floral resources. *The American Naturalist*. 182, 465-473.
- Cushman, S.A., and Landguth, E.L. 2010. Spurious correlations and inference in landscape genetics. *Molecular Ecology*. 19, 3592-3602.

- Cusser, S., Neff, J.L., and Jha, S. 2015. Land-use change and pollinator extinction debt in exurban landscapes. *Insect Conservation and Diversity*. 8, 562-572.
- Cusser, S., and Goodell, K. 2013. Diversity and distribution of floral resources influence the restoration of plant–pollinator networks on a reclaimed strip mine. *Restoration Ecology*. 21.6, 713-721.
- Cusser, S., Neff, J. L., and Jha, S. 2016. Natural land cover drives pollinator abundance and richness, leading to reductions in pollen limitation in cotton agroecosystems. *Agriculture, Ecosystems and Environment*. 226, 33-42.
- Czech, B., Krausman, P.R., and Devers, P.K. 2000. Economic Associations among Causes of Species Endangerment in the United States reflect the integration of economic sectors, supporting the theory and evidence that economic growth proceeds at the competitive exclusion of nonhuman species in the aggregate. *BioScience*. 50, 593-601.
- Daily, G.C., Ceballos, G., Pacheco, J., Suzán, G., and Sanchez-Azofeifa. 2003. Countryside biogeography of neotropical mammals: conservation opportunities in agricultural landscapes of Costa Rica. *Conservation Biology*. 17, 1814-1826.
- Davis M.A., Thompson K., and Philip Grime J. 2005. Invasibility: the local mechanism driving community assembly and species diversity. *Ecography*. 28: 696-704.
- Davis, E.S., Murray, T.E., Fitzpatrick, U., Brown, M.J.F., and Paxton, R.J. 2010. Landscape effects on extremely fragmented populations of a rare solitary bee, *Colletes floralis*. *Molecular Ecology*. 19, 4922-4935.
- De Groot, R. 2006. Function-analysis and valuation as a tool to assess land-use conflicts in planning for sustainable, multi-functional landscapes. *Landscape and Urban Planning*. 75, 175-186.
- De'ath, G., and Fabricius, K.E. 2000. Classification and regression trees: a powerful yet simple technique for ecological data analysis. *Ecology*. 81, 3178-3192.
- Delaplane, K.S., Thomas, P.A., and McLaurin, W.J. 2010. Bee pollination of Georgia crop plants.
- Dunlap, A.S., Nielsen, M.E., Dornhaus, A., and Papaj, D.R. 2016. Foraging bumble bees weigh the reliability of personal and social information. *Current Biology*. 26.9, 1195-1199.

- Dunning, J.B., Danielson, B.J., and Pulliam, H.R. 1992. Ecological processes that affect populations in complex landscapes. *Oikos*. 169-175.
- Dupont YL, Padrón B, Olesen JM, Petanidou T .2009. Spatio-temporal variation in the structure of pollination networks. *Oikos*. 118, 1261-1269.
- Eckert, C. G. 2002. Effect of geographical variation in pollinator fauna on the mating system of *Decodon verticillatus* (Lythraceae). *International Journal of Plant Sciences*. 163.1, 123-132.
- Eickwort, G.C.1986. First steps into eusociality: the sweat bee *Dialictus lineatulus*. *Florida Entomology*. 1:742-754.
- Ellis, C.J., and Coppins, B.J. 2007. 19th century woodland structure controls stand scale epiphyte diversity in present day Scotland. *Diversity and Distributions*. 13, 84-91.
- Elmqvist, T., and Maltby, E., 2010. *The Economics of Ecosystems and Biodiversity*. Earthscan, Oxford.
- Environmental Justice Foundation, 2007. *The deadly chemicals in cotton*. Environmental Justice Foundation in collaboration with Pesticide Action Network UK, London.
- Estrada, A., and Coates-Estrada, R., 2002. Dung beetles in continuous forest, forest fragments and in an agricultural mosaic habitat island at Los Tuxtlas, Mexico. *Biodiversity and Conservation*. 11, 1903-1918.
- Eyhorn, F., and Mäder, P.M. Ramakrishnan, 2005. The impact of organic cotton farming on the livelihoods of smallholders. Evidence from the Maikaal bioRe project in central India. Research Institute of Organic Agriculture FiBL, Frick, Switzerland.
- Fahrig, L. .2003. Effects of habitat fragmentation on biodiversity. *Annual review of ecology, evolution, and systematics*, 487-515.
- Fenster, C.B., Armbruster, W.S., Wilson, P., Dudash, M.R., and Thomson, J.D., 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology, Evolution, and Systematics*. 375-403.
- Firbank, L.G., Petit, S., Smart, S., Blain, A., and Fuller, R.J., 2008. Assessing the impacts of agricultural intensification on biodiversity: a British perspective. *Philosophical Transactions Royal Society B*. 363, 777-787.

- Fischer, J., Lindenmayer, D.B., and Manning, A.D., 2006. Biodiversity, ecosystem function, and resilience: ten guiding principles for commodity production landscapes. *Frontiers in Ecology and the Environment*. 4, 80-86.
- Forrest, J., and Miller-Rushing, A.J. 2010. Toward a synthetic understanding of the role of phenology in ecology and evolution. *Philosophical Transactions Royal Society B*. 365, 3101- 3112.
- Fortuna, M.A., and Bascompte, J. 2006. Habitat loss and the structure of plant–animal mutualistic networks. *Ecology Letters*. 9.3., 281-286.
- Fraterrigo J.M., and Rusak J.A. 2008. Disturbance-driven changes in the variability of ecological patterns and processes. *Ecology Letters*. 11, 756-770.
- Free, J.B. 1993. *Insect pollination of crops*. No. Ed. 2. Academic press, Cambridge.
- Fründ, J., Linsenmair, K. E., and Blüthgen, N. 2010. Pollinator diversity and specialization in relation to flower diversity. *Oikos*. 119.10, 1581-1590.
- Gallai, N., Salles, J.M., Settele, J., and Vaissière, B.E., 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. *Ecological Economics*. 68, 810-821.
- Gardner, T.A., Ferreira J., Barlow J., Lees A.C., Parry L., Vieira I.C.G., and Aragão L.E. 2013. A social and ecological assessment of tropical land-uses at multiple scales: the Sustainable Amazon Network. *Philosophical Transactions Royal Society B*. 368, 20120166.
- Garibaldi, L.A., Carvalheiro, L.G., Leonhardt, S.D., Aizen, M.A., Blaauw, B.R., Isaacs, R., Kuhlmann, M., Kleijn, D., Klein, A.M., Kremen, C., Morandin, L., Scheper, J., and Winfree, R. 2014. From research to action: enhancing crop yield through wild pollinators. *Frontiers in Ecology and the Environment*. 12, 439-447.
- Garibaldi, L.A., Steffan-Dewenter, I., Winfree, R., Aizen, M.A., Bommarco, R., Cunningham, S.A., and Klein, A.M. 2013. Wild pollinators enhance fruit set of crops regardless of honey bee abundance. *Science*. 339, 1608-1611.
- Ghazoul, J. 2005. Buzziness as usual? Questioning the global pollination crisis. *Trends Ecology and Evolution*. 20, 367-373.
- Ghazoul, J. 2006. Floral diversity and the facilitation of pollination. *Journal of Ecology*. 94, 295-304.

- Ghermandi, A., Van Den Bergh, J.C., Brander, L.M., de Groot, H.L., and Nunes P.A. 2010. Values of natural and human-made wetlands: A meta-analysis. *Water Resources*. 46, 12.
- Giller, K.E., Beare, M.H., Lavelle, P., Izac, A., and Swift, M.J., 1997. Agricultural intensification, soil biodiversity and agroecosystem function. *Applied Soil Ecology*. 6, 3-16.
- Goldberg, C.S., and Waits L.P. 2010. Comparative landscape genetics of two pond-breeding amphibian species in a highly modified agricultural landscape. *Molecular Ecology*. 19, 3650-3663.
- Goslee, S.C., Urban D.L. 2007. The ecodist package for dissimilarity-based analysis of ecological data. *Journal of Statistical Software*. 22, 1-19.
- Goulson, D. 2000. Why do pollinators visit proportionally fewer flowers in large patches? *Oikos* . 91, 485-492.
- Goulson, D., Nicholls E., Botías, C., and Rotheray, E.L. 2015. Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. *Science*. 347, 1255-1257.
- Goulson, D. 2000. Why do pollinators visit proportionally fewer flowers in large patches? *Oikos*. 91.3, 485-492.
- Goulson, D. 2003. Effects of introduced bees on native ecosystems. *Annual Review of Ecology, Evolution, and Systematics*. 34.1, 1-26.
- Green, R.E., Cornell, S.J., Scharlemann, J.P., and Balmford, A. 2005. Farming and the fate of wild nature. *Science*. 307, 550-555.
- Greenleaf, S.S., Williams, N.M., Winfree, R., and Kremen, C. 2007. Bee foraging ranges and their relationship to body size. *Oecologia*, 153, 589-596.
- Grime, J.P. 1998. Benefits of plant diversity to ecosystems: immediate, filter and founder effects. *Journal of Ecology*. 86, 902-910.
- Groeneveld, L.F., Lenstra, J.A., Eding, H., Toro, M.A., Scherf, B., Pilling, D., and Weigend, S., 2010. Genetic diversity in farm animals—a review. *Animal Genetics*. 41, 6-31.
- Guillot, G., and Rousset, F. 2013. Dismantling the Mantel tests. *Methods in Ecology and Evolution*. 4, 336-344.

- Gustafson, E.J. 1998. Quantifying landscape spatial pattern: what is the state of the art? *Ecosystems*. 1, 143-156.
- Gustavsson, E., Lennartsson, T., and Emanuelsson, M. 2007. Land-use more than 200 years ago explains current grassland plant diversity in a Swedish agricultural landscape. *Biological Conservation*. 138, 47-59.
- Han, W, Yang, Z, Di, L, Yagci, AL., Han, S .2014. Making cropland data layer data accessible and actionable in GIS education. *Journal of Geography*. 113, 129-138.
- Hansen, A.J., Knight, R.L., Marzluff, J.M., Powell, S., Brown, K., Gude, P.H., and Jones, K. .2005. Effects of exurban development on biodiversity: patterns, mechanisms, and research needs. *Ecological Applications*, 15, 1893-1905.
- Hanski I, Gilpin M .1991. Metapopulation dynamics: brief history and conceptual domain. *Biological Journal of the Linnenan Society*. 42, 3-16.
- Hanski, I., and Ovaskainen, O. .2002. Extinction debt at extinction threshold. *Conservation Biology*. 16, 666-673.
- Harvey, C.A., Medina, A., Sánchez, D. M., Vílchez, S., Hernández, B., Saenz, J. C., and Sinclair, F. L., 2006. Patterns of animal diversity in different forms of tree cover in agricultural landscapes. *Ecological Applications*. 16, 1986-1999.
- Hegland, S.J., and Boeke, L. 2006. Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. *Ecological Entomology*. 31, 532-538.
- Hegland, S.J., Grytnes, J.A., and Totland Ø. 2009. The relative importance of positive and negative interactions for pollinator attraction in a plant community. *Ecological Research*. 24, 929-936.
- Helm, A., Hanski, I., and Pärtel, M. 2006. Slow response of plant species richness to habitat loss and fragmentation. *Ecology Letters*. 9, 72-77.
- Herrera, C.M. 1988. Variation in mutualisms: the spatiotemporal mosaic of a pollinator assemblage. *Biological Journal of the Linnenan Society*. 35, 95-125.
- Hoehn, P., Tscharncke, T., Tylianakis, J.M., and Steffan-Dewenter, I., 2008. Functional group diversity of bee pollinators increases crop yield. *Philosophical Transactions Royal Society B*. 275, 2283-2291.

- Holzschuh, A., Dudenhöffer, J.H., and Tschardtke, T. 2012. Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. *Biological Conservation*. 153, 101-107.
- Homer, C.G., Dewitz, J.A., Yang, L., Jin, S., Danielson, P., Xian, G., Coulston, J., Herold, N.D., Wickham, J.D., and Megown, K. 2015. Completion of the 2011 National Land Cover Database for the conterminous United States-Representing a decade of land cover change information. *Photogrammetric Engineering & Remote Sensing*. 81, 345-354.
- Hothorn, T., Bretz, F., and Westfall, P. .2008. Simultaneous inference in general parametric models. *Biometrical Journal*. 50, 346-363.
- Inari, N., Hiura T., Toda M.J., and Kudo G. 2012. Pollination linkage between canopy flowering, bumble bee abundance and seed production of understorey plants in a cool temperate forest. *Journal of Ecology*. 100,1534–1543.
- IUCN .2016. The IUCN Red List of Threatened Species. Available from: <http://www.iucnredlist.org> .accessed December 2016.
- Ives, A.R., Carpenter, S.R. 2007. Stability and diversity of ecosystems. *Science*. 317, 58-62.
- Jauker, F., Diekötter, T., Schwarzbach, F., and Wolters, V. 2009. Pollinator dispersal in an agricultural matrix: opposing responses of wild bees and hoverflies to landscape structure and distance from main habitat. *Landscape Ecology*. 24, 547-555.
- Jennersten, O., and Nilsson, S. G. 1993. Insect flower visitation frequency and seed production in relation to patch size of *Viscaria vulgaris* (Caryophyllaceae). *Oikos*. 283-292.
- Jha, S., and Kremen, C. .2013a. Urban land-use limits regional bumble bee gene flow. *Molecular Ecology*. 22, 2483-2495.
- Jha, S., and Kremen, C. 2013b. Resource diversity and landscape-level homogeneity drive native bee foraging. *Proceedings of the National Academy of Sciences*. 110, 555-558.
- Jha, S., and Vandermeer, J.H. 2010. Impacts of coffee agroforestry management on tropical bee communities. *Biological Conservation*. 143, 1423-1431.

- Jin, S., Yang, L., Danielson, P., Homer, C., Fry, J., and Xian, G. 2013. A comprehensive change detection method for updating the National Land Cover Database to circa 2011. *Photogrammetric Engineering & Remote Sensing*. 132, 159 – 175.
- Johnson, K.M., and Beale, C.L. 1998. The rural rebound. *The Wilson Quarterly*. 16-27.
- Kadmon, R., and Pulliam, H.R. 1993. Island biogeography: effect of geographical isolation on species composition. *Ecology*. 74, 977-981.
- Kaiser-Bunbury, C.N., Muff, S., Memmott, J., Müller, C.B., and Caflisch, A. 2010. The robustness of pollination networks to the loss of species and interactions: a quantitative approach incorporating pollinator behaviour. *Ecology Letters*. 13, 442-452.
- Kearns, C.A., Oliveras, D.M. 2009. Environmental factors affecting bee diversity in urban and remote grassland plots in Boulder, Colorado. *Journal of Insect Conservation*. 13, 655-665.
- Kearns, C. A., and Inouye, D. W. 1993. *Techniques for pollination biologists*. University press of Colorado, Boulder.
- Kearns, C. A., Inouye, D. W., and Waser, N. M. 1998. Endangered mutualisms: the conservation of plant-pollinator interactions. *Annual Review of Ecology and Systematics*. 29.1, 83-112.
- Kevan, P.G., Clark, E., and Thomas, V.G. 1990. Insect pollinators and sustainable agriculture. *American Journal of Alternative Agriculture*. 5, 13-22.
- Kleijn D., Winfree R., Bartomeus I., Carvalheiro L.G., Henry M., Isaacs R., Klein A.M., Kremen C., M'gonigle L.K., Rader R., and Ricketts T.H. 2015. Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. *Nature Communications*. 6, 7414.
- Klein, A. M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., and Tscharntke, T. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society of London B: Biological Sciences*. 274, 303-313.
- Klein, A.M., Steffan-Dewenter, I., and Tscharntke, T. 2003. Bee pollination and fruit set of *Coffea arabica* and *C. canephora* Rubiaceae. *American Journal of Botany*. 90, 153-157.

- Klein, A.M., Vaissiere, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., and Tscharntke, T. 2007. Importance of pollinators in changing landscapes for world crops. *Proceedings of the Royal Society of London B: Biological Sciences*. 274, 303-313.
- Klinkhamer, P. G., de Jong, T. J., and de Bruyn, G. J. 1989. Plant size and pollinator visitation in *Cynoglossum officinale*. *Oikos*. 201-204.
- Kondoh, M. 2003. Foraging adaptation and the relationship between food-web complexity and stability. *Science*. 299.5611, 1388-1391.
- Krauss, J., Bommarco, R., Guardiola, M., Heikkinen, R.K., Helm, A., Kuussaari, M., Lindborg, R., Ockinger, E., Pärtel, M., Pino, J., Pöyry, J. 2010. Habitat fragmentation causes immediate and time-delayed biodiversity loss at different trophic levels. *Ecology Letters*. 13, 597-605.
- Kremen, C. 2015. Reframing the land-sparing/land-sharing debate for biodiversity conservation. *Annals of the New York Academy of Sciences*. 1355, 52-76.
- Kremen, C., Williams, N.M., Aizen, M.A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., Packer, L., Potts, S.G., Steffan-Dewenter, I., Vázquez, D.P., and Winfree R. 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecology Letters*. 10, 299-314.
- Kremen, C., Williams, N.M., and Thorp R.W. 2002. Crop pollination from native bees at risk from agricultural intensification. *Proceedings of the National Academy of the Sciences*. 99, 16812-16816.
- Kremen, C., Williams, N.M., Aizen, M.A., Gemmill-Herren, B., LeBuhn, G., Minckley, R., and Ricketts, T.H., 2007. Pollination and other ecosystem services produced by mobile organisms: a conceptual framework for the effects of land-use change. *Ecology Letters*. 10, 299-314.
- Kron, P., Stewart, S. C., and Back, A. 1993. Self-compatibility, autonomous self-pollination, and insect-mediated pollination in the clonal species *Iris versicolor*. *Canadian Journal of Botany*. 71.11, 1503-1509.
- Kruess, A., and Tscharntke, T. 2002. Grazing intensity and the diversity of grasshoppers, butterflies, and trap-nesting bees and wasps. *Conservation Biology*. 16, 1570-1580.

- Kunin, W.E. 1993. Sex and the single mustard: population density and pollinator behavior effects on seed-set. *Ecology*. 74, 2145-2160.
- Kuussaari M., Bommarco R., Heikkinen R.K., Helm A., Krauss J., Lindborg R., Öckinger E., Pärtel M., Pino J., Rodà F., Stefanescu C . 2009. Extinction debt: a challenge for biodiversity conservation. *Trends in Ecology and Evolution*. 24, 564-571.
- Langridge, D.F., Jenkins, P.T., and Goodman, R.D. 1977. A study on pollination of dessert peaches cv. Crawford. *Animal Production Science*. 17, 697-699.
- Legendre, P., and Fortin, M.J. 2010. Comparison of the Mantel test and alternative approaches for detecting complex multivariate relationships in the spatial analysis of genetic data. *Molecular Ecological Resources*. 10, 831-844.
- Legendre P., Legendre L 1998. Numerical ecology: second English edition. Developments in environmental modeling. Elsevier, Oxford.
- Leibold, M.A., Holyoak, M., Mouquet, N., Amarasekare, P., Chase, J.M., Hoopes, M.F., Holt, R.D., Shurin, J.B., Law, R., Tilman, D., Loreau, M. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters*. 7, 601-613.
- Lenza, E., Santos, J.O., Marachaiques-Santos, L. 2015. Species composition, diversity and vegetation structure in a gallery forest-cerrado sensu stricto transition zone in eastern Mato Grosso, Brazil. *Acta Bot Brasilica*. 29, 327-338.
- Liu, Y., Shi G., Mao L., Cheng G., Jiang S., Ma X., An L., Du G., Collins Johnson N., Feng H 2012. Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on Glomeromycota in an alpine meadow ecosystem. *New Phytol*. 194, 523-535.
- Lizhong, F.X., and Ning, W. 1997. Study on Using Mason Bees for Peach Pollination in Protective Ground in Winter in Northern China. *Journal of Fruit Science*. 3, 003.
- Lockwood, J.L., Powell, R.D., Nott, M.P., Pimm, S.L. 1997. Assembling ecological communities in time and space. *Oikos*. 80, 549-553.
- Lopezaraiza-Mikel, M.E., Hayes, R.B., Whalley, M.R., and Memmott, J. 2007. The impact of an alien plant on a native plant-pollinator network: an experimental approach. *Ecology Letters*, 10.7, 539-550.

- Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J.P., Hector, A., Hooper, D.U. 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. *Science*. 294, 804-808.
- Losey, J. E., and Vaughan, M., 2006. The economic value of ecological services provided by insects. *Bioscience*. 56, 311-323.
- Losey, J., and Vaughan, M. 2008. Conserving the ecological services provided by insects. *American Entomologist*, 54, 113-114.
- Luck, G.W., Daily, G.C., and Ehrlich, P.R. 2003. Population diversity and ecosystem services. *Trends in Ecology and Evolution*. 18, 331-336.
- Lunau, K. 2004. Adaptive radiation and coevolution—pollination biology case studies. *Organisms Diversity and Evolution*. 4.3, 207-224.
- Magurran, A.E., and Henderson, P.A. 2010. Temporal turnover and the maintenance of diversity in ecological assemblages. *Proceedings of the Royal Society of London B: Biological Sciences*. 365, 3611-3620.
- Manning, A.D., Fischer, J., and Lindenmayer, D.B., 2006. Scattered trees are keystone structures—implications for conservation. *Biological Conservation*. 132, 311-321.
- Margo, R.A. 1992. Explaining the postwar suburbanization of population in the United States: The role of income. *Journal of Urban Economics*. 31, 301-310.
- Martin, S.L., Hayes, D.B., Kendall, A.D., and Hyndman, D.W. 2017. The land-use legacy effect: Towards a mechanistic understanding of time-lagged water quality responses to land-use/cover. *Science of the Total Environment*. 579, 1794-1803.
- Marzluff, J.M., and Ewing, K. 2001. Restoration of fragmented landscapes for the conservation of birds: a general framework and specific recommendations for urbanizing landscapes. *Restoration Ecology*. 9, 280-292.
- Mato Grosso Institute of Agricultural Economics .2014..
<http://www.imea.com.br/imea-site/> Accessed in 12 October 2017
- Mayfield, M.M., and Daily, G.C., 2005. Countryside biogeography of neotropical herbaceous and shrubby plants. *Ecological Applications*. 15, 423-439.

- McCoy, E.D., Bell, S.S., and Walters, K., 1986. Identifying biotic boundaries along environmental gradients. *Ecology*. 67, 749-759.
- McCune, B., Grace, J.B., and Urban, D.L 2002. MRPP .multi-response permutation procedures. in analysis of ecological communities. MjM Software Design, Oregon.
- Memmott, J., Craze, P. G., Waser, N. M., and Price, M. V. 2007. Global warming and the disruption of plant–pollinator interactions. *Ecology Letters*. 10.8, 710-717.
- Memmott, J., Waser, N. M., and Price, M. V. 2004. Tolerance of pollination networks to species extinctions. *Proceedings of the Royal Society of London B: Biological Sciences*. 271.1557, 2605-2611.
- Merten, G.H., and Minella, J.P.G. 2013. The expansion of Brazilian agriculture: soil erosion scenarios. *International Soil and Water Conservation Research*. 1, 37-48.
- Metzger, J.P. 2000. Tree functional group richness and landscape structure in a Brazilian tropical fragmented landscape. *Ecological Applications*. 10, 1147-1161.
- Michener, C.D., 2007. *The bees of the world*. John Hopkins University Press, Baltimore.
- Minckley, R.L., Weislo, W.T., Yanega, D., Buchmann, S.L. 1994. Behavior and phenology of a specialist bee *Dieunomia* and sunflower *Helianthus* pollen availability. *Ecology*. 75, 1406-1419.
- Mittermeier, R.A., Mittermeier, C.G., Brooks, T.M., Pilgrim, J.D., Konstant, W.R., Da Fonseca, G.A., and Kormos, C., 2003. Wilderness and biodiversity conservation. Proceeding of the National Academy of the Sciences, U.S.A. 100, 10309-10313.
- Mladenoff, D.J., Sickley, T.A., Haight, R.G, and Wydeven, A.P. 1995. A regional landscape analysis and prediction of favorable gray wolf habitat in the northern Great Lakes region. *Conservation Biology*. 279-294.
- Moeller, D. A., and Geber, M. A. .2005. Ecological context of the evolution of self-pollination in *Clarkia xantiana*: population size, plant communities, and reproductive assurance. *Evolution*. 59.4, 786-799.

- Moen, J., De Clercq, P., and Tirry, L. 2011. Side effects of pesticides on the larvae of the hoverfly *Episyrphus balteatus* in the laboratory. *Phytoparasitica*. 39, 1-9.
- Moffett, J. O., Stith, L. S., and Shipman, C. W., 1976. Influence of distance from pollen plant on seed produced by male-sterile cotton. *Crop Science*. 16, 765-766.
- Moldenke, A.R. 1975. Niche specialization and species diversity along a California transect. *Oecologia* 21: 219-242.
- Munyuli, M.T. 2012. Micro, local, landscape and regional drivers of bee biodiversity and pollination services delivery to coffee *Coffea canephora* in Uganda. *International Journal of Biodiversity Science, Ecosystem Services and Management*. 8, 190-203.
- Murtaugh, P.A. 2009. Performance of several variable-selection methods applied to real ecological data. *Ecology Letters*. 12, 1061-1068.
- Myers, N. 1990. The biodiversity challenge: expanded hot-spots analysis. *Environmentalist*. 10, 243-256.
- Myster, R.W. 2016. The physical structure of forests in the Amazon basin: a review. *Botanical Reviews*. 82, 407-427.
- Naimi B. 2013. usdm: Uncertainty analysis for species distribution models. R package version 1, 1-12.
- National Agriculture Statistics Service, 2013, January 1. Retrieved April 16, 2015, from <http://www.nass.usda.gov/>
- National Cotton Council of America, 2014, January 1. Retrieved April 16, 2015, from <http://www.cotton.org/>
- National Research Council, 2007. *Status of pollinators in North America*. National Academies Press, Washington, DC.
- Nyéki, J., Szabó, Z., Andrásfalvy, A., Soltész, M., and Kovács, J. .1997. Open pollination and autogamy of peach and nectarine varieties. *IV International Peach Symposium*. 465, 279-284.
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Stevens, M.H.H., and Oksanen, M.J., Suggests MASS, 2007. The vegan package. *Community ecology package* 10, 631-637.

- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., O'Hara, R.G., Simpson, G.L., Solymos, P., Stevens, M.H.H., and Wagner, H. .2010. Vegan: Community Ecology Package. R package version 1.18-0/r1102.
- Ollerton, J., Winfree, R., and Tarrant, S. 2011. How many flowering plants are pollinated by animals? *Oikos* 120: 321-326.
- Packer, L., and Owen, R.E. 1994. Relatedness and sex ratio in a primitively eusocial halictine bee. *Behavior Ecology and Sociobiology*. 34, 1-10.
- Palmer, M.W. 1988. Fractal geometry: a tool for describing spatial patterns of plant communities. *Plant Ecology*. 75, 91-102.
- Paltto, H., Nordén, B., Götmark, F., and Franc, N. 2006. At which spatial and temporal scales does landscape context affect local density of Red Data Book and Indicator species? *Biological Conservation*. 133, 442-454.
- Paquette S.R., and Lapointe, F.J. 2009. A statistical procedure to assess the significance level of barriers to gene flow. *Journal of Genetics and Genomics*. 36, 685-693.
- Parker, I.M. 1997. Pollinator limitation of (*Cytisus scoparius*) Scotch broom., an invasive exotic shrub. *Ecology*. 78, 1457-1470.
- Pedro, S.R.M. 2014. The stingless bee fauna in Brazil (Hymenoptera: Apidae). *Sociobiology*. 61: 348-354.
- Perez, A., and Pollack, S. 2003. Fruit and tree nuts outlook. USDA Electronic report from the economic research service, <
<http://www.ers.usda.gov/publications/fts-fruit-and-tree-nuts-outlook.aspx>>
10th January 2014.
- Perfecto, I., and Vandermeer, J. 2008. Spatial pattern and ecological process in the coffee agroforestry system. *Ecology*. 89, 915-920.
- Perfecto, I., and Vandermeer, J., 2008. Biodiversity conservation in tropical agroecosystems. *Annals of the New York Academy of Science*. 1134, 173-200.
- Perfecto, I., and Vandermeer, J., 2010. The agroecological matrix as alternative to the land-sparing/agriculture intensification model. *Proceedings of the National Academy of Science, U.S.A.* 107, 5786-5791.

- Petanidou, T., Kallimanis, A. S., Tzanopoulos, J., Sgardelis, S. P., and Pantis, J. D. .2008. Long-term observation of a pollination network: fluctuation in species and interactions, relative invariance of network structure and implications for estimates of specialization. *Ecology Letters*. 11.6, 564-575.
- Peterson, G., Allen, C. R., and Holling, C. S., 1998. Ecological resilience, biodiversity, and scale. *Ecosystems*. 11, 6-18.
- Piha, H., Luoto, M., and Merilä, J. 2007. Amphibian occurrence is influenced by current and historic landscape characteristics. *Ecological Applications*. 17, 2298-2309.
- Pires, V.C., Silveira, F.A., Sujii, E.R., Torezani, K.R.S., Rodrigues, W.A., Albuquerque, F.A., and Pires, C.S.S. 2014. Importance of bee pollination for cotton production in conventional and organic farms in Brazil. *Journal of. Pollination Ecology*. 13, 151-160.
- Potts, S.G., Biesmeijer, J.C., Kremen, C., Neumann, P., Schweiger, O., Kunin, W.E. 2010. Global pollinator declines: trends, impacts and drivers. *Trends in Ecology and Evolution*. 25, 345-353.
- Potts, S.G., Vulliamy B., Roberts S., O'Toole C., Dafni A., Ne'eman G., Willmer P. 2005. Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecological Entomology*. 30, 78-85.
- Potts, S. G., Vulliamy, B., Dafni, A., Ne'eman, G., and Willmer, P. 2003. Linking bees and flowers: how do floral communities structure pollinator communities? *Ecology*. 84, 2628–2642.
- Potts, S.G., Vulliamy, B., Roberts, S., O'Toole, C., Dafni, A., Ne'eman, G., and Willmer, P. .2005. Role of nesting resources in organising diverse bee communities in a Mediterranean landscape. *Ecological Entomology*. 30, 78-85.
- Pretty, J.N., Morison, J.I., and Hine, R.E. 2003. Reducing food poverty by increasing agricultural sustainability in developing countries. *Agriculture Ecosystem and the Environment*. 95, 217-234.
- Price, M.V., Waser, N.M., Irwin, R.E., Campbell, D.R., and Brody, A.K. 2005. Temporal and spatial variation in pollination of a montane herb: a seven-year study. *Ecology*. 86, 2106-2116.

- Qian H., Ricklefs R.E. 2012. Disentangling the effects of geographic distance and environmental dissimilarity on global patterns of species turnover. *Global Ecology and Biogeography*. 21, 341-351.
- Quantum GIS Development Team. 2015. Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. <http://www.qgis.org/>
- Racey, G.D., and Euler, D.L. 1982. Small mammal and habitat response to shoreline cottage development in central Ontario. *Canadian Journal of Zoology*. 60, 865-880.
- Raine, N.E., Ings, T.C., Dornhaus, A., Saleh, N., and Chittka, L. 2006. Adaptation, genetic drift, pleiotropy, and history in the evolution of bee foraging behavior. *Advances in the Study of Behavior*. 36, 305-354.
- Rathcke, B. J., and Jules, E. S. 1993. Habitat fragmentation and plant–pollinator interactions. *Current Science*. 273-277.
- Ratter, J.A., Ribeiro, J.F., and Bridgewater, S. 1997. The Brazilian Cerrado vegetation and threats to its biodiversity. *Annals of Botany*. 80, 223-230.
- Rhodes, J., 2002. Cotton pollination by honeybees. *Animal Production Science*. 42, 513-518.
- Richards, A.J., 2001. Does low biodiversity resulting from modern agricultural practice affect crop pollination and yield? *Annals of Botany*. 88, 165-172.
- Ricketts, T.H., Regetz, J., Steffan-Dewenter, I., Cunningham, S.A., Kremen C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, SS, Klein, A.M., Mayfield, M.M., Morandin, L.A., Ochieng, A., and Viana, B.F. 2008. Landscape effects on crop pollination services: are there general patterns? *Ecology Letters*. 11, 499–515.
- Ricketts, T.H., Daily, G.C., Ehrlich, P.R., and Michener, C.D. 2004. Economic value of tropical forest to coffee production. *Proceedings of the National Academy of the Science, U.S.A.* 101, 12579-12582.
- Ricketts, T.H., Regetz, J., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Bogdanski, A., and Viana, B.F., 2008. Landscape effects on crop pollination services: are there general patterns? *Ecology Letters*. 11, 499-515.
- Ricklefs, R.E. 2004. A comprehensive framework for global patterns in biodiversity. *Ecology Letters*. 7, 1-15.

- Rioux Paquette, S., Talbot, B., Garant, D., Mainguy, J., and Pelletier, F. 2014. Modelling the dispersal of the two main hosts of the raccoon rabies variant in heterogeneous environments with landscape genetics. *Evolutionary Applications*. 7, 734-749.
- Ritchie, A. D., Ruppel, R., and Jha, S. 2016. Generalist Behavior Describes Pollen Foraging for Perceived Oligolectic and Polylectic Bees. *Environmental entomology*. 45.4, 909-919.
- Robinson, R.A., and Sutherland, W.J., 2002. Post-war changes in arable farming and biodiversity in Great Britain. *Journal of Applied Ecology*. 39, 157-176.
- Roland, J., and Taylor, P.D. 1997. Insect parasitoid species respond to forest structure at different spatial scales. *Nature*. 386, 710-713.
- Root, R.B. 1973. Organization of a plant-arthropod association in simple and diverse habitats: the fauna of collards *Brassica oleracea*. *Ecological Monographs* 43, 95-124.
- Rosenzweig, M.L. 1995. *Species diversity in space and time*. Cambridge University Press, Cambridge.
- Roubik, D.W. 1980. Foraging behavior of competing Africanized honeybees and stingless bees. *Ecology*. 61, 836-845.
- Roubik, D.W. 1992. *Ecology and natural history of tropical bees*. Cambridge University Press, New York.
- Roubik, D.W. 1995. *Pollination of cultivated plants in the tropics*. No. 118. FAO, Rome.
- Roubik, D.W. 2000. Deceptive orchids with *Meliponini* as pollinators. *Plant Systematics Evolution*. 222, 271-279.
- Rudzitis, G. 1999. Amenities increasingly draw people to the rural west. *Rural Development Perspectives*. 14, 9-13.
- Sadeghi, H., and Gilbert, F. 2000. Oviposition preferences of aphidophagous hoverflies. *Ecological Entomology*. 25, 91-100.
- Sang, A., Teder, T., Helm, A., and Pärtel, M. 2010. Indirect evidence for an extinction debt of grassland butterflies half century after habitat loss. *Biological Conservation*. 143, 1405-1413.

- Schroth, G., and Harvey, C. A. 2007. Biodiversity conservation in cocoa production landscapes: an overview. *Biodiversity Conservation*. 16, 2237-2244.
- Schweiger, O., Maelfait JP, Wingerden WV, Hendrickx F, Billeter R, Speelmans M, Augenstein I, Aukema B, Aviron S, Bailey, and Bukacek, R. 2005. Quantifying the impact of environmental factors on arthropod communities in agricultural landscapes across organizational levels and spatial scales. *Journal of Applied Ecology*. 42, 1129-1139.
- Scialabba, N.H., and Williamson, D. 2004. The scope of organic agriculture, sustainable forest management and ecoforestry in protected area management. FAO, Rome.
- Scobell, S. A., and Scott, P. E. 2002. Visitors and floral traits of a hummingbird-adapted cactus (*Echinocereus coccineus*) show only minor variation along an elevational gradient. *The American Midland Naturalist*. 147.1, 1-15.
- Scott, A.J., Shorten, J., Owen, R., and Owen, I. 2011. What kind of countryside do the public want: community visions from Wales UK? *GeoJournal*. 76, 417-436.
- Scott, J.A. 1992. *The butterflies of North America: a natural history and field guide*. Stanford University Press, Redwood City.
- Senapathi, D., Carvalheiro, L.G., Biesmeijer, J.C., Dodson, C.A., Evans, R.L., McKerchar, M., and Potts, S.G. 2015. The impact of over 80 years of land cover changes on bee and wasp pollinator communities in England. *Proceedings of the Royal Society of London B: Biological Sciences*. 282, 20150294.
- Shmida, A., Ellner, S. 1984. Coexistence of plant species with similar niches. *Plant Ecology*. 58, 29-55.
- Shuler, R.E., Roulston, T.H., Farris, G.E. 2005. Farming practices influence wild pollinator populations on squash and pumpkin. *Journal of Economic Entomology*. 98, 790-795.
- Shuler, R.E., T'ai, H.R., and Farris, G.E., 2005. Farming practices influence wild pollinator populations on squash and pumpkin. *Journal of Economic Entomology*. 98, 790-795.
- Steffan Dewenter, I., Münzenberg, U., Bürger, C., Thies, C., and Tschardtke, T. 2002. Scale-dependent effects of landscape context on three pollinator guilds. *Ecology*. 83, 1421-1432.

- Steffan-Dewenter, I., Kessler, M., Barkmann, J., Bos, M.M., Buchori, D., Erasmi, S., and Tscharntke, T., 2007. Tradeoffs between income, biodiversity, and ecosystem functioning during tropical rainforest conversion and agroforestry intensification. *Proceedings of the National Academy of Science*, U.S.A. 104, 4973-4978.
- Steffan-Dewenter, I., Münzenberg, U., Bürger, C., Thies, C., and Tscharntke, T., 2002. Scale-dependent effects of landscape context on three pollinator guilds. *Ecology*. 83, 1421-1432.
- Stevens, V.M., Turlure, C., and Baguette, M. .2010. A meta analysis of dispersal in butterflies. *Biological Reviews*. 85, 625-642.
- TAMU .2013. General Production. Available from:
<http://cotton.tamu.edu/GeneralProduction.html> .accessed December 2016.
- TAMU Extension .2013. <<http://agrilifeextension.tamu.edu/>> 10th January 2014.
- Temple, S.A., and Cary, J.R. .1988. Modeling dynamics of habitat interior bird populations in fragmented landscapes. *Conservation Biology*. 2, 340-347.
- Tepedino, V.J., and Stanton, N.L.1981. Diversity and competition in bee-plant communities on short-grass prairie. *Oikos*. 36, 35-44.
- Thomson, J. D. 1978. Effects of stand composition on insect visitation in two-species mixtures of *Hieracium*. *American Midland Naturalist*, 431-440.
- Tilman, D.1982. *Resource competition and community structure*. Princeton University Press, Princeton.
- Tilman, D. 1986. Nitrogen-limited growth in plants from different successional stages. *Ecology*. 67, 555-563.
- Tilman, D., May, R.M., Lehman, C.L., and Nowak, M.A. 1994. Habitat destruction and the extinction debt. *Nature*. 371, 65-66.
- Tobler, W.R. 1970. *Spectral analysis of spatial series*. Library Photographic Service, University of California, Riverside, CA.
- Triplehorn, C.A.J., Borror, N.F., Triplehorn, D.J.C.A., and Johnson, N.F. 2005. *Borror and DeLong's Introduction to the Study of Insects*, Cengage Learning, Boston, MA.

- Tscharntke, T., and Brandl, R., 2004. Plant-insect interactions in fragmented landscapes. *Annual Review of Entomology*. 49, 405-430.
- Tscharntke, T., Clough, Y., Wanger, T.C., Jackson, L., Motzke, I., Perfecto, I., and Whitbread, A. 2012. Global food security, biodiversity conservation and the future of agricultural intensification. *Biological Conservation*. 151, 53-59.
- Tscharntke, T., Klein, A.M., Kruess, A., Steffan-Dewenter, I., and Thies, C. 2005. Landscape perspectives on agricultural intensification and biodiversity–ecosystem service management. *Ecology Letters*. 8, 857-874.
- Tuomisto, H., Ruokolainen, K. 2006. Analyzing or explaining beta diversity? Understanding the targets of different methods of analysis. *Ecology*. 87, 2697-2708.
- Turner, M.G. 1989. Landscape ecology: the effect of pattern on process. *Annual review of ecology and systematics*. 20.1, 171-197.
- Turner, M.G., and Bratton, S.P., 1987. *Landscape heterogeneity and disturbance*. Springer, New York, NY.
- Tylianakis, J.M., Didham, R.K., Bascompte, J., Wardle, D.A. 2008. Global change and species interactions in terrestrial ecosystems. *Ecology Letters*. 11, 1351-1363.
- Tylianakis, J.M., Klein, A.M., Tscharntke, T. 2005. Spatiotemporal variation in the diversity of Hymenoptera across a tropical habitat gradient. *Ecology*. 86, 3296-3302.
- Tylianakis, J.M., Klein, A.M., Lozada, T., and Tscharntke, T. 2006. Spatial scale of observation affects α , β and γ diversity of cavity nesting bees and wasps across a tropical land-use gradient. *Journal of Biogeography*. 33, 1295-1304.
- United Nations News Centre, 2013, June 14. Retrieved April 16, 2015, from <http://www.un.org/news/>
- USDA ERS, 2014, March 5. Retrieved April 16, 2015, from <http://www.ers.usda.gov/>
- USDA, National Agricultural Statistics Service, 2009. <http://www.nass.usda.gov/Publications/Ag_Statistics/2009/> 10th January 2014.

- Vázquez, D.P., Morris, W.F., and Jordano, P. 2005. Interaction frequency as a surrogate for the total effect of animal mutualists on plants. *Ecology Letters*. 8:1088-1094.
- Veddeler, D., Klein, A.M., and Tschardt, T. 2006. Contrasting responses of bee communities to coffee flowering at different spatial scales. *Oikos*. 112, 594-601.
- Vellend, M. 2010. Conceptual synthesis in community ecology. *Quarterly Review of Biology*. 85,183-206
- Wagner, H.H., Fortin, M.J. 2013. A conceptual framework for the spatial analysis of landscape genetic data. *Conservation Genetics*. 14, 253-261.
- Waser, N.M. 1983. The adaptive nature of floral traits: ideas and evidence. *Pollination Biology*. 1, 241-285.
- Waser, N. M. 1986. Flower constancy: definition, cause, and measurement. *The American Naturalist*. 127.5, 593-603.
- Waser, N. M., Chittka, L., Price, M. V., Williams, N. M., and Ollerton, J. 1996. Generalization in pollination systems, and why it matters. *Ecology*. 77.4, 1043-1060.
- Weddell, B.J. 1991. Distribution and movements of Columbian ground squirrels (*Spermophilus columbianus*): are habitat patches like islands? *Journal of Biogeography*. 18, 385-394.
- Westphal, C., Steffan-Dewenter, I., and Tschardt, T. 2003. Mass flowering crops enhance pollinator densities at a landscape scale. *Ecology Letters*. 6, 961-965.
- Westrich, P. 1996. *The Conservation of Bees*. Academic Press for the Linnean Society of London and IBRA, London.
- Whittaker, R.H., and Klomp, H. 1975. The design and stability of plant communities. Unifying concepts in ecology. Springer, Netherlands
- Whittingham, M.J., Stephens, P.A., Bradbury, R.B., and Freckleton, R.P. 2006. Why do we still use stepwise modelling in ecology and behaviour? *Journal of Animal Ecology*. 75:1182-1189.

- Wiens, J.A., Crawford, C. S., and Gosz, J.R. 1985. Boundary dynamics: a conceptual framework for studying landscape ecosystems. *Oikos*. 45, 421-427.
- Wilkins, N., Hays, A., Kubenka, D., Steinbach, D., Grant, W., Gonzalez, E., and Shackelford, J. 2003. Texas rural lands: trends and conservation implications for the 21st century. Texas A&M University, College Station.
- Williams NM, Crone EE, Roulston TH, Minckley RL, Packer L, Potts SG .2010. Ecological and life-history traits predict bee species responses to environmental disturbances. *Biological Conservation*. 143:2280-2291.
- Williams, N. M., Crone, E. E., T'ai, H. R., Minckley, R. L., Packer, L., and Potts, S. G., 2010. Ecological and life-history traits predict bee species responses to environmental disturbances. *Biological Conservation*. 143, 2280-2291.
- Williams, N.M., and Kremen, C. .2007. Resource distributions among habitats determine solitary bee offspring production in a mosaic landscape. *Ecological Applications*. 17, 910-921.
- Williams, N.M., and Winfree, R. .2013. Local habitat characteristics but not landscape urbanization drive pollinator visitation and native plant pollination in forest remnants. *Biological Conservation*. 160, 10-18.
- Williams, N.M., Crone, E.E., Minckley, R.L., Packer, L., and Potts, S.G. .2010. Ecological and life-history traits predict bee species responses to environmental disturbances. *Biological Conservation*. 143, 2280-2291.
- Williams, N.M., Minckley, R.L., and Silveira, F.A. 2001. Variation in native bee faunas and its implications for detecting community changes. *Conservation Ecology*. 5, 7.
- Winfree, R., and Kremen, C. 2009. Are ecosystem services stabilized by differences among species? A test using crop pollination. *Proceedings of The Royal Society: B*. 276, 229-237.
- Winfree, R., Bartomeus I., and Cariveau D. .2011. Native pollinators in anthropogenic habitats. *Annual Review of Ecology, Evolution and Systematics*. 42, 1-22.
- Winfree, R., Griswold, T., and Kremen, C. 2007. Effect of human disturbance on bee communities in a forested ecosystem. *Conservation Biology*, 21, 213-223.

- Woods, M. 2011. The local politics of the global countryside: boosterism, aspirational ruralism and the contested reconstitution of Queenstown, New Zealand. *GeoJournal*. 76, 365-381.
- Xie Z, Williams PH, Tang Y .2008. The effect of grazing on bumblebees in the high rangelands of the eastern Tibetan Plateau of Sichuan. *Insect Conservation and Diversity*. 12, 695-703.
- Yang, C.F. Sun, S.G, and and Guo, Y.H., 2005. Resource limitation and pollen source self and outcross. affecting seed production in two louseworts, *Pedicularis siphonantha* and *P. longiflora* Orobanchaceae. *Botanical Journal of the Linnean Society*. 147, 83-89.
- Zappi, D.C., Filardi, F.L.R., Leitman, P., Souza, V.C., Walter, B.M.T., Pirani, J.R., Morin, M.P., Queiroz, L.P., Cavalcanti, T.B., Mansano, V.F., and Forzza, R.C. 2015. Growing knowledge: an overview of seed plant diversity in Brazil.
- Zurbuchen, A., Landert, L., Klaiber, J., Müller, A., Hein, S., and Dorn, S. 2010. Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. *Biological Conservation*. 143.3, 669-676.