BEE ASSEMBLAGE AND VEGETATION ACROSS A SUITE OF RESTORATION CONDITIONS IN A FIRE-MAINTAINED LONGLEAF PINE SAVANNA

by

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(Under the Direction of L. Katherine Kirkman and C. Ronald Carroll)

ABSTRACT

Pollinator populations are declining worldwide. Habitat restoration may mitigate declines in wild pollinators. I examined the effects of a suite of longleaf pine savanna restoration conditions on bee (Hymenoptera: Apoidea: Anthophila) and vegetation assemblages with particular focus on the practice of prescribed burning. I found that bee assemblages in restored and degraded longleaf savannas differed compositionally from those in reference savannas. Restoration stages in which the forest structure was most similar to reference sites resulted in similar bee and vegetation assemblage composition. Within the burn cycle of reference conditions, bee species richness was found to be higher in the year immediately after fire than two years later partially as the result of an increase in floral abundance after burning. These results suggest that restoration of diverse ground cover plant species and the use of prescribed burning will promote conservation of diverse bee assemblages in longleaf savannas.

INDEX WORDS: pollinators, native bees, longleaf pine restoration, prescribed fire and bee habitat, vegetation and bee species richness

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Project Overview

There is growing evidence of population declines in several North American pollinator species. Loss of appropriate native habitat for some pollinators may be contributing to these declines. Global concern over the status of pollinators has sparked a general interest in pollinator conservation efforts at national and international levels. Such efforts have largely focused on enhancement of degraded plant communities to encourage habitat for native pollinator populations. Specific strategies include removal of non-native species, establishment of corridors of appropriate vegetation to provide refugia and re-introduction of native plant communities that harbor an abundance of bee pollinated species. While interest in restoring pollinator habitat extends to that of the species rich, fire-maintained longleaf pine ecosystem in the southeastern US, little is known about the response of the bee assemblage to such restoration efforts or how restoration sites should be monitored to determine effectiveness of restoration efforts over time. Fire-maintained longleaf pine savannas historically covered the majority of the Southeastern coastal plain of the United States, but most of this ecosystem has been lost because of fire exclusion, land conversion to agricultural use, and urbanization. The goal of the proposed study is to compare bee and plant communities among habitats undergoing longleaf pine savanna restoration and through a biannual fire-cycle in a benchmark longleaf pine ecosystem. This study will assess how restoration trajectories influence bee pollinator communities, and identify indicator species that may be useful for monitoring restoration success.

The following sections review pertinent literature on the general status of pollinator communities with an emphasis on bee species, ecology of bee pollinators in fire-prone habitats, the objectives and approaches associated with restoration of vegetation of the species-rich longleaf pine ecosystem, and pollinator response to restoration projects.

Literature Review

Status of pollinator species

Although numerous studies have found evidence of declines among pollinator species in North America, consensus regarding the implications of these findings in terms of threats to regional biodiversity or reduction in pollinator services remains elusive. Declines in the non-native European honey bees (*Apis mellifera* Linnaeus) are of particular interest because of their importance in agricultural pollination. Data collected by the USDA National Agricultural Statistics Service reveal a decrease in the number of managed honey bee hives from 5.9 million in 1947 to 2.4 million in 2005, while the number of managed hives in Canada has not significantly decreased in that time. Data was only available from 1990-2003 for Mexico, but hive numbers dropped from 2.1 to 1.7 million during that time (NRC-USA 2007). There is far less information on the status of feral honey bees, but some studies have shown catastrophic colony losses in parts of Arizona and California in the 1990's due to infestations of varroa (*Varroa destructor*

Anderson and Trueman) and tracheal (*Acarapis woodi* Reeni) mites (Kraus and Page 1995; Loper et al. 2006). Another study conducted in Arnot Forest, NY showed no significant change in the number of feral hives between surveys conducted in 1978 and 2002, despite reports of varroa mite infestation (Seeley 2007).

Long-term information on the status of other native pollinators in North America is sparse. However, the National Research Council compiled quantitative and qualitative evidence suggesting declines in three species of bumble bees (*Bombus spp.*), two genera of stingless bee (*Melipona* and *Trigona*), one species of butterfly (*Euphydryas editha bayensis* Boisduval), four species of bats (*Leptonycteris, Choeronycteris,* and *Musonycteris spp.*), and two species of hummingbirds (*Selaphorus spp.*) in North America. The authors of the report stressed that without organized long-term monitoring, there is no way of verifying the status of most pollinator species. A monitoring project in Europe (ALARM—Assessing Large Scale Risks for Biodiversity with Testing Methods) found significant declines in native bees and pollinating flies in the United Kingdom and the Netherlands when data collected before and after 1980 were compared (NRC-USA 2007)

Different factors have been linked to declines in managed versus wild pollinator populations. Managed honey bee colonies face a variety of threats, including parasites (varroa and tracheal mites), pathogens (ex. *Paenibacillus larvae* which leads to a disease known as American foulbrood), the widespread use of pesticides, competition with invasive Africanized honey bees (*A. mellifera scutellata* Lepeletier), and small hive beetles (*Aethina tumida* Murray). Parasites, pathogens, and insecticides also threaten the handful of native bee species that are managed for pollination services. Declines in wild pollinators are thought to be due to not only pathogen spillover from managed populations, but also to competition from introduced species, habitat loss and fragmentation, pesticide use, and climate change (NRC-USA 2007).

Although fears of a large-scale collapse in pollinator populations with potential shortages and price increases in many food crops commonly grown in North America have been projected, there is little evidence to date that pollinator declines are strongly affecting production in crops. Regardless of losses in managed pollinator colonies, pollination services appear to be sufficient, even in times of especially high demand (NRC-USA 2007). Linkages between pollinator abundance and reproduction in natural plant populations is not as well understood as in agricultural crops; however, there is some evidence that pollinator declines, coupled with other factors such as fragmentation and competition with invasive species, are having negative effects on wild plant populations (Aguilar et al. 2006). Biesmeijer et al. (2006) suggested a link between observed declines in some pollinator species in Western Europe and declines in obligate outcrossing insect-pollinated plants. Reproduction in this group of plants has been found particularly sensitive to habitat fragmentation and loss of pollinator services, whereas little evidence has been detected of a similar effect on plants with specialized pollinator requirements, another group of species that might be particularly vulnerable (Aguilar et al. 2006). The architecture of natural plant-pollinator communities provides a great deal of resilience to pollinator losses (Ghazoul 2005). Generally, these communities are structured asymmetrically such that specialist pollinators are not necessarily associated with specialist plants; therefore, losses of a few pollinator species should not result in cascading effects on the survival of plant species (Bascompte et al. 2006). Furthermore, some investigators have pointed out that if natural plant communities are resource limited rather than pollination limited, it is unclear how much of a pollinator decline would be necessary to affect reproduction in natural plant communities (Ghazoul 2005). Of course, without baseline assemblage data and monitoring programs, there is no way to predict how many pollinators have been or soon could be lost from plant-pollinator communities.

In many pollinator studies and monitoring efforts, the primary focus is on bee taxa as opposed to pollinator assemblages that include other orders of insects such as beetles, butterflies, moths, as well as other animals; the reason for this is two-fold. First, bees are the most efficient and effective pollinators, and the majority of pollination is carried out by bees worldwide (Michener 2007). Secondly, groups of insect pollinators are often taxonomically diverse and require different trapping methods, so a subset of pollinators is usually sampled because of time, funding, and personnel constraints. This study was focused on bee taxa not only because of their importance, but also to facilitate comparison between the longleaf-wiregrass ecosystem and similar systems in which bee communities have been studied.

Pollinators in fire-maintained ecosystems

Fire is likely to have a complex relationship to bee assemblage dynamics because of immediate direct effects (mortality) and secondary indirect effects on habitat availability and floral abundance. Response to fire is likely to vary among bee species based on life history characteristics, the intensity and frequency of the fire regime, as well as the structural characteristics of the vegetation (Swengel 2001).

A large amount of insect pollinator mortality typically occurs in prescribed fires in prairies and savannas (Anderson et al. 1989), although recovery of these populations usually occurs in 1-2 years post fire (Panzer 2002). The indirect effects of fires are likely to be more influential in shaping bee communities. Most bees nest in places where many forms of disturbance, even low intensity fire, would affect them. Bees that nest above-ground usually build their nests in perennial grasses, forbs, shrubs, and decaying wood, while a larger subset of bees requires access to bare ground to excavate below ground nests (Williams et al. 2010). Only a small proportion of ground nesting bees are likely to die due to soil heating even in more intense fires. The most vulnerable species, mostly in the family Megachilidae, are species that excavate very shallow nests (>10cm) (Cane and Neff 2011). However, data from a series of studies in Mediterranean ecosystems indicated that above-ground nesting bees were 15% less abundant in recently burned areas relative to unburned sites presumably because of habitat destruction. While fire had no significant effect on below-ground nesting bees, above-ground nesting bees were 8 % more abundant in older burn sites compared to unburned sites. It was suggested that below-ground nesting bees were affected more negatively by time since burn because the accumulation of biomass at these sites limited their access to bare ground (Williams et al. 2010). In another study bee species richness was found to be positively correlated with the abundance of nesting resources for above-ground nesting bees (Grundel et al. 2010).

Given that flight distances vary with bee body size (Greenleaf et al. 2007) ranging from less than 200 m (Zurbuchen et al. 2010) to over a kilometer (Osborne et al. 2008) for native bees, the patchiness of burned sites may play a role in species assemblages following fire. The area of the patch burned will presumably influence how quickly that area is recolonized and the composition of the post-fire bee assemblage. When relatively small areas are burned within a matrix containing unburned areas, the composition of the post-fire bee assemblage may be more influenced by indirect effects resulting from changes in vegetation rather than the direct effects of the fire (Swengel 2001).

The severity of the fire is also an important variable controlling composition of post-fire bee assemblages. In Mediterranean studies, sites were designated as recently burned for 2-5 years post-fire (Williams et al. 2010). This ecosystem is considered mature between 30 and 40 years post-fire, and fires are intense and consume almost all canopy trees (Potts et al. 2003). Moretti et al. (2009) compared taxonomic and functional trait differences in bee communities along a time-since-fire gradient in Mediterranean and temperate forests located in Israel and Switzerland, respectively. In both ecosystems, the species composition of the bee assemblage changed along the gradient; however, time-since-fire was found to affect functional aspects of the bee assemblage only in the temperate ecosystem. For example, in recently burned plots a higher proportion of bees that nest in wood and existing cavities occurred than in earlier burns. This effect was not observed in the Mediterranean ecosystem where more intense fires result in more complete combustion of these nesting resources.

Evidence indicating that bee assemblage composition in response to fire may also be highly dependent on structural and taxonomic aspects of the plant assemblage was provided by Grundel et al. (2010). They investigated bee distribution across a canopy-cover gradient in a fire-maintained ecosystem in Northwest Indiana. This study site consisted of a mosaic of anthropogenically disturbed sites, prairie remnants, oak-savannas, woodlands with intermediate canopy cover, oak scrub habitats, and mature hardwood forests. Bee abundance was negatively correlated with canopy cover and positively associated with recent fire frequency, indicating that more bees were found in open habitats, which in this area are managed with frequent prescribed burning. Bee species richness was positively associated with plant richness as well as habitat availability, based on the amount of dead woody vegetation present. Bee assemblage composition was influenced by a combination of these factors. In the grassland ecosystem of Northwest Indiana, there is little to no canopy cover or woody vegetation in the most frequently burned, open plots. Therefore, nesting resources were not abundant for above-ground nesting bees in these areas, and, in fact, the proportion of wood nesting bees was lowest in open habitats.

This finding contrasts with a study in Switzerland where, because of the vegetation structure, recent fire was associated with more above-ground nesting bees (Moretti et al. 2009). It also highlights the importance of addressing fire frequency and intensity and how historic fire regimes and land use have shaped the present-day ecosystem when considering the effects of fire on bees and other organisms. Another aspect of the bee assemblage composition that varied along the habitat gradient in the grassland ecosystem was the percentage of oligolectic bees, or diet-specialists (restricted to a few genera of plants). Higher proportions of oligoleges were associated with open habitats, recent fire (< 2years), abundant native flowering plants, and perennials with 11% of bees in open habitats being diet-specialist compared to only 2% in forests. The proportion of oligoleges was also found to be positively related to proximity of human development.

Grundel et al. (2010) concluded that their findings suggested a positive link between disturbance, in the form of both fire and anthropogenic disturbance, and oligolectic bees.

Longleaf pine woodlands groundcover restoration

Fire-dependent longleaf pine woodlands was once the dominant forest type in the southeastern Coastal Plain, extending between southern Virginia and eastern Texas, covering about 30 million ha. Today, about 97% of the forest in the original range has been lost to land conversion to agriculture and commercial pine plantations and region-wide fire exclusion. The area that remains in natural longleaf pine stands is highly fragmented (Van Lear et al. 2005). The canopy of these forests is typically dominated by widely spaced longleaf pines, and the groundcover is extremely diverse for a temperate forest, with species densities reaching 40-50 m^{-2} (Kirkman et al. 2001). Groundcover species are mostly perennial herbaceous forbs or low woody shrubs interspersed within a matrix of perennial grasses. The grasses, along with fallen pine needles, act as fuel for the frequent low-intensity prescribed fires that are crucial for the maintenance of this ecosystem (Hendricks et al. 2002). These fires control the encroachment of woody species that would otherwise shade out groundcover (Kirkman et al. 2004). In the preceding two decades, awareness of the degradation of this unique ecosystem has grown and numerous restoration efforts in the Southeast have been implemented (ALRI 2011).

Starting conditions of longleaf pine restoration range from cultivated agricultural fields, off-site planted pine stands to fire-suppressed pine forests with hardwood encroachment, or even hardwood dominated forests. The diverse groundcover plant community that is characteristic of frequently burned longleaf pine woodlands and savannas is not likely to be present in any of these degraded land cover types. Trusty and Ober (2011) found that the factors most commonly associated with successful groundcover restoration in southeastern forests were season of planting, the presence of an intact tree canopy, and burning after plantings had time to become established. Given that much of the area that was historically longleaf savanna has now been converted to planted slash (*Pinus elliottii* Engelm.) or loblolly (*Pinus taeda* L.) plantations, some researchers recommend using these undesirable species as a structural and functional bridge as part of a gradual restoration plan, rather than removing the forest canopy and starting from scratch. There is evidence that this approach results in less hardwood dominance, increased fuel from needle cast, and more effective prescribed fire necessary to promote and retain herbaceous ground cover (Kirkman et al. 2007). Depending on the pre-restoration state of groundcover, the restoration objectives and the availability of seed, practitioners may opt to plant a mix of legumes, asters, and grasses. Alternatively, they may plant only grasses if it is determined that the most important goal is returning fire to the area. Often the establishment of wiregrass (*Aristida stricta* Michx.) is emphasized because it is thought to promote even, low-intensity fires, and it is usually absent in areas that have been cultivated.

I have found no reports where a return of healthy pollination function was considered as a specific goal or metric in assessing the success of a longleaf grassland restoration project. However, there is emerging interest in pollinator conservation in this region through land owner incentive programs for reforestation of longleaf pine stands (USDA-NRCS 2011a). In addition, conservation activities are being actively funded and promoted by non-profit organizations for establishment of habitat for pollinators and other beneficial insects in restored longleaf pine forests (The Xerces Society; USDA-NRCS 2011b). Longleaf restoration initiatives in general have made progress in the last decade with 350,000 acres planted or maintained through the Conservation Reserve Program (CRP). The Federal Coordinating Committee recently finalized a plan involving multiple agencies and programs to increase the area of longleaf pine forests in the Southeast from 3.4 million acres to 8 million acres in 15 years (ALRI 2011).

Pollinator assemblages in restored ecosystems

Conservation of native pollinator species is often proposed as a way of buffering against possible shortages in agricultural pollinators. Healthy wild bee populations are in many cases crucial for agricultural pollination in the surrounding landscape (Garibaldi et al. 2013), so restoring habitat for these organisms in landscapes used for agriculture can provide an important ecosystem service. Until recently honey bee pollination was thought to adequately substitute for pollination by wild insects for crops which require animal pollination, but Garibaldi et al. (2013) found that in all 41 crop systems studied wild pollinators had a positive association with fruit set compared. This association only existed in 14% of crop systems for honey bees. These results suggest that communities of wild pollinators are actually more effective at crop pollination than wild or managed honey bees.

Promoting native pollinators is also seen as a means of assuring the health and proliferation of wild plant communities. Most restoration efforts focus on reestablishing the plant community with the assumption that non-target species, such as pollinators, will recolonize on their own. Studies investigating bee assemblage response to restored forest vegetation have yielded mixed results, suggesting that the rate at which bee assemblages recover to a reference state likely varies considerably depending on the structure and complexity of the ecosystem. For example, Fiedler (2010) investigated the effects of the removal of invasive *Frangula alnus* Mill. from a Michigan prairie fen habitat and found that while pollinator assemblages were depauperate in invaded areas, once the invasive species was removed, pollinators seemed to recolonize the area quickly. Similarly, Hanula and Horn (2011) found that two years after the removal of Chinese privet (*Ligustrum sinense* Lour.), bee assemblages were similar to those found in reference plots. In both studies, post-restoration plant assemblages were significantly different than reference plant communities suggesting that the bee response was more directly tied to the structure of the vegetation rather than to the successional development of the plant communities (Hanula et al. 2009; Fiedler 2010). These studies employed restoration practices that resulted in a substantial disturbance, freeing areas from invasive shrubs that had excluded most other plant species. It cannot be concluded that the transitional ecosystem which emerged immediately following restoration will persist into the future, thus the long-term composition of pollinator communities in these systems remains unclear.

Analyses of plant-pollinator networks and function in older restoration sites have also yielded varied results. Williams (2011) concluded that while pollinator function had been restored in several California riparian restoration plots, it was still less robust than at reference sites based on the finding that there was lower redundancy of bees visiting plants at restored sites. By studying the level of connectance in plant-pollinator networks, or the number of actual connections as a proportion of all possible pairwise connections, similar studies concluded that plant-pollinator networks were very similar in restored and reference hay meadows (Forup and Memmott 2005) and, conversely that networks were significantly less complex in restored compared to reference heathlands (Forup et al. 2008).

As part of a study which compared bee communities in upland and wet flatwood longleaf pine forests, Bartholomew and Prowell (2006) found that bee diversity in recently restored sites was similar to that in sites which had been restored less recently. The researchers compared bee communities between sites which had been managed for longleaf for about 2 decades to a more recently acquired site which had been restored about a decade before the study. Although these sites were returned to a frequent burn regime, little information about the vegetation structure was provided; thus the relationship of these efforts to the various restoration trajectories discussed above is unclear. It was noted that the study was not designed to investigate the effects of restoration on bee communities.

Objectives

The objectives of this study were (1) to compare bee abundance and composition among degraded longleaf forests and those undergoing various types of restoration management to that of reference longleaf pine savannas, (2) to assess the effect of frequent, low-intensity prescribed burning on bee communities, (3) to examine the influence of vegetative structure and floral resources on bee assemblage composition, (4) to identify indicator species of the reference and restoration sites that may be useful for monitoring restoration progress, and (5) to compare relative abundance of functional guilds of species (food specialists and generalists, above-ground and below-ground nesting) across a longleaf pine restoration treatments and through the fire-cycle in reference sites.

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CHAPTER 2

BEE ASSEMBLAGES AND VEGETATION IN REFERENCE, RESTORED, AND DEGRADED LONGLEAF PINE SAVANNAS[1]

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Abstract

There is growing concern about population declines in several North American pollinator species, and loss of appropriate native habitat for some pollinators may be a contributing factor. While interest in restoring pollinator habitat extends to the species rich, fire-maintained longleaf pine ecosystem in the southeastern US, little is known about the response of the bee communities to such restoration efforts or how restoration sites should be monitored to determine effectiveness over time. Fire-maintained longleaf pine (*Pinus palustris*) savannas historically covered the majority of the Southeastern coastal plain of the United States, but most of this ecosystem has been lost because of fire suppression, land conversion, and urbanization. We investigated the effects of fire and forest cover on bee communities in a suite of restoration conditions. Specifically, we measured bee assemblage composition in reference longleaf savannas, fire suppressed longleaf forests, young longleaf plantations with and without ground cover reintroduction, and mature slash pine (*Pinus elliottii*) forests that are being converted to longleaf pine. Bee sampling was conducted using a combination of pan trapping and standardized netting transects. We also assessed how factors such as plant assemblage characteristics, habitat availability, and forest structure affect bee assemblages.

Bee and plant communities found in 75 year old frequently burned slash pine stands were most similar to those found in longleaf reference sites likely because of the similarities in percent canopy cover and ground cover composition. Although bee species richness in young planted pine stands was similar to that of reference longleaf pine savannas, plant species richness was significantly lower in these habitats compared to

reference plots, indicating that the plant-pollinator communities in these sites had not reached a reference condition.

Introduction

In recent decades, population declines of pollinators have been documented in ecosystems all over the world (Biesmeijer et al. 2006; NRC-USA 2007). Bees are generally considered the most efficient and effective pollinators (Kearns et al. 1998, Michener 2007); thus declines in both native bees as well as European honey bees (*Apis mellifera*) are of particular economic and conservation concern. In addition to widespread use of pesticides, changing climate, parasites, disease, and competition from introduced species, habitat loss is likely a significant cause of population declines of bees. Urban development, landscape fragmentation, as well as intensive silvicultural and agricultural practices tend to decrease the abundance and diversity of floral resources and the availability of some types of nesting sites (NRC-USA 2007). As a result of the reported declines, habitat enhancement for wild, unmanaged bees that may mitigate population decline has emerged as regional, national and global conservation priorities (Vanbergen et al. 2013).

Management strategies to directly promote pollinators have included the introduction of carefully selected plant species to aid in attracting and supporting specific pollinators (Molina-Montenegro et al. 2008; Dixon 2009), selection of target restoration plant species based on functional complementarity or redundancy with respect to pollination services (Devoto et al. 2012), and the creation or maintenance of habitat corridors between habitat fragments (Townsend and Levey 2005). In practice, though, managers usually lack the funds and background knowledge to incorporate specific practices focused on promoting pollinators into restoration plans (Dixon 2009).

Natural community restoration success is typically measured by monitoring the establishment of plants or other target organisms. However, some non-target organisms, such as pollinators, could be important to consider not only as a measure of restoration success but because of their likely role in propagating the system through promoting plant reproduction. Facilitating wild bee populations through restoration may also have a secondary positive effect on pollination in surrounding agricultural systems. Though managed honey bee colonies are usually assumed to provide adequate or even superior pollination services to crops which require animal pollination (Ghazoul 2005), Garibaldi et al. (2013) found that in crop systems on six continents, wild pollinators actually provided more effective pollination, measured by their association with increased fruit set, than honeybees.

Where native bees have been monitored following natural plant community restoration projects, they have been found to recolonize quickly (Fiedler 2010; Hanula and Horn 2011), but bee communities in restored habitats sometimes differ in important ways from those found in reference conditions. For example, Williams (2011) concluded that while pollinator function had been restored in several California riparian restoration sites, it was still less robust than at reference sites. In other studies conducted in Great Britain, the degree of reassembly of plant-pollinator networks varied strongly by habitat. Plant pollinator networks of restored hay meadows attained similar structure to that of reference sites (Forup and Memmott 2005) whereas, networks were significantly less complex in restored compared to reference heathlands (Forup et al. 2008).

In the southeastern United States considerable restoration efforts have been initiated in recent decades in the longleaf pine (*Pinus palustris*) savanna ecosystems. Longleaf savannas are fire-maintained ecosystems characterized by a canopy of widely spaced longleaf pine, little to no shrub layer, and a diverse groundcover layer of perennial forbs, shrub, and grasses (Kirkman et al. 2001). Historically the dominant forest type on the southeastern Coastal Plain, longleaf savannas have been destroyed or degraded on 97% of their original range due to fire exclusion, land conversion, and urbanization (Van Lear et al. 2005). America's Longleaf Restoration Initiative is a collaboration of public and private sector partners with the goal of restoring 4 million acres of longleaf savannas (ALRI 2011). These initiatives have made considerable progress with the help of federally incentivized programs such as the USDA Conservation Reserve Program (CRP), and recently non-profit and government organizations have been promoting and funding the establishment of habitat for pollinators and other beneficial insects in restored longleaf pine forests (The Xerces Society; USDA-NRCS 2011b). Conservation and restoration of plant species rich ecosystems with abundant floral resources, such as longleaf savannas, is especially important for maintaining pollinator communities. Ebeling et al. (2008) found that high flowering plant species richness was associated with high pollinator species richness and high frequency and stability of floral visitation, which may contribute to functional redundancy in pollinator assemblages and stability of pollination services. I have found no report in the scientific literature in which a return of healthy pollinator function was considered as a specific goal or metric in assessing the success of a longleaf restoration project.

The goal of this study was to determine the relationship between bee assemblages and vegetation of natural fire-maintained longleaf pine stands representative of reference condition with that of longleaf pine stands degraded by fire exclusion and disturbed sites in various stages of restoration. Specifically, I compared the number bee captures and assemblage composition among degraded longleaf forests, reference sites, and prior agricultural sites undergoing different phases of restoration management. I examined the plant assemblages at these sites to determine the influence of floral resource availability and vegetation structure on bee species assemblages. Lastly, I identified indicator species of reference and restoration sites that may be useful for monitoring restoration progress.

Methods

Study Area

To compare bee assemblage composition, I sampled bees in 15 locations in southwestern Georgia (Baker, Calhoun, and Decatur Counties), which represented a range of pre-restoration conditions or stages in the process of reassembling components of longleaf pine assemblages. Five habitat types with vegetation varying in structural complexity and species composition of the canopy, midstory and groundcover included: 1) reference longleaf savannas with intact, high-diversity groundcover community, open canopy, and no mid-story cover (REF); 2) slash pine (*Pinus elliottii*) to longleaf pine conversion plots with restoration groundcover community, open canopy, and no mid-story cover (SLR); 3) old field restoration plots managed under the Conservation Reserve

Program with planted longleaf pines <10 years old, restoration groundcover community, no canopy, and no mid-story cover (CRP); 4) immature planted longleaf pine stands (10-15 years old) with suppressed groundcover community, open canopy, and no mid-story cover (LLP); and 5) fire-excluded, mature longleaf stands with suppressed groundcover community, closed canopy, and dense mid-story cover which had not been burned within the last 10 years (FEX) (Table 2.1). All sites except fire-excluded sites, had been burned historically on a 2-3 year rotation and were burned during the winter or spring before the study began (between January and April 2013). Reference sites had been burned frequently for over 80 years as part of northern bobwhite quail management efforts on the property.

With the exception of CRP sites, three replicates of each stand type were located at the Joseph W. Jones Ecological Research Center at Ichauway, an 11,700 ha privately owned property located on the Coastal Plain of southwestern Georgia (Baker County). This property includes large tracts of 100 year old, second growth natural longleaf pine forests managed with frequent prescribed fire. A diverse ground cover dominates much of the longleaf pine uplands, representing desired future conditions of restoration, or reference conditions. Restoration efforts on degraded upland sites have been implemented throughout to promote the re-establishment of multi-aged and species diverse longleaf pine-wiregrass savannas. These efforts include conversion of young planted longleaf pine stands, mature planted slash pine stands, and fire-suppressed mature natural longleaf pine stands. Former agricultural lands undergoing restoration as part of the CRP Longleaf Initiative were located on private properties in adjacent counties. In compliance with the CRP programs, these former fields had been planted with longleaf pine seedlings and seeded with grasses and forbs. This restoration activity has been adopted on over 84,000 ha in Georgia (USDA-FSA 2014).

The prescription of a particular strategy for restoration of a longleaf pine stand depends on the initial condition of a site and overall objectives. In the case of young planted longleaf pine stands on previously cultivated sites, the pines are generally planted at a density of 1000-2000 trees per ha and burned regularly to discourage ruderal plants and hardwoods. On these sites where the groundcover plant community has been damaged or destroyed by tillage for agriculture, the first objective is to restore a multi-age longleaf canopy over time. Sites managed under the CRP take this strategy one step further by incorporating ground cover plant reestablishment into the early stages of restoration. The CRP provides landowners with federal financial incentives to plant longleaf pine seedlings on former cropland which is located within the historic range of the longleaf pine ecosystem. Through a cost-share arrangement, landowners are required to manage lands using prescribed burning and are encouraged to plant native grasses such as wiregrass (*Aristida stricta*), Indian grass (*Sorghastrum spp.*), switchgrass (*Panicum virgatum*), and little bluestem (*Schizachyrium scoparium*), and native forbs such as partridge pea (*Chamaecrista fasciculata*) and lespedeza (*Lespedeza spp.*) between rows of planted longleaf pine seedlings (USDA-NRCS 2006). The strategy with mature planted slash pine stands is characterized by a gradual conversion to longleaf pine-dominated assemblages. Instead of clear-cutting the slash pine canopy and planting longleaf pines, slash pines were periodically harvested using a combination of harvesting methods, single-tree selection and gap harvesting. In the canopy gaps created by harvesting, longleaf pines and wiregrass were planted. Land managers have employed prescribed burning and hardwood removal and suppression throughout this gradual restoration process (see Kirkman et al. 2007 for detailed description of slash pine conversion study). The fire-excluded sites represent natural stands of longleaf pine in which fire has been excluded for more than a decade resulting in establishment of an understory of deciduous hardwoods.

I based study site selection at Ichauway on land cover type and spatial distribution across the entire property depending on the total area of land cover type available. Selection of study sites on privately owned lands off of Ichauway was determined by proximity to Ichauway, date of CRP planting, and landowner cooperation. At each study site location, I established a 100 x 200 m plot for sampling bees, flowering plants, and vegetation structure (Figure 2.1). Because some bee species can forage long distances (Greenleaf et al. 2007), I located bee sampling transects at least 150 m from any area with a significantly different land cover type whenever possible to ensure that the bees collected were associated with the focus habitat type. In the case of burn excluded areas and CRP sites, the tracts were not large enough to accommodate a 150 m buffer, so sampling transects were located at least 50 m from adjacent land cover types. With the exception of SLR sites, all plots were at least 700 m from each other. The slash pine restoration project (Kirkman et al. 2007) was conducted in three adjacent stands, so plots were located in the interior of these as far apart as possible (about 300 m).

Bee sampling

To sample bee assemblages at each study site, I used standard pan trapping and aerial netting techniques (Droege 2012). These two methods are considered complementary because pan trapping tends to over sample small bees and under sample larger bees, whereas, small and fast-flying bees are often missed with aerial netting (Droege 2012), and they have been used in many similar studies, including one in longleaf savannas (Bartholomew 2004). In each 0.2 ha plot, I established a 200 m sampling transect.

Within the 200 m transect, I placed 15 pan traps equidistant along a 100 m sub-transect located in the center of the larger transect. Pan traps consisted of white Solo brand 96 ml plastic cups (stock number P325w-0007). At each sub-transect, I used 15 traps (five each of white, fluorescent blue, and fluorescent yellow; fluorescent paint source: Guerra paint and pigment, guerrapaint.com). I placed each trap in a 9 gauge steel wire loop with a stem which held it 30 cm above the ground. For each sampling event, I filled trapping pans ¾ full with a solution of soapy water (1.3 ml of Dawn brand dish soap per liter of water). Bees are attracted to these traps, and the soap reduces surface tension causing the bees to sink and drown. Traps were left out for 24 hours, and then bees in all traps along a sub-transect were collected in one filter and rinsed with water. Filters containing the bees were allowed to air-dry for a few hours and stored in a freezer (-12°C). Specimens were pinned within a few days of capture.

During each sampling event, I also collected bees by aerial netting. Walking slowly along the 200 m netting transect, I caught all bees observed for two periods (30 minutes each), once between 8:00 a.m. and 12:00 p.m. and secondly between 12:00 p.m. and 3:00 p.m. Netted bees were killed using ethyl acetate and pinned. I conducted bee sampling once per month in all sites between May and September 2013 and in April

2014. To avoid effects of adverse weather on bee capture rates (Droege 2012), I conducted bee sampling only on sunny or partly cloudy days with low wind speeds (<13 mph). I identified all specimens to species or the lowest taxonomic level possible using all available keys appropriate to the region (Mitchell 1960, 1962; Gibbs 2010, 2011; Ascher and Pickering 2014). Bee species identifications were verified by Sam Droege (USGS Patuxent Wildlife Research Center). Male *Lasioglossum* specimens were not identified and were excluded from analysis. A few other specimens which could not be positively indentified to species were categorized by morphospecies and included in the analysis. I classified each bee species relative to nesting preference (above- or below-ground) and degree of feeding specialization (oligolectic or polylectic) based on life history data in published literature (Mitchell 1960, 1962; Michener 2007). Bees were classified as oligolectic if their floral visitation is restricted to one plant family, while all other bees with more generalized feeding practices were classified as polylectic. Voucher specimens for all bee species collected are archived at the Georgia Museum of Natural History (University of Georgia, Athens, GA).

Vegetation Sampling

To determine the relationship of floral resources with bee assemblages, I recorded all open flowers in a 1 m strip along the middle 50 m of the 200 m transect in each plot at each bee sampling event. For each flowering plant present, I recorded number of individuals per species, and abundance class of flowers per plant (1-10, 11-100, 101-1000). Floral abundance was estimated by multiplying the number of plants in flower by the midpoint of the abundance class of flowers per plant. For any plant species that could not be identified in the field, I collected a specimen for verification in the lab.

To characterize other vegetation composition and structural variables that might influence bee nesting resources among habitat types, I sampled vegetation of each plot in fall 2013. I established five circular subplots (radius=11.3 m) in each main plot (10% of the total area of the plot), one at the center and one located in each corner. In each subplot, I measured six metrics. To estimate percent canopy cover I used a hand-held convex spherical densiometer (Forestry Suppliers, Jackson, MS) positioned in the center of each subplot to obtain readings in 4 opposing directions (Strickler 1959). I measured tree diameter at 1.4 m height (diameter at breast height, DBH) for all of trees greater than 10 cm DBH to obtain total basal area. To quantify abundance of trees and shrubs between 2.5 and 10 cm DBH, I recorded the number of individuals present by species. The presence of dead woody vegetation on the ground (coarse woody debris, CWD) was recorded by size class (1=2.5-5 cm, 2=5-10 cm, 3=10-20 cm, 4=20-40 cm, and 5=40-80 cm) Maser et al. (1979). CWD index was calculated by multiplying the midpoint of size classes by the abundance as an approximation of CWD volume. Within each circular subplot, I recorded all plant species present in 4 randomly located 1 x 1m frames. I also recorded percent cover of woody species, forbs, graminoids, and bare ground/litter (cover classes: 0=0%, 1=1-25%, 2=26-50%, 3=51-75%, and 4=76-100%).

Statistical Analyses

I compared bee species richness (number of species), captures, evenness, Shannon-Wiener diversity, and Chao 1 estimated species richness among habitat types using analysis of variance (ANOVA) with R package stats and carried out post hoc means comparisons using Tukey's HSD test in R package agricolae (De Mendiburu 2014; R Development Core Team 2014). Bee counts were natural-log transformed so that assumptions of normality and homogeneity of variance were met. Shannon-Wiener diversity and Chao 1 estimated species richness were calculated in R package vegan (Oksanen et al. 2013). The Chao 1 estimator was chosen because it accounts for a large number of rare species with a correction factor (Chao 1984). I also compared proportions of bee captures and bee species richness within important functional groups (diet specialization, nesting preference) among habitat types using ANOVA on arc-sine square root transformed data. I examined monthly patterns in bee captures and species richness with a repeated measures ANOVA in R package car (Fox and Weisburg 2011). This analysis employs the Mauchly test of sphericity and applies Greenhouse-Geisser p-value correction (G-G) if there is a departure from sphericity. Rarefaction curves were calculated on bee samples to investigate the effect of number of individuals sampled on the number of species caught. For this analysis, we combined all data recorded at each habitat type (18 samples with three replicates each sampled six times over the course of the study). Rarefaction curves were generated with EstimateS software, 9.1.0 (Colwell 2013).

To examine bee assemblage composition among restoration habitat types I conducted non-metric multidimensional scaling (NMDS) analysis based on Bray-Curtis (Sørenson) distances using R package vegan (McCune and Grace 2002, Oksanen et al. 2013). I used Multi-response Permutation Procedure (MRPP) followed by pair-wise t-tests to determine if bee assemblage composition differed significantly among habitat types (PC-ORD, McCune and Mefford 2011). Bee count data were natural-log transformed for NMDS and MRPP to decrease the influence of rare species. I examined the influence of vegetation and forest structure metrics on bee assemblage composition by generating environmental fit vectors for bee assemblage ordinations in R package vegan (Oksanen et al. 2013). Forest structure data were examined to determine if assumptions of normality and homogeneity of variance were met and transformed when necessary. Indicator species analysis was used to identify indicators of reference or restoration sites (R package indicspecies, De Caceres and Legendre 2009). This analysis was also used to identify indicator species of habitat pairs. For that analysis, I was especially interested in pairs of habitats with similar forest structure or management history. In indicator species analysis, *A* is the probability that a site belongs to the target site group given that a species is present. *B* is the probability of finding a species in the sites belonging to a site group. *P*-values were calculated using the Monte Carlo method with 999 permutations. Bee species were considered indicators only if they represented more that 3% of the total individuals sampled.

I performed ANOVA on all vegetation and forest structure metrics, including the species richness of plants in flower and floral abundance pooled over all sampling months, to characterize differences among sites. Floral abundance data were log transformed for analysis. I examined monthly patterns in floral abundance and species richness with a repeated measures ANOVA in R package car (Fox and Weisburg 2011). An ordination was also generated for plants in flower (Bray-Curtis) using the log transformed number of plants of each species in R package vegan (Oksanen et al. 2013). Differences in ordination space among habitat types were analyzed using MRPP in R package vegan (PC-ORD, McCune and Mefford 2011).

Results

Bee richness and number of captures

Over the course of this study, I collected 3083 bees from 96 species, 34 genera, and all 6 North American families. Halictidae was the dominant family (73.9% of individuals) followed by Apidae (22.5%), Megachilidae (3.8%), Andrenidae, Colletidae, and Melittidae (each <1%). The most common species was *Lasioglossum reticulatum* (566 individuals), and seven out of the ten most common species were *Lasioglossum*. Only three species were found in all sites: *Lasioglossum tegulare*, *Bombus impatiens*, and *Melissodes communis*. About 30% of species (29 species) were only collected once, and 14% (13 species) were only collected twice.

Collectively for the study period, bee captures differed by habitat type ($F_{4, 10} = 8.2$, $P = 0.003$, Figure 2.2a), with more bee captures in CRP and LLP than in FEX or SLR. On a monthly basis, bee captures differed among habitat type, sampling month, and habitat x month interaction ($F_{4, 10} = 3.6$, $P = 0.045$, $F_{5, 50} = 7.0$, $P > 0.0001$; and $F_{20, 50} = 2.1$, $P = 0.019$, respectively, Figure 2.3a). Bee captures differed among habitat types in

May, June, and September of 2013 ($F_{4, 10} = 5.3$, $P = 0.015$; $F_{4, 10} = 21.1$, $P < 0.0001$; and $F_{4, 10} = 5.5$, $P = 0.013$, respectively, Figure 2.3a, Table 2.2a).

No differences in total bee species richness (Figure 2.2b), Chao1 estimated species richness (Figure 2.2c), Shannon-Wiener diversity, and evenness occurred among habitats. Likewise, no differences occurred among habitats for the estimated number of species compared at a common number of individuals sampled (n=335) based on 95% confidence intervals (Figure 2.4). Further, none of the rarefaction curves reached an asymptote, which suggests that the sampling may under-represent total potential species richness (Figure 2.4, Gotelli and Colwell 2011). Bee richness varied by month ($F_{5, 50} = 7.8$, $P < 0.0001$, Figure 2.3b), but not among site types. No interaction occurred by month and site. Bee richness was higher in late spring to early summer, peaking in all habitat types in May or June 2013, and lower in September 2013 and April 2014 ($F_{5,84} = 5.1$, $P = 0.0004$).

The relative abundance of oligolectic bees captured was higher in SLR and REF plots and lower in CRP plots ($F_{4, 10} = 4.5$, $P = 0.025$, Figure 2.2d). A similar, but weak trend occurred for the proportion of oligolectic bee species sampled by habitat ($F_{4,10} = 2.7$, $P = 0.095$). In contrast, the proportion of species and individuals represented by nesting guilds did not differ among habitat types ($F_{4, 10} = 1.2$, $P = 0.387$ and $F_{4, 10} = 1.9$, $P = 0.186$, respectively).

Habitat characteristics and bee composition

Habitat types differed in tree basal area, percent canopy cover, shrub abundance, percent cover of woody plants, CWD index, groundcover plant species richness, the percentage of exotic and annual species of plants in flower, and the relative abundance of exotic and annual flowers (selected data in Figure 2.6). CRP sites had very low canopy cover, cover of woody plants, CWD abundance (index), and plant species richness as well as high proportions of exotic and annual flowers. LLP sites were somewhat similar to CRP, except that canopy cover was higher in these sites and, while there was an equally high proportion of annual flowers, LLP sites had few exotic flowers. REF and SLR sites were similar in canopy cover, basal area, and shrub abundance and SLR was the only group with equally high floral and plant species richness as REF. SLR sites did have significantly higher amounts of CWD compared to REF, which was the result of a recent pine tree harvest in these sites. FEX sites were characterized by very high canopy cover, shrub abundance, and CWD index, low plant species richness, and low proportions of exotic and annual flowers.

Bee composition based on species abundances differed by habitat in ordination space (MRPP: $A = 0.23$, $P < 0.0001$, Figure 2.5). The MRPP and pair-wise analyses of dispersion of sample units in ordination space confirms that site of the same habitat type were more similar to each other in bee composition than to other habitat types (Table 2.3). Based on species presence-absence, bee composition of site types also differed in ordination space (Jaccard; 2-dim; stress=0.17; axis1= 36.9%; axis2 = 24.4%); MRPP results were also similar ($A = 0.12$, $P = 0.001$).

Mean values of percent canopy cover, CWD index, percent cover of woody plants, the relative abundance of annual flowers, species richness of plants in flower, and floral abundance are correlated with the bee abundance based ordination (Figure 2.5).

Bee assemblages in FEX habitat were correlated with high abundance of CWD, high percent canopy cover, and high percentage of woody groundcover plants. Floral abundance was inversely related to CWD and canopy cover and was most closely associated with CRP habitat. The relative abundance of annual flowers was associated with CRP and LLP bee assemblages, while high floral species richness was most closely associated with REF bee assemblages. Nine indicator species associated with four of the 5 site types (Table 2.4a) and 5 indicators of 5 habitat pairs (Table 2.4b) were identified.

Floral abundance, species richness, and composition

The number of species and total abundance of flowers (pooled data for all months differed by habitat type ($F_{4, 10} = 19.3$, $P = 0.001$ and $F_{4, 10} = 7.5$, $P = 0.005$, respectively, Figure 2.2) with higher species richness in REF sites than CRP and LLP sites (Figure 2.2e) and floral abundance was less in FEX sites relative to all other habitat types (Figure 2.2f). Differences in flower abundance occurred by habitat type and month and no interaction occurred ($F_{4, 10} = 6.9$, $P = 0.006$, $F_{5, 50} = 4.4$, G-G corrected $P = 0.016$; and $F_{20, 50} = 1.7$, G-G corrected $P = 0.131$, respectively, Figure 2.3c). Flower abundance was highest in September and lowest in May ($F_{5, 84} = 2.7$, $P = 0.028$, Figure 2.3c). Overall, this pattern is inverse of that observed in bee captures; however, when pooled over sampling months, bee and floral abundance exhibit a weak positive relationship ($R^2 = 0.292$, $P = 0.046$). Flowering plant species richness differed by habitat type, month, and the habitat type x month interaction ($F_{4, 10} = 7.1$, $P =0.005$, $F_{5, 50} = 3.9$, $P = 0.005$; and $F_{20, 50} = 2.2$, $P = 0.013$, respectively, Figure 2.3d). Differences in richness occurred between habitats only in June and July. Most notably in these months, the number of species in REF exceeded that of FEX (Figure 2.3d, Table 2.2b). The composition of species in flower based on abundances differed among habitat types in ordination space (MRPP: $A = 0.29$, $P < 0.0001$, Table 2.3, Figure 2.5). The habitat types most similar compositionally to each other in floral species were consistent with that of bee composition.

Discussion

The similarity in bee species richness and number of captures in restoration and degraded habitats to that of reference habitats in this study provides evidence that these disturbed habitats support diverse bee assemblages. However, distinct differences in assemblages of bee species are present among habitat types. The observation that habitats with most similar floral assemblages also had similar bee assemblages could not fully be explained by variations in floral reward throughout the sampling season. Although bee and floral abundance were weakly, positively correlated when data were pooled over the months, the peak in bee captures in spring and peak floral abundance and species richness later in the year suggests that factors other than floral reward influence seasonal bee captures. Furthermore, the finding that seasonal patterns in bee captures in structurally similar habitat types were similar implies that such factors affect bee assemblages differently in different habitat types. Given that no differences in bee nesting guild composition among habitats was observed and seasonal differences in nesting habitat were not measured, it is not clear that seasonal differences in the availability of nesting habitat is an explanatory factor.

It should also be noted that the bee assemblage observed in CRP habitat is likely to be dependent on the selection of the few plant species included in the restoration seed mixes. Generally, these sites were dominated by exotic and annual plants instead of fire-adapted native perennials that are characteristic of longleaf savannas. In the region where this study was performed, local commercially available seed sources and species are limited. As was the case with two of the three CRP sites in this study, a non-native, such as *Lespedeza bicolor* and an aggressive cultivar of *Chamaecrista fasciculata*, have been used in restoration plantings in lieu of native legume species. Often these plants have abundant flowers and bees were especially abundant and species rich in these sites. However, in these sites, the bee assemblage differed from another CRP site in which only native species were used. Although this latter site was also dominated by ruderal plant species it supported a lower bee abundance and richness and different assemblage of bee species than other CRP sites thus, presence of non-native species in the seed mix seems to have a large effect on bee assemblages in CRP restoration projects. Further, given the aggressive and competitive characteristics of species such as the non-native planted species in this study, the implications for future groundcover composition in a restoration context and relationship to bee use in these CRP sites is uncertain. Additional long term investigation of the response of bee assemblages to plant community restoration changes will be required to understand the successional relationships between bee and plant taxa over time.

Of the restoration habitat types, SLR sites were most similar to reference sites in vegetation structure. These sites are equal to reference sites in canopy cover, basal area, shrub abundance, and floral abundance, and they were the only group with equally high floral and plant species richness as reference habitats. Futher, ordinations indicated that although bee assemblage composition differed between them, REF and SLR were more similar to each other than other habitat types. While it remains unclear what accounts for the difference in the bee composition between these two similar habitats, it is important to note that the close spatial proximity of SLR sites (about 300 m apart) may partially explain the greater similarity among SLR sites relative to similarity with that of REF sites. The facts that in both REF and SLR habitats, bee assemblages were characterized by a high percentage of oligolectic bees, and one oligolectic bee species (*Cemolobus ipomoeae*) was found to be an indicator of this habitat pair are particularly noteworthy in regard to the frequent fire, presence of numerous native plant species, and relatively open canopy in these two habitat types. Oligolectic bees have been associated with open habitats, recent fire (< 2 years), and native, perennial flowers, as well as proximity to human development in a gradient of prairie to closed forest (Grundel et al. 2010), suggesting a link between disturbance (fire and soil disturbance) and oligolectic bees. In my study the associations among canopy openness, disturbance, and oligoletic bees are not as clear. Even though other habitat types also had open canopies and recent fire, they lacked the abundance of native perennial species, suggesting that link between native perennial flowering plants and oligoleges may be of significance. Interestingly, the majority of oligolectic bees in SLR and REF sites were collected while foraging on *Ipomoea pandurata* (bee species: *Melitoma taurea* and *Cemolobus ipomoeae*) and *Vaccinium* species (bee species: *Habropoda laboriosa*); these plants were not found in the LLP or CRP sites. This finding indicates a potential need to include plant hosts in restoration seed mixes for targeted conservation of oligolectic bees.

Although this study did not reveal differences in bee nesting guilds, there may be important differences in soil types or above-ground nesting resources which were not measured in this study. Measurement of nesting habitat could be improved by carrying out a more detailed assessment of which soil types provide the best habitat for bees which excavate nests and including fine scale classifications of CWD and plant stem habitat. In addition, the specific nesting habitats of a several species captured in this study were poorly known limiting the detail of nesting guild classification. There were also constraints on the size and location of restoration habitats for the study.

In summary, the results of this study indicate that restored and degraded longleaf savannas can support diverse bee assemblages; however, these assemblages may differ from reference longleaf bee assemblages in substantial ways, such as the proportion of oligoletic bees. Oligolectic bees are often rarer than polylectic bees (Grundel 2010; Goulson and Darvill 2004), and declines in diet specialists have been tied to decreasing species rich grasslands in the UK (Goulson et al. 2005). Our study provides evidence that conservation and restoration of this the diverse ground cover of the longleaf pine ecosystem may be especially important for maintaining bee diversity. It also strongly identifies the need to examine restoration practices such as the CRP over time to determine the changes in bee and plant relationships in a restoration context.

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Table 2.1. Site descriptions

	REF	*SLR*	*CRP*	*LLP*	*FEX*
Age of dominant pines	80-100 yr	75 yr	<10 yr	10-15 yr	80-100 yr
Prescribed burn return interval	2 yr	2 yr	2 yr	2 yr	>10 yr
Groundcover condition	dense, native grass-forb species	dense, native grass-forb species	planted grass-forb and ruderal species	sparse ruderal species	sparse native species
Midstory	open	open	open	open	shrubs and vines

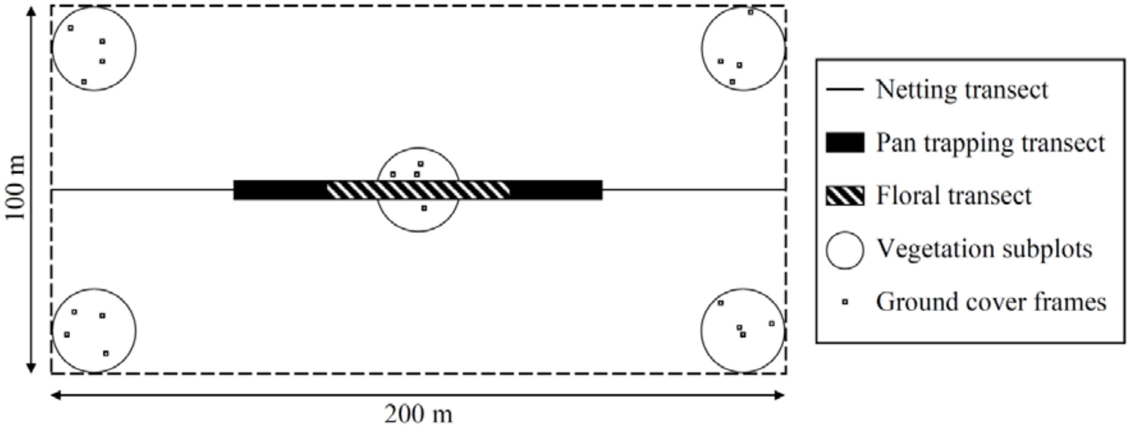

Figure 2.1. Plot diagram for vegetation and bee sampling

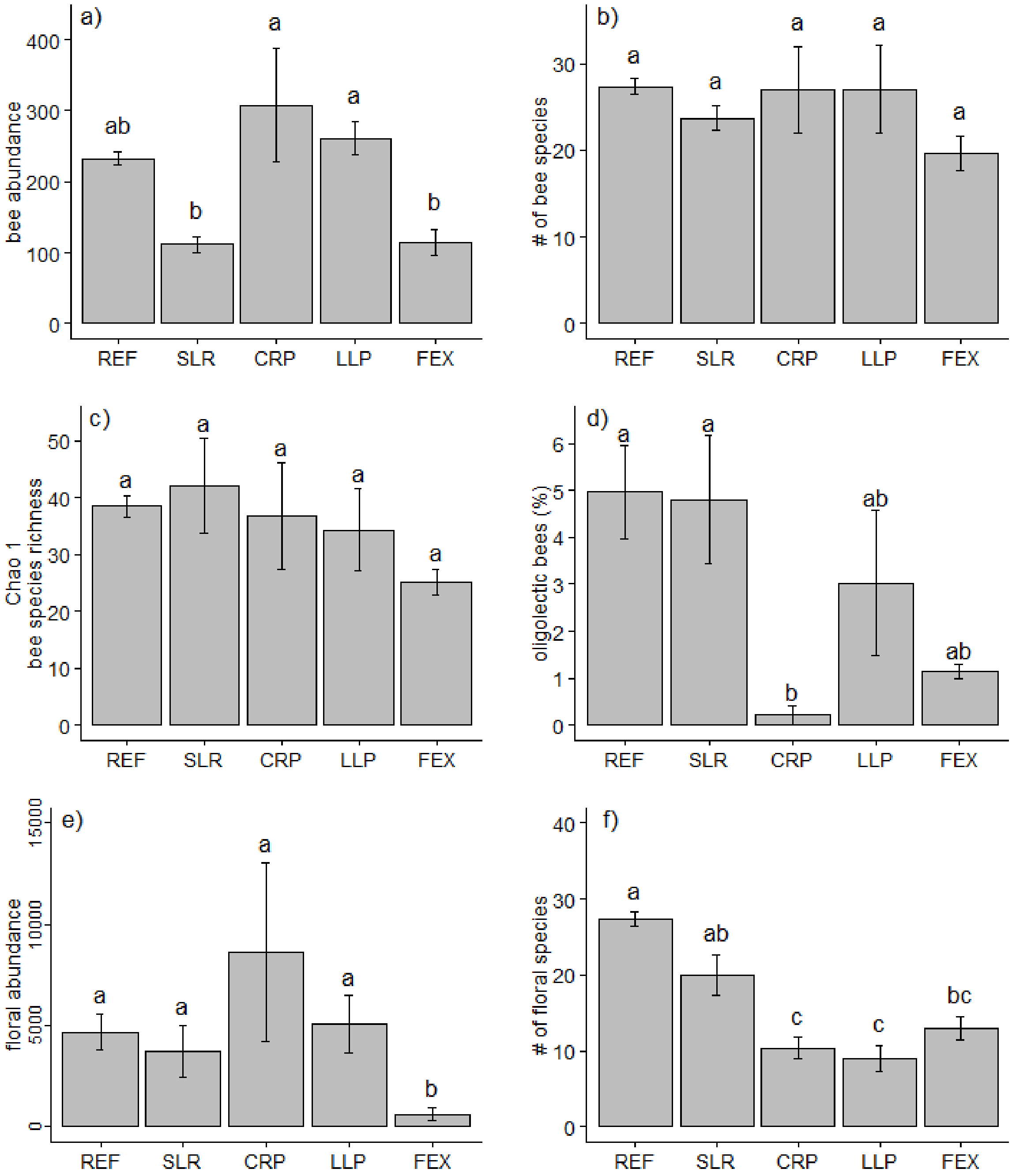

Figure 2.2. Means, standard errors, and statistical differences based on Tukey's tests for a) total number of bees captured, b) total number of bee species, c) Chao 1 estimated bee species richness, d) percentage of oligolectic bees captured, e) floral abundance, and d) total number of floral species. These values are pooled over the sampling season. Untransformed data are shown but ANOVA was run on natural-log transformed bee (a) and floral (e) abundances and on arcsine square root transformed proportions of oligolectic bees (d).

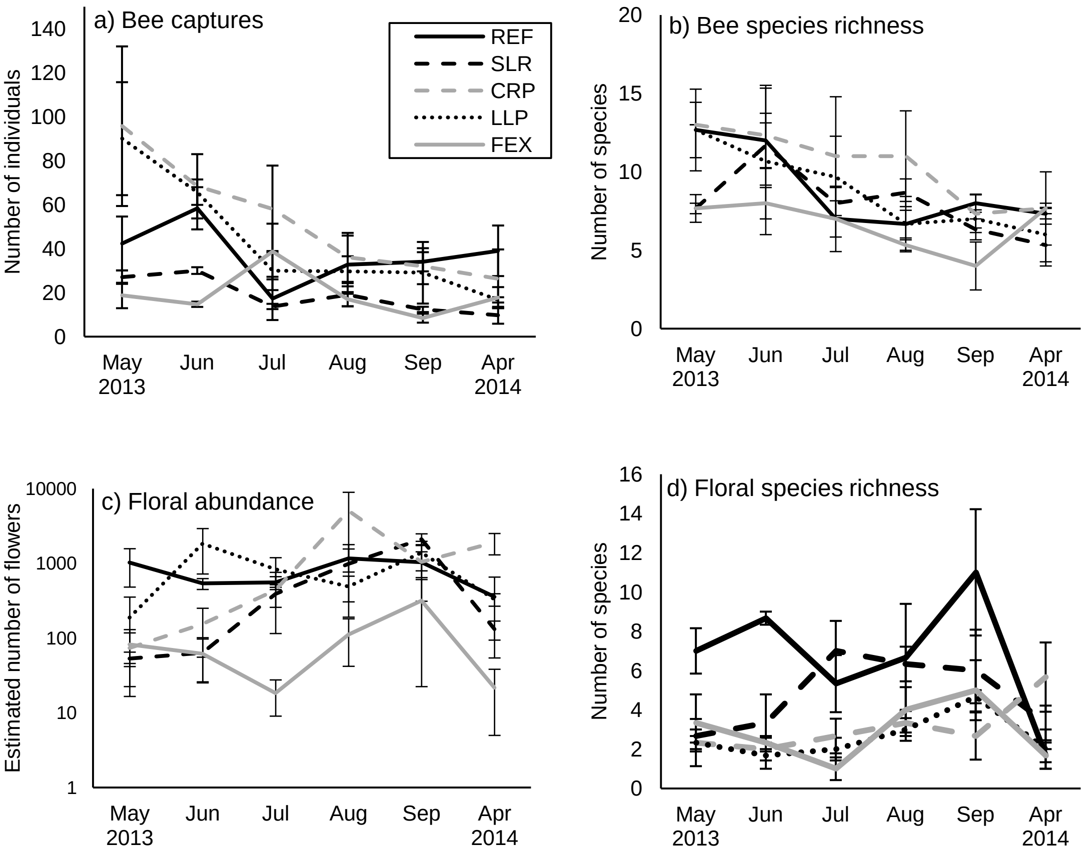

Figure 2.3. Changes in bee and floral abundance and number of species captured through the sampling season. Error bars are standard error. Untransformed data are shown, but natural log transformed data was used in ANOVA. Floral abundance (c) is displayed on a log scale because the data was recorded using log scale classes.

Table 2.2. Mean bee captures and floral species richness comparison between habitats by month. Mean differences based on Tukey’s tests between habitat types indicated by different letters (α < 0.05).

a) Bee captures

	May 2013	*Jun*	*Jul*	*Aug*	*Sep*	*Apr 2014*
REF	ab	ab	——	——	a	——
SLR	ab	b	——	——	a	——
CRP	a	a	——	——	ab	——
LLP	a	a	——	——	ab	——
FEX	b	c	——	——	b	——

b) Floral species richness

	May 2013	*Jun*	*Jul*	*Aug*	*Sep*	*Apr 2014*
REF	——	a	a	——	——	——
SLR	——	ab	a	——	——	——
CRP	——	b	ab	——	——	——
LLP	——	b	ab	——	——	——
FEX	——	b	b	——	——	——

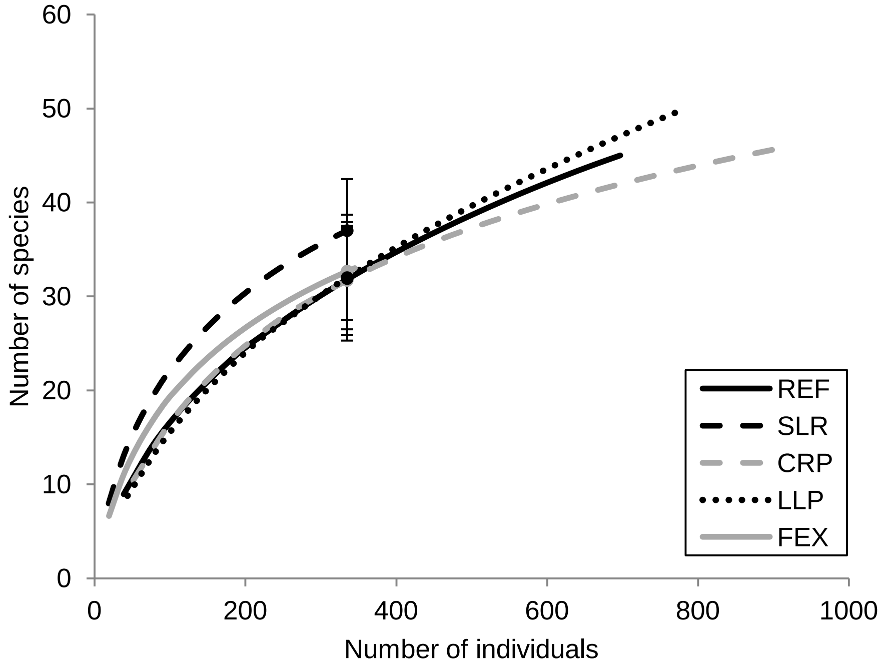

Figure 2.4. Individual-based rarefaction curves for total bee species richness. Error bars are 95% confidence intervals at n= 335 individuals.

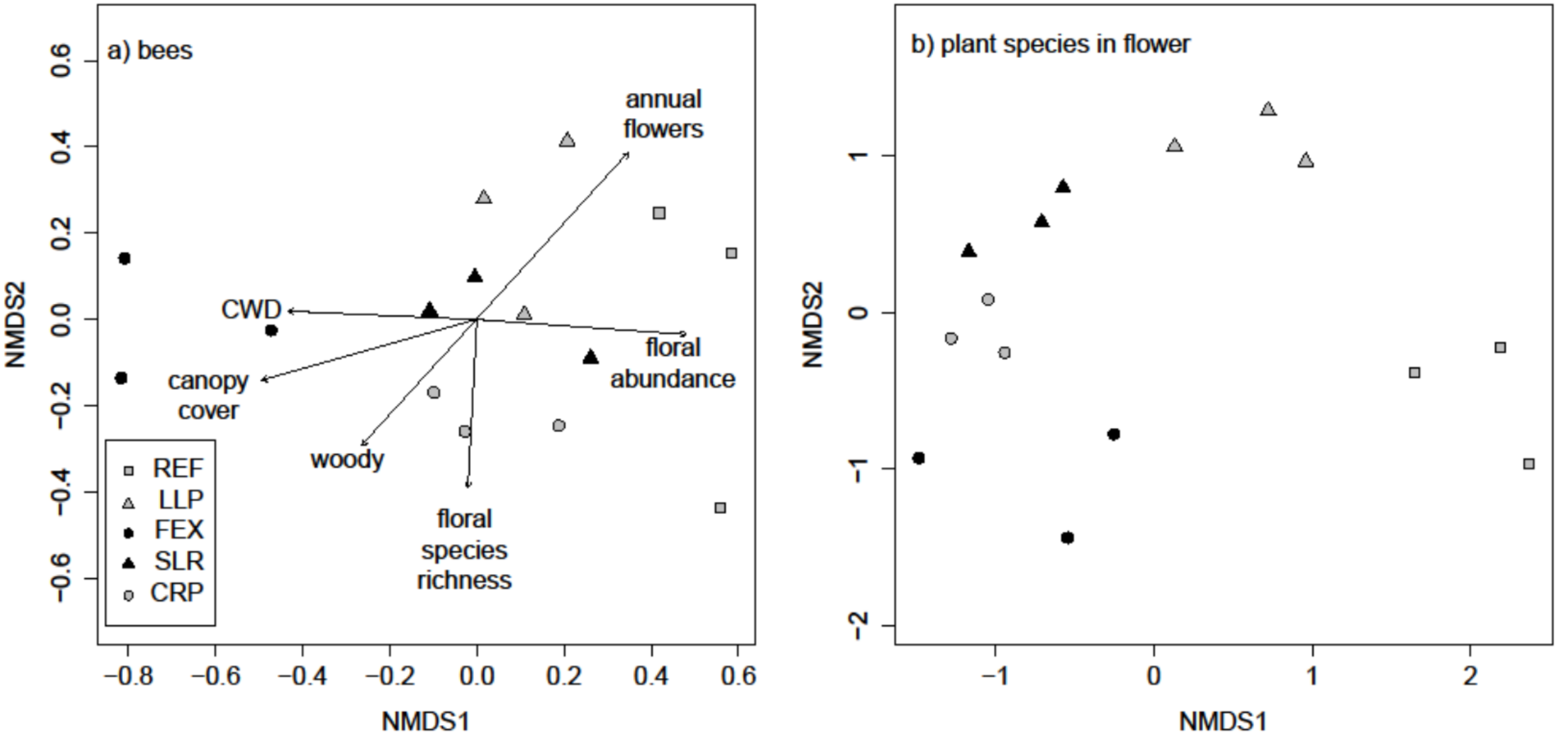

Figure 2.5. Two-dimensional NMDS ordinations of site types based on Bray-Curtis distances. a) Bee assemblages: NMDS ordination has a stress value of 0.13 and explains 75.0% of the among-site variation (axis 1: 68.1%, axis 2: 6.9%). b) Composition of plants in flower: NMDS ordination has a stress value of 0.05 and explains 76.5% of the variation (axis 1: 54.4%, axis 2: 22.1%).

Table 2.3. *P*-values associated with MRPP on pair-wise comparisons of composition of abundance-based bee (white boxes) and plants in flower (gray boxes) by site types. All comparisons were considered different at α < 0.05.

	REF	*SLR*	*CRP*	*LLP*	*FEX*
REF		0.031	0.026	0.033	0.024
SLR	0.026		0.026	0.023	0.024
CRP	0.022	0.022		0.03	0.023
LLP	0.022	0.023	0.022		0.023
FEX	0.023	0.023	0.022	0.022	

Table 2.4. Indicator species of habitat types and habitat pairs. ^{1}A =1 indicates that the species was only present in the target site type. ^{2}B = 1 indicates that the species was found in all of the replicates of the target site type. Significance is determined at $\alpha < 0.05$.

a) Individual habitats	*Indicator species*	A^1	B^2	*P-value*
REF	*Lasioglossum hitchensi*	0.5357	1	0.044
LLP	*Lasioglossum nymphale*	0.9767	1	0.016
	Lasioglossum vierecki	0.9744	1	0.007
	Melissodes communis	0.4538	1	0.018
FEX	*Augochlorella aurata*	0.9787	1	0.016
	Melissodes bimaculata	0.7265	1	0.033
CRP	*Ceratina cockerelli*	1	1	0.011
	Apis mellifera	0.8043	1	0.047
	Lasioglossum callidum	0.7447	1	0.02
b) Habitat pairs	*Indicator species*	A^1	B^2	*P-value*
REF and LLP	*Lasioglossum reticulatum*	0.7155	1	0.012
REF and SLR	*Cemolobus ipomoea*	0.8125	1	0.01
REF and CRP	*Lasioglossum callidum*	0.9078	1	0.012
LLP and CRP	*Lasioglossum floridanum*	0.8333	1	0.01
FEX and SLR	*Lasioglossum raleighense*	1	0.8333	0.013

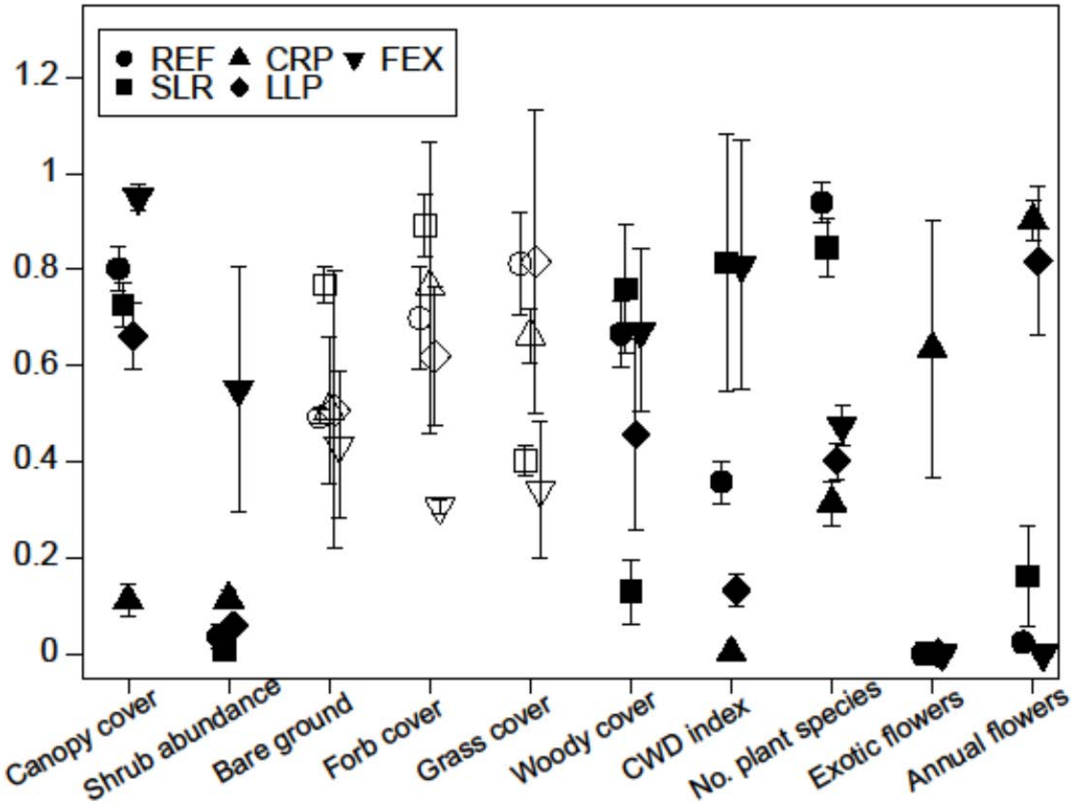

Figure 2.6. Forest structure metrics each scaled by dividing untransformed data by its maximum value among each of the 15 total sites. Error bars represent standard error. Variables with solid symbols have significant differences among habitat types ($\alpha < 0.05$). All proportion data including canopy cover, the cover of bare ground, forbs, grass, and woody groundcover plants, and the proportion of exotic and annual flowers were arcsine square root transformed for ANOVA. CWD index was natural log transformed.

CHAPTER 3

THE EFFECT OF PRESCRIBED BURNING ON FLOWERING AND BEE ASSEMBLAGES IN A LONGLEAF PINE SAVANNA[2]

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[2] Breland, S. B., Kirkman, L. K. To be submitted to the *Journal of Insect Conservation*.

Abstract

There is growing concern about population declines in several North American pollinator species, and loss of appropriate native habitat for some pollinators may be a contributing factor. Prescribed burning is an integral part of restoring and maintaining the longleaf pine savannas of the Southeastern United States, and the effects of frequent prescribed burning in this ecosystem on bee assemblages is largely unknown. I investigated the changes in bee and plant assemblages over a two year burn interval. Bee species richness and diversity was highest in the year of the burn, but there was no measurable effect on bee captures or the relative abundances of focal bee functional groups. The effect of fire on bee richness may be partially explained by an increase in floral abundance after prescribed burning. More investigation is needed to account for the lack of a differential response among bee function groups. These results suggest that prescribed burning is consistent with management goals that include the conservation of diverse bee assemblages in this fire-maintained ecosystem.

Introduction

In recent decades, population declines of pollinators have been documented in ecosystems all over the world (Biesmeijer et al. 2006, NRC-USA 2007). Bees are generally considered the most efficient and effective pollinators (Kearns et al. 1998, Michener 2007); thus declines in both native bees, as well as European honey bees (*Apis mellifera*), are of particular concern. In addition to widespread use of pesticides, changing climate, parasites, disease, and competition from introduced species, habitat loss or degradation is likely a significant cause of population declines of bees. Urban development, landscape fragmentation, as well as intensive silvicultural and agricultural practices tend to decrease the abundance and diversity of floral resources and the availability of some types of nesting sites (NRC-USA 2007). As a result of the reported declines, habitat enhancement for wild, unmanaged bees that may mitigate population decline has emerged as regional, national and global conservation priorities.

Recent conservation interest in pollinators associated with fire-dependent habitats has emerged, particularly in understanding how bee assemblages respond to fire regimes (Potts et al. 2003, Moretti et al. 2009, Grundel et al. 2010). Burning is likely to affect bee assemblages in direct ways, including mortality of adults, larvae and eggs, and indirectly, such as changes in habitat and food availability. High insect pollinator mortality typically occurs in fires in prairies and savannas (Anderson et al. 1989), although only a small proportion of below-ground nesting bees are likely to die due to soil heating, even in more intense fires (Cane and Neff 2011). The most vulnerable species, mostly in the family Megachilidae, are those that excavate very shallow nests (<10 cm). In addition to direct mortality of above ground nesting bees, indirect effects of fires are likely to be highly influential in shaping bee assemblages, particularly in regard to disturbances to nesting habitat. Bees that nest above-ground usually build their nests in perennial grasses, forbs, shrubs, and decaying wood. Data from a series of studies in Mediterranean phrygana ecosystems indicated that above-ground nesting bees were less abundant in recently burned areas relative to unburned sites presumably because the vegetation which provides nesting habitat is disturbed by fire (Williams et al. 2010). However, this condition can benefit a larger subset of bees that requires access to bare ground to excavate below-ground nests (Williams et al. 2010).

The severity of the fire (degree of vegetation consumed), the period of vegetation recovery, and the area of the patch burned are all important variables controlling composition of post-fire bee assemblages. For example, in Mediterranean phrygana, in which the scrubland vegetation is completely consumed and killed by fire, the vegetation recovery and reassembly process occurs over a 30-40 year interval (Potts et al. 2003). Moretti et al. (2009) compared taxonomic and functional trait differences in bee assemblages along a time-since-fire gradient in both Mediterranean phrygana habitats and hardwood temperate forests of Israel and Switzerland. Regardless of the ecosystem, the species composition of the bee assemblage changed along the post-fire chronosequence; however, guild composition varied with time-since-fire only in the temperate ecosystem. In recently burned temperate forests, a higher proportion of bees that nest in wood and existing cavities occurred than in earlier burns. The intense fires in this ecosystem result in complete combustion of these nesting resources which remain absent from the site for long periods. The patchiness of burned sites may also play a role in species assemblages following fire given that flight distances vary with bee body size (Greenleaf et al. 2007) ranging from less than 200 m (Zurbuchen et al. 2010) to over a kilometer (Osborne et al. 2008) for native bees. The area of the patch burned will presumably influence how quickly that area is recolonized and the composition of the post-fire bee assemblage. When relatively small areas are burned within a matrix containing unburned areas, the composition of the post-fire bee assemblage may be more influenced by indirect effects resulting from changes in vegetation rather than the direct effects of the fire (Swengel 2001).

Because fire plays an important role in the maintenance of the structure and function of many ecosystems (Bond and Keeley 2005), the exclusion of fire can be disruptive to successional processes and community assemblages. In the southeastern United States, fire suppression is a major cause of habitat degradation for many plant and animal species. Fire-dependent longleaf pine forests and woodlands was once the dominant vegetation type in the southeastern Coastal Plain, extending between southern Virginia and eastern Texas, covering about 30 million ha. Today it is considered an endangered ecosystem (Noss et al. 1995), and about 97% of the forest in the original range has been lost from land conversion to agriculture and commercial pine plantations and region-wide fire exclusion. The area that remains in natural longleaf pine stands is highly fragmented (Van Lear et al. 2005).

The longleaf pine ecosystem evolved with exposure to frequent low-intensity burning through anthropogenic intervention as well as lightning ignitions. In the absence of frequent burning, less fire adapted species, particularly hardwoods, tend to invade the ecosystem (Engstrom et al. 2001). Frequent disturbance by fire has contributed to the characteristically high biodiversity in the ground cover (Peet and Allard 1993), with groundcover plant assemblages reaching species densities of 40-50 m^{-2} in some sites (Kirkman et al. 2001). Numerous fire-adapted rare plants are associated with longleaf pine savannas, 27 of which are federally threatened or endangered (Van Lear et al. 2005). Because the herbaceous ground cover is highly dependent on frequent consumption of aboveground biomass (ground cover vegetation, litter, pine needles) by fire, it is likely that the fire regime regulates insects, such as pollinators, which are closely associated with plants (Folkerts et al. 1993).

Until the last decade, relatively little was known about bee assemblages in fire-maintained longleaf pine savannas. A few recent species surveys in longleaf pine habitat representing various physiographic regions of the southeastern coastal plain have been conducted in Louisiana, Florida, North Carolina and Georgia (Bartholomew 2006; Hall and Ascher 2014; Moylett 2014). Relative to time since fire in longleaf pine habitats, Moylett (2014) found that bee captures and estimated species richness in the North Carolina study were lower in sites which had been unburned for 50 years compared to recently burned sites but found no effect of burning on the relative abundance of bee functional groups based on floral or nesting preference.

Previous studies on the dynamics of bee assemblages in fire-adapted systems have included sites which have not been burned for decades (Potts et al. 2003; Williams 2010; Moylett 2014). In systems which have adapted with long fire return intervals, fires tend to be stand-replacing, and therefore are likely to have drastic effects on bee mortality and especially above-ground nesting resources (Williams 2010); therefore, comparisons of freshly burned sites to long unburned sites (30-50 years) are appropriate and necessary for understanding the effects of burning on bees in these ecosystems. The fact that longleaf savannas are adapted to frequent, low-intensity burning with rapid regrowth of perennial vegetation may explain why differences in the proportions of bee nesting guilds in response to fire have not been found in these ecosystems (Moylett 2014). In a longleaf savanna, burning not only removes the standing ground cover vegetation, but also may stimulate flowering in many bee-pollinated forbs in the growing season following fire (Robbins and Myers 1992, Hiers et al. 2000). The effect of periodic flushes in floral resources in response to fire on bee assemblages has received little attention in the scientific literature.

The goal of this study was to determine the effect of frequent, low-intensity prescribed burning on bee assemblages in the longleaf pine savanna ecosystem through a two year burn cycle. Specifically, I examined the influence of floral resource availability and vegetation structure on bee species assemblages and the effect of burning on the relative abundances of bee functional groups based on feeding and nesting preferences.

Methods

Study Area

To study the effects of frequent, low-intensity prescribed burning on bee pollinators, I sampled bee and vegetation assemblages from sites located at the Joseph W. Jones Ecological Research Center at Ichauway. Ichauway is an 11,700 ha privately owned property located on the Coastal Plain of southwestern Georgia (Baker County). This property includes large tracts of 100 year old, second growth natural longleaf pine forests managed with frequent prescribed fire (two to three year fire interval). I selected six sites representing alternate years of a two year burn cycle (burned versus unburned). Three sites had been burned in winter 2011/2012 and were next burned in the winter of 2013/2014 and three sites were burned in winter of 2012/2013 and were not burned in the winter of 2013/2014. All sites were monitored over the course of two years, 2013 and 2014, I defined burn cycle 1 as unburned before 2013 sampling season (unburned, UB) → burned immediately before 2014 sampling season (burned, B) and burn cycle 2 as B 2013 → UB 2014. The sites were characterized by an open canopy dominated by mature longleaf pine, sparse to no mid-story cover, and a dense high-diversity groundcover plant community with no evidence of widespread historical soil disturbance. These study sites have been burned on a two to three year fire return interval for over 8 decades. At each study site location, I established a 100 x 200 m plot for sampling bees, flowering plants, and vegetation structure (Figure 3.1). Because some bee species can forage long distances (Greenleaf et al. 2007), I located bee sampling transects at least 150 m from any area with a significantly different land cover type to ensure that the bees collected were associated with the focus habitat type. All plots were at least 1000 m from each other.

Bee sampling

I used standard pan trapping and aerial netting techniques to sample bee assemblages at each study site (Droege 2012). These two methods are considered complementary because pan trapping tends to over sample small bees and under sample larger bees, whereas, small and fast-flying bees are often missed with aerial netting (Droege 2012), and they have been used in many similar studies including three in longleaf savannas (Bartholomew 2006; Hall and Ascher 2014; Moylett 2014). In each 0.2 ha plot, I established a 200 m sampling transect.

Within the 200 m transect, I placed 15 pan traps equidistant along a 100 m sub-transect located in the center of the larger transect. Pan traps consisted of white Solo brand 96 ml plastic cups (stock number P325w-0007). At each sub-transect, I used 15 traps (five each of white, fluorescent blue, and fluorescent yellow; fluorescent paint source: Guerra paint and pigment, guerrapaint.com). I placed each trap in a 9 gauge steel wire loop with a stem which held it 30 cm above the ground. For each sampling event, I filled trapping pans ¾ full with a solution of soapy water (1.3 ml of Dawn brand dish soap per liter of water). Bees are attracted to these traps, and the soap reduces surface tension causing the bees to sink and drown. Traps were left out for 24 hours, and then bees in all traps along a sub-transect were collected in one filter and rinsed with water. Filters containing the bees were allowed to air-dry for a few hours and stored in a freezer (-12°C). Specimens were pinned within a few days of capture.

During each sampling event, I also collected bees by aerial netting. Walking slowly along the 200 m netting transect, I caught all bees observed for two periods (30 minutes each), once between 8:00 a.m. and 12:00 p.m. and secondly between 12:00 p.m. and 3:00 p.m. Netted bees were killed using ethyl acetate and pinned. I conducted bee sampling once per month in all sites between May and October 2013 and between May and October 2014 for a total of 12 sampling events. To avoid effects of adverse weather on bee capture rates (Droege 2012), I conducted bee sampling only on sunny or partly cloudy days with low wind speeds (<13 mph). I identified all specimens to species or the lowest taxonomic level possible using all available keys appropriate to the region (Mitchell 1960, 1962; Gibbs 2010, 2011; Ascher and Pickering 2014). Bee species identifications were verified by Sam Droege of USGS Patuxent Wildlife Research Center. I classified each bee species relative to nesting preference (above- or below-ground) and degree of feeding specialization (oligolectic or polylectic) based on life history data in published literature (Mitchell 1960a, 1962a, Michener 2007). Bees were classified as oligolectic if their floral visitation is restricted to one plant family. Voucher specimens for all bee species collected are archived at the Georgia Museum of Natural History (University of Georgia, Athens, GA).

Vegetation Sampling

To determine floral resource differences at the time of bee sampling, I recorded all open flowers in a 1 m strip along the middle 50 m of the 200 m transect in each plot during sampling events. For each flowering plant present, I recorded number of individuals per species, and abundance class of flowers per plant (1-10, 11-100, 101-1000). Floral abundance was estimated by multiplying the number of plants in flower by the midpoint of the abundance class of flowers per plant. For any plant species that could not be positively identified in the field, I collected a specimen for verification in the lab.

To characterize other vegetation composition and structural variables that might influence bee nesting resources as well as floral resource differences among site types, I sampled vegetation in fall 2013. I established five circular subplots (radius=11.3 m) in each main plot (10 percent of the total area of the plot), one at the center and one located in each corner. In each subplot, I measured six metrics. To estimate percent canopy cover I used a convex densitometer (Forestry Suppliers stock number 43887) positioned in the center of each subplot to obtain readings in 4 opposing directions. I measured tree diameter at 1.4 m height (diameter at breast height, DBH) for all of trees greater than 10 cm DBH to obtain total canopy basal area. To quantify abundance of trees and shrubs between 2.5 and 10 cm DBH, I recorded the number of individuals present by species. The presence of dead woody vegetation lying on the ground (coarse woody debris, CWD) was recorded by size class (1=2.5-5 cm, 2=5-10 cm, 3=10-20 cm, 4=20-40 cm, and 5=40-80 cm) according to Maser et al. (1979). Within each circular subplot, I recorded all plant species present in 4 randomly located 1 x 1m frames. I also recorded percent cover of woody species, forbs, graminoids, and bare ground/litter (cover classes: 0=0%, 1=1-25%, 2=26-50%, 3=51-75%, and 4=76-100%). Bare ground cover was re-measured in during the summer 2014 to exclude litter, so that areas with a thick layer of litter which may be undesirable bee nesting habitat would not be included.

Statistical Analysis

I compared mean bee species richness (number of species), number of captures, evenness, Shannon-Wiener diversity, and Chao1 estimated species richness with *t* tests in R package stats (De Mendiburu 2014; R Development Core Team 2014). Shannon-Wiener diversity and Chao1 estimated species richness were calculated in R package vegan (Oksanen et al. 2013). The Chao 1 estimator was chosen because it accounts for a large number of rare species with a correction factor (Chao 1984). I also compared proportions of bee captures and bee species richness within important functional groups (diet specialization, nesting preference) using *t* tests. Means of these variables were compared with *t* tests between B and UB sites within each sampling year. In order to test the effect of burn cycle without regard to the effect of sampling year, *t* tests were also run on the difference, or change, in these response variables within burn cycles between the 2013 and 2014 sampling seasons. All response variables were tested for the assumptions of normality and equal variances, and these assumptions were met.

To examine bee assemblage composition between years and burn cycles, I conducted non-metric multidimensional scaling (NMDS) analysis based on Bray-Curtis (Sørenson) and Jaccard distances using R package vegan (McCune and Grace 2002; Oksanen et al. 2013). I used Multi-response Permutation Procedure (MRPP) to determine if bee assemblage composition differed significantly among burn cycles, 1 vs. 2, or time since burn, B vs. UB (Oksanen et al. 2013). Bee count data were natural-log transformed for NMDS and MRPP to decrease the influence of rare species.

I performed *t* tests on differences in floral species richness and abundance between 2013 and 2014 in the two burn cycles and between B and UB sites within each sampling year. Ordinations were also generated for the assemblage of plants in flower (Bray-Curtis) using the log transformed number of plants of each species and the presence-absence of floral species (Jaccard) in R package vegan (Oksanen et al. 2013). Differences in ordination space between burn cycles and times since burn were analyzed using MRPP in R package vegan (Oksanen et al. 2013). I compared basal area, % canopy cover, shrub abundance, CWD index, plant species richness, forb, grass, and woody plant cover measurements from fall of 2013 and the bare ground cover measurements from summer 2014 between burn cycles with *t* tests (De Mendiburu 2014; R Development Core Team 2014). Prior to analyses, vegetation response variables were tested for the assumptions of normality and equal variances, and these assumptions were met.

Results

Bee assemblage

Over the course of this study, I collected 3540 bees from 69 species, 30 genera, and 5 families. Halictidae was the dominant family (86.3% of individuals) followed by Apidae (10.1%), Megachilidae (2.9%), Andrenidae, and Colletidae (both <1%). The most common species, *Lasioglossum reticulatum,* accounted for nearly half the individuals collected (n=1712 individuals), and eight out of the ten most common species were

Lasioglossum. About 36% of species (25 species) were collected only once, and 22% (15 species) were collected twice.

Prescribed burning had a significant effect on bee richness and Shannon-Wiener diversity (Table 3.1). I collected an average of 17.40% (± 5.7%) more bee species in the first year after a prescribed burn compared to the second year after a burn (Figure 3.3a). Diversity was similarly higher in the first year after a fire (Table 3.1). No significant effects of burning on total bee captures (Figure 3.2b), Chao 1 estimated bee species richness (Table 3.1), or the proportions of oligolectic or above-ground nesting bees were found. The effect of burning on bee evenness was inconsistent in the two sampling years with greater evenness in UB plots in 2013 and greater evenness in B plots in 2014 (Table 3.1). Based on clustering in abundance-based ordination space, bee composition was more similar with regard to site identity than time since burn (MRPP: A = 0.04, p = 0.029, Figure 3.4a). In contrast, the presence-absence based ordination, bee assemblages with similar time since burn had greater similarity (MRPP: A = 0.02, p = 0.043, Figure 3.4b). Two-dimensional solutions are presented for both ordinations, however based on Monte-Carlo test p-values, neither ordination reached a strong low dimensional solution (abundance: p = 0.0677, presence-absence: p = 0.0717). The similarity of bee assemblages included in the ordinations, the fact that the same sites from different years were included, and the low number of sites may explain the marginal ordination solution.

Floral assemblage and vegetation

Overall, prescribed burning had a significant effect on floral abundance (Table 3.1) with an average of more than twice as many flowers in the first year after a prescribed burn compared to the second year after a burn (Figure 3.3b). In 2013, burned sites had more flowers present than unburned sites; however, this effect was not observed in 2014 (Table 3.1, Figure 3.2d). There was no significant effect of prescribed burning on floral richness for either year (Table 3.1, Figure 3.2c). The floral assemblage clustering from year to year was influenced more by site than time since burn in both abundance-based and presence-absence based ordinations (Bray-Curtis MRPP: A = 0.05, $p = 0.029$ and Jaccard MRPP: A = 0.03, $p = 0.008$, Figure 3.5.)

Burn cycles differed in the amount of bare ground measured in summer 2014 ($t = 11.1$, $df = 4$, $p = 0.0002$). Plots that had been burned the previous winter (2014) had an average of 39.17% (± 2.2%) bare ground cover compared to 14.17% (± 0.4%) in plots which had been burned more than a year before (winter 2013). As expected, there were no differences in basal area, % canopy cover, shrub abundance, CWD index, total plant species richness, or forb, grass, or woody plant cover between burn cycles ($p>0.05$).

Discussion

In this study, the greater bee richness and diversity observed in plots burned less than one year is consistent with other studies indicating a negative relationship between time since fire and bee species richness (Potts et al. 2003; Moretti et al. 2009; Moylett 2014) and diversity (Moretti et al. 2009) in fire-dependent communities. Even though floral abundance was not consistently higher in burned plots from year to year in my study, the tendency of this pattern in 2014, coupled with an overall lower number of flowers in 2014 regardless of burn cycle, suggests that precipitation, temperature, or other environmental factors may explain the greater variation in floral abundance observed the second year. Though generally, greater floral abundance of the ground cover vegetation in longleaf pine savannas occurs in the growing season after a prescribed fire, some species do not necessarily exhibit this response. Some differences in flowering response also may be attributable to life history characteristics of the particular plant species present in a given site. In addition, abundance and duration of flowering can also be influenced by timing of fire (Robbins and Myers 1992; Hiers et al. 2000). Despite these possible confounding influences on flowering response, the results of my study suggest that there was an overall positive effect of recent burning on floral abundance. Accordingly, differences in floral abundance may partially account for higher bee richness and diversity in recently burned plots, an observation consistent with that of Ebeling et al. (2008), who found that pollinator richness across a variety of taxa was clearly positively correlated with percent blossom cover.

The absence of an effect of burning on relative abundances of bee functional groups or families contrasts with that of other studies in ecosystems with less frequent fire as well as those with fire of greater intensity and severity (Potts et al. 2003; Moretti et al. 2009), but is similar to the findings of Moylett (2014), also in a longleaf pine habitat, but in a sandhill community. It is probable that the low-intensity, frequent burns characteristic of longleaf savannas cause less bee mortality and instability in nesting resources. Admittedly, a greater number of functional groups were examined in Moretti et al. (2009) including differences in tongue lengths, dispersal abilities, pollen transport, and generation length in response to time since burn. Possibly, the compositional differences we observed in the presence-absence bee ordination, which was associated with recent burn history, could reflect differences among guilds that I did not examine.

Further insights on the effects of fire on bee functional groups in longleaf savannas may be gained from including these groups in analysis.

The absence of a difference in ground nesting resources relative to timing of fire was an unexpected outcome and may reflect the fact that bare ground measurements were made in summer 2014, rather than continuously over the course of the study. Quantification of the change in nesting resources as a site progresses through the burn interval will be necessary to link changes in these resources to bee assemblage composition.

In summary, the results of this study indicate that bee richness and diversity is highest in longleaf pine savannas which are burned on a short interval in the same year as burning. More investigation is needed to understand how floral and nesting resource dynamics affect bee assemblages over this short burn interval. However, my results suggest that despite any immediate negative effects on bee mortality and nesting resources, prescribed burning promotes bee species richness within one year of the fire in association with an increase in flower production.

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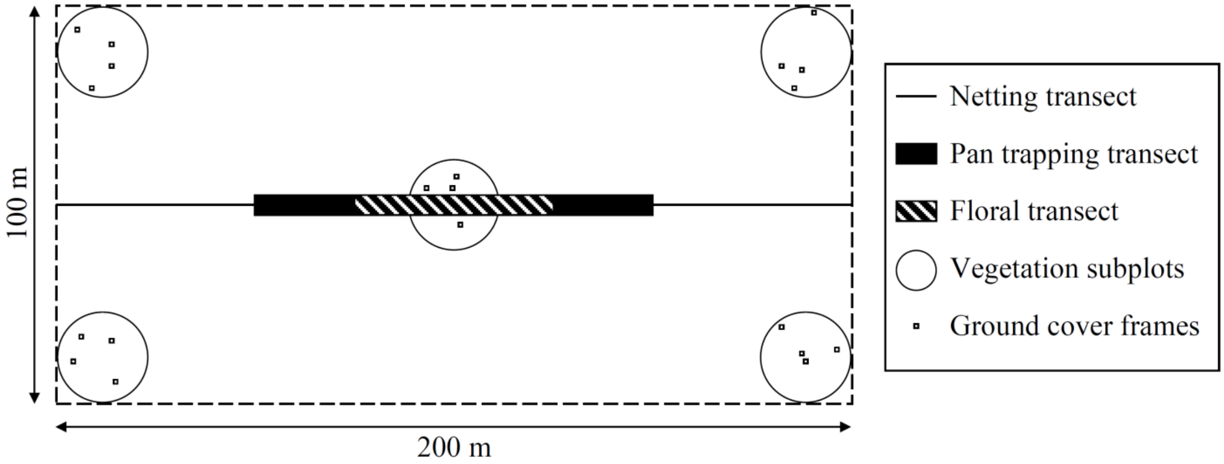

Figure 3.1. Plot diagram

Table 3.1. Hypotheses, means, standard error, *t*-values, degrees of freedom, and *P*-values of bee and floral variables compared between burn cycles and burn conditions. Cycle 1 and 2 refer to the change between 2013 and 2014 in burn cycle 1 and burn cycle 2 plots. † Groups 1 and 2 refer to the groups which are being compared in the *t*-test as indicated by the hypothesis (For example: where the hypothesis states cycle 1 > cycle 2, group 1 is the cycle 1 mean and group 2 is the cycle 2 mean). *P*-values in bold are significant at $\alpha < 0.05$.

	Hypothesis	*Group 1 mean (± SE)†*	*Group 2 mean (± SE)†*	*t-val*	*df*	*P-value*
Bee captures	cycle 1 < cycle 2	-10.67 (± 102.59)	110.67 (± 87.19)	-0.90	4	0.2092
	UB 2013 > B 2013	276.00 (± 46.03)	263.67 (± 25.90)	0.23	4	0.4134
	UB 2014 > B 2014	374.33 (± 67.22)	265.33 (± 83.38)	1.02	4	0.1832
Number of bee species	cycle1 > cycle 2	3.67 (± 2.19)	-3.67 (± 1.45)	2.79	4	**0.0246**
	UB 2013 < B 2013	21.00 (± 1.73)	26.00 (± 1.15)	-2.40	4	**0.0371**
	UB 2014 < B 2014	22.33 (± 1.20)	24.67 (± 2.60)	-0.81	4	0.2307
Shannon-Weiner bee diversity	cycle 1 > cycle 2	0.12 (± 0.08)	-0.11 (± 0.04)	2.63	4	**0.0292**
	UB 2013 > B 2013	1.62 (± 0.07)	1.37 (± 0.13)	1.69	4	0.0835
	UB 2014 < B 2014	1.26 (± 0.09)	1.74 (± 0.14)	-2.91	4	**0.0219**
Bee evenness	cycle 1 > cycle 2	0.011 (± 0.008)	-0.015 (± 0.016)	1.48	4	0.1062
	UB 2013 > B 2013	0.52 (± 0.02)	0.41 (± 0.03)	2.78	4	**0.0250**
	UB 2014 < B 2014	0.40 (± 0.02)	0.54 (± 0.03)	-3.70	4	**0.0104**
Chao 1 estimated bee species richness	cycle 1 > cycle 2	-4.90 (± 3.78)	-7.73 (± 4.35)	0.49	4	0.3242
	UB 2013 < B 2013	36.78 (± 2.12)	37.08 (± 3.66)	-0.07	4	0.4729
	UB 2014 < B 2014	29.35 (± 1.40)	31.88 (± 5.66)	-0.43	4	0.3433
Floral abundance	cycle 1 > cycle 2	-693.33 (± 1612.47)	-5051.67 (± 275.10)	2.66	4	**0.0281**
	UB 2013 < B 2013	3753.33 (± 166.44)	6435.00 (± 171.56)	-11.22	4	**0.0002**
	UB 2014 < B 2014	1383.33 (± 429.04)	3060.00 (± 1447.77)	-1.11	4	0.1645
Number of floral species	cycle 1 > cycle 2	-5.67 (± 4.18)	-10.33 (± 1.86)	1.02	4	0.1825
	UB 2013 < B 2013	30.67 (± 4.06)	33.00 (± 1.15)	-0.55	4	0.3047
	UB 2014 < B 2014	22.67 (± 2.85)	25.00 (± 6.24)	-0.34	4	0.3755

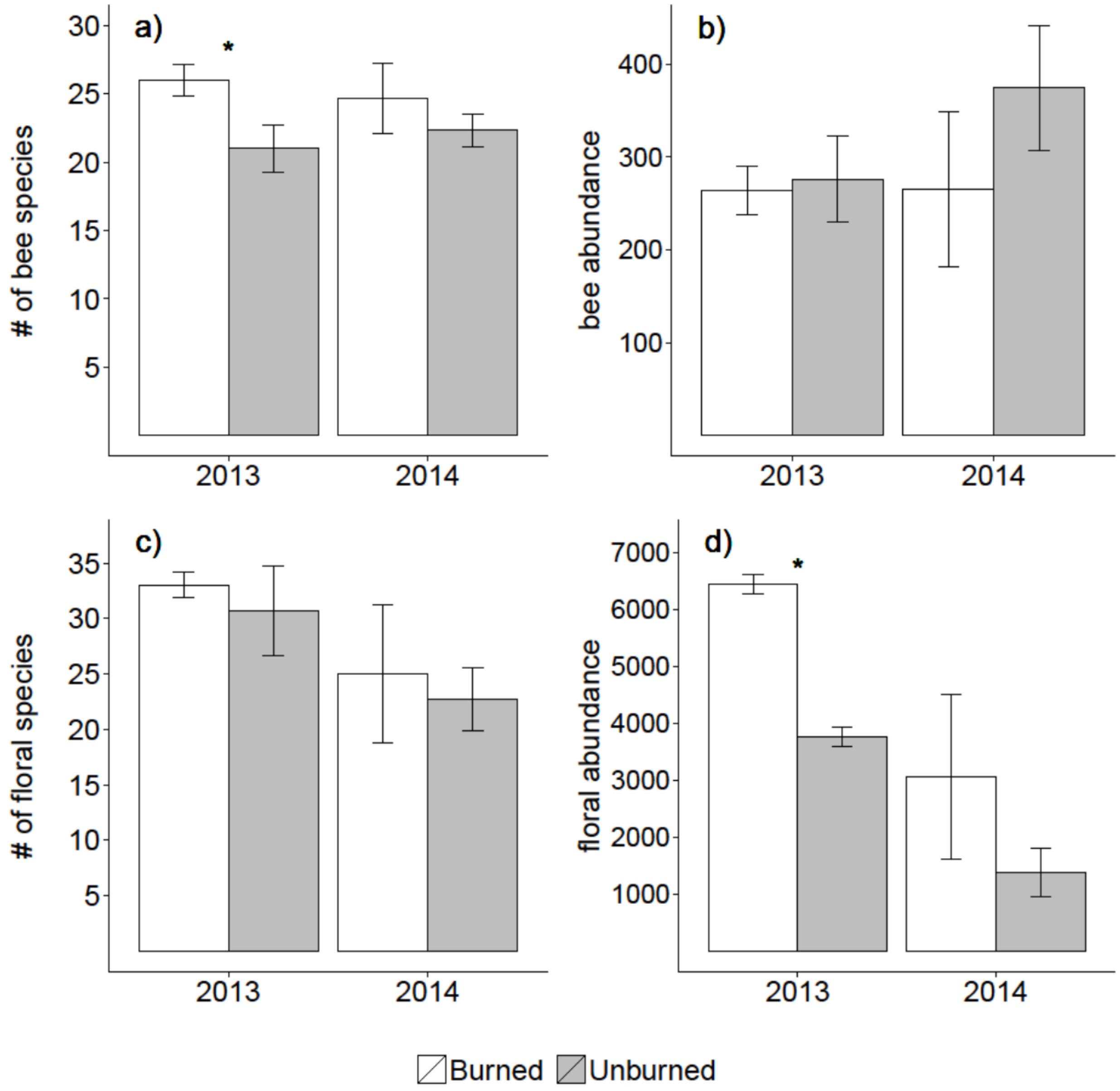

Figure 3.2. The mean (a) number of bee species, (b) bee captures, (c) number of floral species, and (d) floral abundance in 2013 and 2014 sampling seasons. Error bars represent standard error. Asterisks indicate that the number of (a) bee species and (d) floral abundance was significantly higher in burned compared to unburned plots in 2013 at $\alpha < 0.05$.

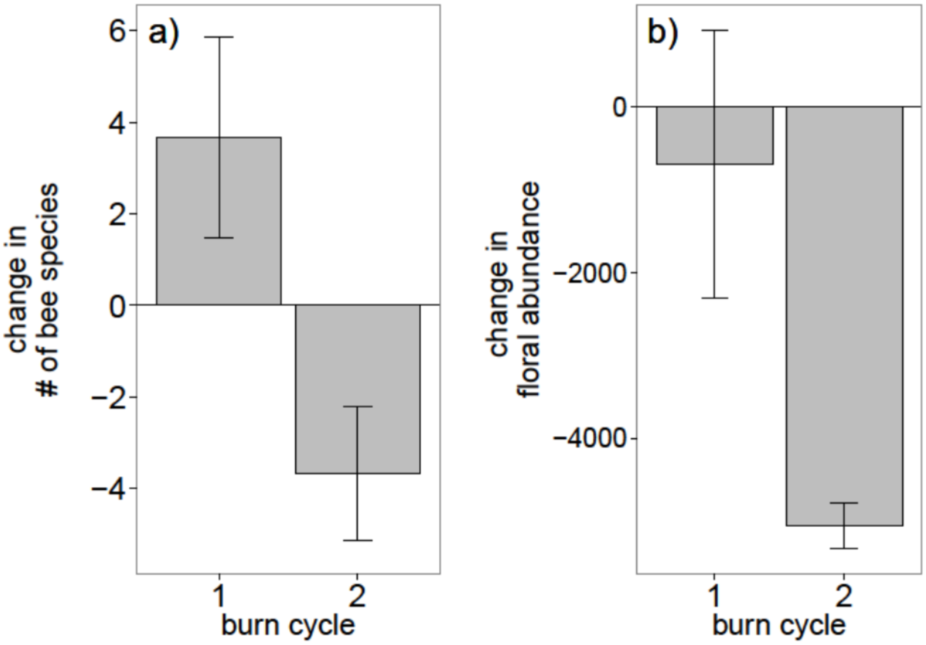

Figure 3.3. Mean change in (a) bee species richness and (b) floral abundance between 2013 and 2014 sampling seasons in burn cycle 1 (UB → B) and 2 (B → UB). Both mean differences are significantly greater in burn cycle 1 than burn cycle 2 at $\alpha < 0.05$.

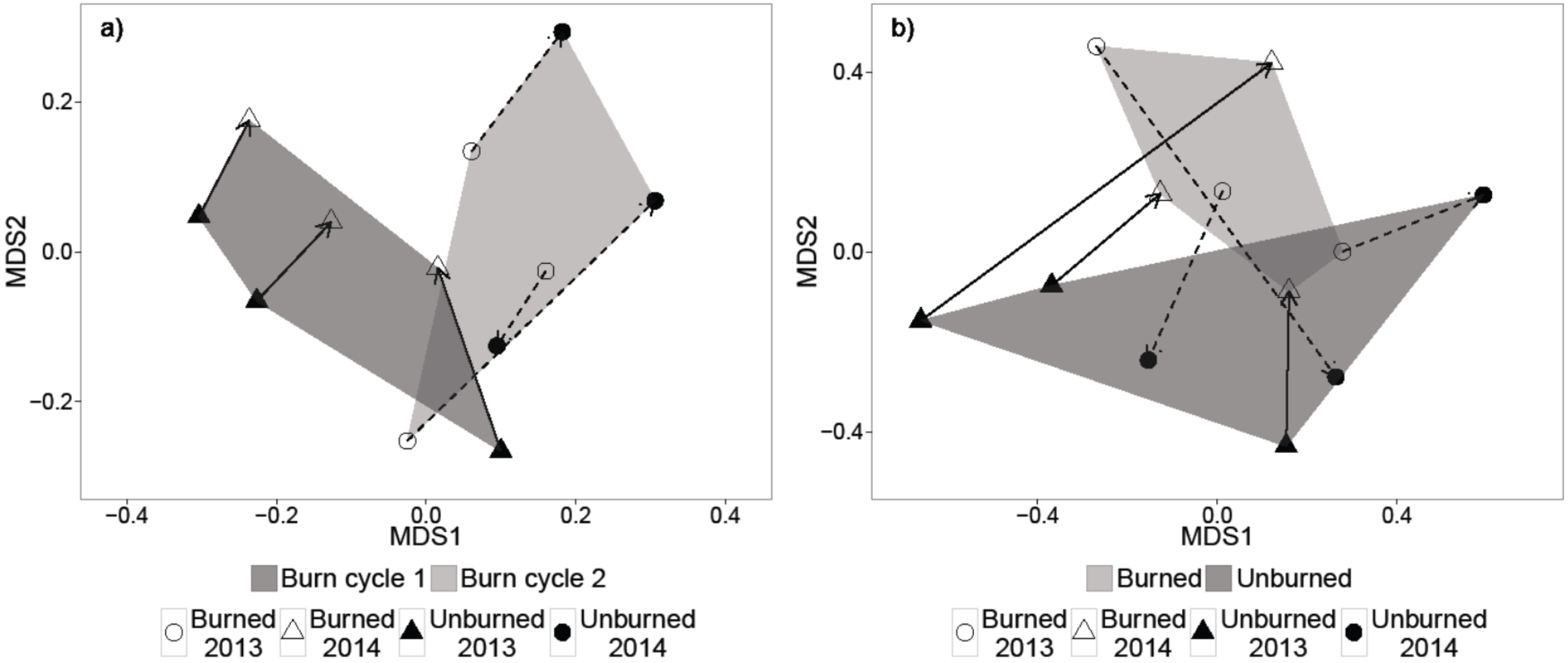

Figure 3.4. Two-dimensional NMDS ordinations of bee assemblages: a) Bray-Curtis ordination based on abundance data has a stress value of 0.10 and explains 88.8% of the among-site variation (axis 1: 86.3%, axis 2: 2.5%) and b) Jaccard ordination based on presence-absence data has a stress value of 0.15 and explains 51.7% of the variation (axis 1: 40.1%, axis 2: 11.6%). Arrows connect the same plots from the 2013 to the 2014 sampling season. Groups highlighted in gray in represent (a) burn cycle 1 (UB → B) vs. burn cycle 2 (B → UB) and (b) B vs. UB. These respective groups were found to be more similar to each other in bee composition than other groups by MRPP.

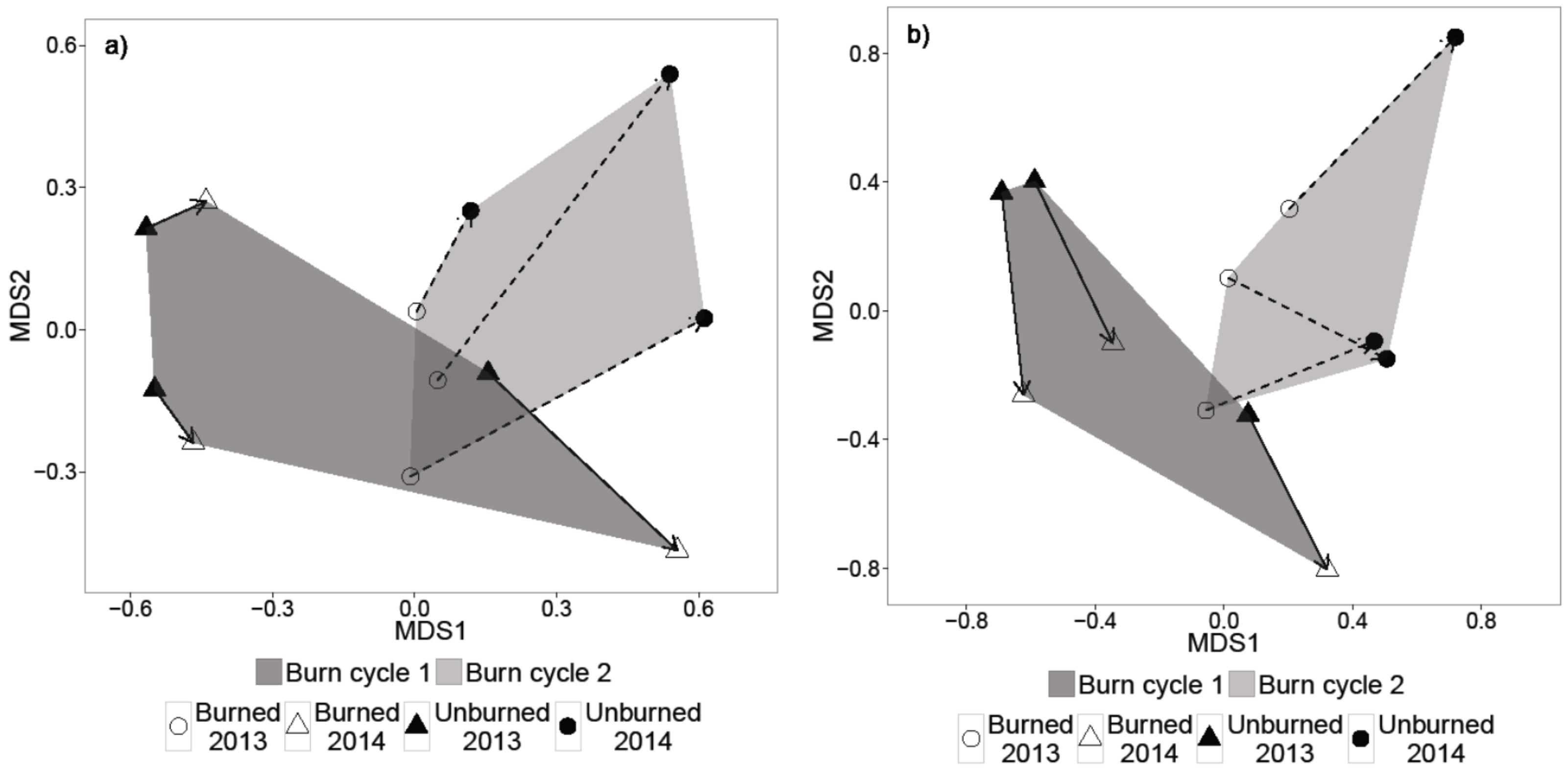

Figure 3.5. NMDS ordinations of floral assemblages: a) The two dimensional Bray-Curtis ordination based on abundance data has a stress value of 0.14 and explains 68.9% of the among-site variation (axis 1: 56.4%, axis 2: 12.5%) and b) and the three dimensional Jaccard ordination based on presence-absence data has a stress value of 0.08 and explains 61.3% of the variation (axis 1: 36.6%, axis 2: 18.2%, axis 3: 6.5%). For the Jaccard ordination (b) only axes 1 and 2, which explain most of the variation, are shown. Arrows connect the same plots from the 2013 to the 2014 sampling season. Groups highlighted in gray in represent burn cycle 1 (UB → B) vs. burn cycle 2 (B → UB). These groups were found to be more similar to each other in floral composition than other groups by MRPP.

CHAPTER 4

CONCLUSIONS

The decline in pollinator abundance and diversity, particularly of bee species, is a topic of increasing conservation concern. The National Research Council has recently highlighted the need for further inquiry into the severity, causes, and consequences of these declines, along with the need for basic research into the ecology of wild pollinators in the US. About three quarters of angiosperms benefit from animal pollination (NRC-USA 2007). Because of their role in plant reproduction, pollinators likely contribute to the success of restored ecosystems. Though interest in restoring pollinator habitat is growing, traditional restoration plans have focused on the reestablishment of plant species while ignoring the status of important ecosystem functions such as pollination in natural assemblages as well as agricultural crops. In the southeastern coastal plain of the U. S., the fire-maintained longleaf pine savanna ecosystem was historically the predominant forest type. Today it is considered an endangered ecosystem, and the patches that remain only cover a tiny fraction of the original range (Noss 1989). Often in longleaf groundcover restoration projects, a main objective is returning a regular fire regime to a fire-suppressed area. However, it is unclear how this and other restoration practices affect pollinator assemblages and function. This thesis addresses the effects of common restoration practices and trajectories on bee and plant assemblages in longleaf savannas with a particular focus on the effects of frequent prescribed burning.

In Chapter 2, I found that both degraded and restored longleaf pine savannas could support bee assemblages that were as diverse as those found in reference sites. However, the bee assemblages in these sites did differ compositionally, and sites displayed different seasonal patterns in bee captures. Of restoration site types, slash pine restoration (SLR) had the bee assemblage that was most similar to that of reference longleaf savanna. The bee assemblage composition and season pattern of bee captures in SLR sites were also closest to that of reference sites. These two site types supported similarly high percentages of oligolectic bees and shared an oligolectic indicator bee species. The similarity between SLR and restoration sites in vegetation structure and plant assemblage may explain the similarity among bee assemblages. During the restoration of SLR sites, dense mid-story hardwoods were removed, their regrowth was suppressed with herbicides, and frequent prescribed fire was reintroduced. Overstory slash pines were selectively harvested, but not clear-cut, resulting in a forest which is structurally similar to longleaf pine savannas. This gradual restoration strategy has been found to promote reintroduction of desirable groundcover plant species (Kirkman et al. 2007), and the preservation of forest structure and presence of diverse groundcover plants may help support a healthy bee assemblage as well.

One theme that stands out from this work is the importance of the presence or absence of just a few plant species. Oligolectic bees in reference and SLR sites were supported by only a few species of plants, and the very high numbers of bees in two of the CRP sites was the result of one prolifically flowering plant species. This prolific species, a particularly aggressive cultivar of *Chamaecrista fasciculata* Greene (Partridge Pea), attracted an abundance of mostly polylectic bee species. If the goal of restoration is to provide habitat for diverse and abundant bees, the inclusion of this type of species in a restoration seed mix seems reasonable. Several studies have found that high pollination function depends more on bee abundance than diversity. However, if the goals of restoration include preserving pollinator species richness, restoration practitioners may consider including a wider range of plant species, particularly ones that host oligolectic bees. In both my study and a similar study by Bartholomew (2004), plants in Asteraceae and in the genera *Ipomoea* and *Vaccinium* were identified among others as common hosts to oligoleges in the longleaf pine ecosystem. The presence of these plants in restored longleaf pine savannas may contribute to their suitability as habitat for a wider range of bee species.

The focus of Chapter 3 was on the impact of prescribed burning on bee and floral assemblages in longleaf savannas. Prescribed burning is considered integral to restoring a longleaf savanna and must be administered regularly to maintain the open midstory and sparse canopy that is characteristic of the ecosystem (Kirkman et al. 2007). Fire has been found to have a complex and conflicting impacts on insect populations. Initially, fire removes most floral food resources and above-ground nesting resources besides those that are too large to burn such as large rotting logs. Below-ground nesting resources, or exposed soil, should increase in response to burning. As time progresses the relative abundance and characteristics of these resources change in different ways. Since it is generally recommended that land managers burn on a two to three year interval in longleaf savannas, I chose to study bee and floral dynamics over a two year burn interval. I found that bee species richness was higher in the year immediately after fire than two years after fire. This can be partially explained by the positive effect of fire on floral abundance.

The effect of frequent prescribed burning on bee functional groups is a research topic which merits further study. In both my study and a recent study from Moylett (2014), prescribed burning in longleaf savannas was found to have no effect on the relative abundance of bee functional groups. This may indicate that unlike in other fire-prone ecosystems (Potts et al. 2006; Williams et al. 2010), burning in longleaf savannas is a less disruptive form of disturbance to habitat and thus to the composition of bee assemblages.

During the course of this study, I collected two bee species that had not been seen in over twenty years (*Trachusa dorsalis* Lepeletier and *Triepeolus monardae* Mitchell; Colla et al. 2012). This and other evidence from analysis of bee collections indicates that the southeastern U. S. has been undersampled for bees in the past (Colla et al. 2012). The bee community present in longleaf pine savannas is likely to be diverse because of the high diversity of plant species (Ebeling et al. 2008). It also faces the threats of habitat fragmentation, degradation, and loss which have reduced this ecosystem to 3% of its historical range (Van Lear et al. 2005). As is the case over much of the world, bees in this ecosystem require more extensive monitoring if we are to be aware of emerging threats to their biodiversity. However, active steps to restore longleaf pine savannas regionally and to improve and maintain this habitat with prescribed burning may help to mitigate losses in bee biodiversity in the future.

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Appendix A. List of bee species found over the course of both thesis sections.

species	*early and late date of collection*	*pan traps*	*netting*	*total*	*CRP*	*FEX*	*LLP*	*REF*	*SLR*	*above-ground nesting*	*oligolectic*
ANDRENIDAE											
Andrena (Trachandrena) alleghaniensis (Viereck, 1907)	1-Apr, 30-Apr	2	2	4	*			*			
Andrena (Scrapteropsis) atlantica (Mitchell, 1960)	23-Apr		1	1			*				
Andrena (Melandrena) confederata (Viereck, 1917)	24-Apr	2		2			*				
Andrena (Holandrena) cressonii (Robertson, 1891)	22-Apr		1	1			*				
Andrena (Callandrena sensu lato) fulvipennis (Smith, 1853)	9-Oct, 26-Oct	1	2	3				*			*
Andrena (Melandrena) hilaris (Smith, 1853)	1-Apr		1	1				*			
Andrena sp. (Fabricius)	21-Apr, 22-Apr	1	1	2					*		
Andrena (Larandrena) miserabilis (Cresson, 1872)	11-Apr	1		1		*					
Perdita (Hexaperdita) bishoppi (Cockerell, 1906)	10-Sep, 26-Oct	3	11	14				*	*		*
Perdita (Hexaperdita) boltoniae (Robertson, 1902)	20-Sep		1	1				*			*

species	*early and late date of collection*	*pan traps*	*netting*	*total*	*CRP*	*FEX*	*LLP*	*REF*	*SLR*	*above-ground nesting*	*oligolectic*
Pseudopanurgus labrosiformis (Robertson, 1898)	16-Oct		2	2				*			*
APIDAE											
Apis (Apis) mellifera (Linnaeus, 1758)	23-Apr, 14-Oct	3	48	51	*	*	*	*	*	*	
Bombus (Cullumanobombus) fraternus (Smith, 1854)	22-Jul, 24-Jul		2	2	*						
Bombus (Cullumanobombus) griseocollis (DeGeer, 1773)	30-Apr, 24-Jul	1	9	10	*				*		
Bombus (Pyrobombus) impatiens (Cresson, 1863)	1-Apr, 26-Oct	32	219	251	*	*	*	*	*		
Bombus (Thoracobombus) pensylvanicus (DeGeer, 1773)	18-Jun, 11-Sep	7	9	16	*		*	*			
Cemolobus ipomoeae (Robertson, 1891)	20-May, 15-Jul	7	15	22	*		*	*	*		*
Ceratina (Zadontomerus) calcarata (Robertson, 1900)	11-Apr, 16-Oct	30		30		*				*	
Ceratina (Ceratinula) cockerelli (Smith, 1907)	28-May, 26-Jul	1	14	15	*					*	
Ceratina (Zadontomerus) floridana (Mitchell, 1962)	11-Apr, 16-Oct	8	2	10	*	*				*	

species	*early and late date of collection*	*pan traps*	*netting*	*total*	*CRP*	*FEX*	*LLP*	*REF*	*SLR*	*above-ground nesting*	*oligolectic*
Ceratina (Zadontomerus) strenua (Smith, 1879)	29-May	1		1	*						
Epeolus lectoides (Robertson, 1901)	17-Jun		3	3		*					
Eucera (Synhalonia) dubitata (Cresson, 1878)	1-Apr, 30-Apr	7		7	*	*	*	*			
Habropoda laboriosa (Fabricius, 1804)	1-Apr, 24-Apr	1	10	11		*	*	*			*
Melissodes (Melissodes) bimaculata (Lepeletier, 1825)	13-Jun, 26-Oct	334	7	341	*	*	*	*	*		
Melissodes (Melissodes) communis (Cresson, 1878)	20-May, 12-Sep	167	17	184	*	*	*	*	*		
Melissodes (Eumelissodes) dentiventris (Smith, 1854)	7-Oct, 14-Oct		4	4		*		*			*
Melissodes (Eumelissodes) druriella (Kirby, 1802)	26-Oct	1		1				*			
Melissodes (Apomelissodes) mitchelli (LaBerge, 1956)	31-May		2	2			*				*
Melissodes (Melissodes) tepaneca (Cresson, 1878)	24-May, 26-Oct	39	1	40	*	*	*	*			
Melissodes (Eumelissodes) tincta (LaBerge, 1961)	5-Oct, 8-Oct		3	3				*			*

species	*early and late date of collection*	*pan traps*	*netting*	*total*	*CRP*	*FEX*	*LLP*	*REF*	*SLR*	*above-ground nesting*	*oligolectic*
Melissodes (Eumelissodes) trinodis (Robertson, 1901)	12-Jun	1		1				*			*
Melissodes sp.1 (Latreille)	17-Jun, 24-Jun	4		4			*	*			
Melissodes sp.2 (Latreille)	18-Sep	1		1	*						
Melitoma taurea (Say, 1837)	16-May, 15-Jul	24	22	46		*	*	*	*		*
Peponapis (Peponapis) pruinosa (Say, 1837)	12-Jun		1	1					*		*
Ptilothrix bombiformis (Cresson, 1878)	18-Jun, 24-Jul	3		3	*		*	*			*
Svastra (Epimelissodes) aegis (LaBerge, 1956)	8-Aug, 9-Aug		3	3				*	*		*
Svastra (Epimelissodes) atripes (Cresson, 1872)	24-Jul, 14-Oct	31	15	46	*			*	*		
Trachusa (Legnanthidium) ridingsii (Cresson, 1878)	22-Aug, 11-Sep		9	9				*			
Triepeolus monardae (Mitchell, 1962)	16-Oct		1	1				*			
Xylocopa (Schonnherria) micans (Lepeletier, 1841)	22-Jul, 13-Aug		11	11	*					*	

species	*early and late date of collection*	*pan traps*	*netting*	*total*	*CRP*	*FEX*	*LLP*	*REF*	*SLR*	*above-ground nesting*	*oligolectic*
Xylocopa (Xylocopoides) virginica (Linnaeus, 1771)	17-Jun, 5-Sep		5	5	*		*	*		*	
COLLETIDAE											
Caupolicana electa (Cresson, 1878)	9-Oct, 14-Oct		3	3				*			*
Colletes distinctus (Cresson, 1868)	14-May		1	1				*			
Colletes latitarsis (Robertson, 1891)	17-Jun		8	8			*				
Colletes mandibularis (Smith, 1853)	31-May		1	1			*				
Hylaeus (Paraprosopis) floridanus (Robertson, 1893)	22-Apr		1	1			*			*	
HALICTIDAE											
Agapostemon (Agapostemon) splendens (Lepeletier, 1841)	22-May, 21-Aug	11	14	25	*	*	*	*	*		
Agapostemon (Agapostemon) virescens (Fabricius, 1775)	16-May, 7-Sep	14		14	*	*		*			
Augochlora (Augochlora) pura (Say, 1837)	2-Apr, 27-Aug	4		4		*		*		*	

species	*early and late date of collection*	*pan traps*	*netting*	*total*	*CRP*	*FEX*	*LLP*	*REF*	*SLR*	*above-ground nesting*	*oligolectic*
Augochlorella aurata (Smith, 1853)	11-Apr, 10-Sep	126	6	132		*		*	*		
Augochloropsis (Paraugochloropsis) anonyma (Cockerell, 1922)	30-Apr, 28-Jun	31		31	*			*			
Augochloropsis (Paraugochloropsis) metallica (Fabricius, 1793)	14-May, 26-Oct	6	14	20		*	*	*			
Augochloropsis (Paraugochloropsis) sumptuosa (Smith, 1853)	2-Apr, 17-Jul	3		3			*	*			
Halictus (Odontalictus) poeyi (Lepeletier, 1841)	30-Apr, 13-Aug	8	13	21	*		*	*	*		
Lasioglossum (Dialictus) admirandum (Sandhouse, 1924)	21-Jun		1	1				*			
Lasioglossum (Dialictus) apopkense (Robertson, 1892)	1-Apr, 16-Oct	758	35	793	*	*	*	*	*		
Lasioglossum (Dialictus) batya (Gibbs, 2011)	9-Jul, 10-Sep	1	1	2				*			
Lasioglossum (Hemihalictus) birkmanni (Crawford, 1906)	11-Apr	1		1		*					
Lasioglossum (Dialictus) coreopsis (Robertson, 1902)	14-May, 30-May	4	2	6	*			*			
Lasioglossum (Dialictus) creberrimum (Smith, 1853)	29-May	1		1	*						

species	*early and late date of collection*	*pan traps*	*netting*	*total*	*CRP*	*FEX*	*LLP*	*REF*	*SLR*	*above-ground nesting*	*oligolectic*
Lasioglossum (Dialictus) floridanum (Robertson, 1892)	1-Apr, 16-Oct	697	22	719	*	*	*	*	*		
Lasioglossum (Dialictus) hitchensi (Gibbs, 2012)	11-Apr, 15-Oct	58	33	91	*	*	*	*	*		
Lasioglossum (Dialictus) illinoense (Robertson, 1892)	22-Apr, 16-Oct	159	15	174		*	*	*	*		
Lasioglossum (Dialictus) imitatum (Smith, 1853)	2-Apr, 20-Sep	6	5	11		*	*	*	*		
Lasioglossum (Dialictus) longifrons (Baker, 1906)	16-May, 8-Oct	64	35	99	*		*	*	*		
Lasioglossum (Dialictus) nymphale (Smith, 1853)	21-May, 5-Sep	42	2	44			*	*	*		
Lasioglossum (Hemihalictus) pectorale (Smith, 1853)	11-Apr, 27-Aug	44	10	54	*	*	*	*	*		
Lasioglossum (Dialictus) pilosum (Smith, 1853)	18-Sep	1		1	*						
Lasioglossum (Dialictus) platyparium (Robertson, 1895)	22-Aug, 10-Sep	1	1	2			*	*			
Lasioglossum (Dialictus) puteulanum (Gibbs, 2009)	31-May, 18-Sep	1	1	2	*		*				
Lasioglossum (Dialictus) raleighense (Crawford, 1932)	22-Apr, 17-Sep	22	5	27		*			*		

species	*early and late date of collection*	*pan traps*	*netting*	*total*	*CRP*	*FEX*	*LLP*	*REF*	*SLR*	*above-ground nesting*	*oligolectic*
Lasioglossum (Dialictus) reticulatum (Robertson, 1892)	1-Apr, 26-Oct	2048	140	2188	*	*	*	*	*		
Lasioglossum (Dialictus) tamiamense (Mitchell, 1960)	17-Jul	1		1			*				
Lasioglossum (Dialictus) tegulare (Robertson, 1890)	2-Apr, 15-Oct	192	16	208	*	*	*	*	*		
Lasioglossum (Dialictus) vierecki (Crawford, 1904)	24-Apr, 17-Sep	22	17	39	*		*				
Lasioglossum (Dialictus) weemsi (Mitchell, 1960)	2-Apr, 15-Oct	37	115	152	*	*	*	*	*		
Lasioglossum sp.1 (Curtis)	28-May		1	1	*						
Lasioglossum sp.2 (Curtis)	18-Jun	1		1			*				
Lasioglossum sp.3 (Curtis)	22-Aug		1	1			*				
Lasioglossum (Dialictus) callidum (Sandhouse, 1924)	1-Apr, 26-Oct	148	53	201	*	*	*	*	*		
Nomia (Acunomia) nortoni (Cresson, 1863)	13-Aug, 19-Sep		3	3	*						

species	*early and late date of collection*	*pan traps*	*netting*	*total*	*CRP*	*FEX*	*LLP*	*REF*	*SLR*	*above-ground nesting*	*oligolectic*
MEGACHILIDAE											
Anthidiellum (Loyolanthidium) notatum (Latreille, 1809)	31-May, 8-Oct		9	9		*		*	*	*	
Anthidiellum (Loyolanthidium) perplexum (Smith, 1854)	7-Oct, 8-Oct		3	3				*		*	
Coelioxys (Boreocoelioxys) octodentata (Say, 1824)	11-Jul		1	1					*		
Coelioxys (Boreocoelioxys) sayi (Robertson, 1897)	17-Oct		1	1		*					
Dianthidium (Dianthidium) curvatum (Smith, 1854)	18-Jul, 16-Oct	3	1	4				*			
Heriades (Neotrypetes) variolosa (Cresson, 1872)	14-May, 13-Sep		3	3		*		*		*	
Hoplitis (Alcidamea) producta (Cresson, 1864)	23-Apr		1	1			*			*	
Hoplitis (Alcidamea) truncata (Cresson, 1878)	14-May, 16-May	1	1	2				*		*	
Lithurgus gibbosus (Smith, 1853)	30-May, 31-May	1	4	5			*			*	*
Megachile (Xanthosarus) addenda (Cresson, 1878)	20-May, 31-May	3	1	4			*	*	*		

species	*early and late date of collection*	*pan traps*	*netting*	*total*	*CRP*	*FEX*	*LLP*	*REF*	*SLR*	*above-ground nesting*	*oligolectic*
Megachile (Acentron) albitarsis (Cresson, 1872)	18-Jun, 10-Sep	1	4	5	*			*	*		
Megachile (Chelostomoides) campanulae (Robertson, 1903)	12-Jun	1		1				*		*	
Megachile (Sayapis) frugalis (Cresson, 1872)	21-May		2	2		*				*	
Megachile (Chelostomoides) georgica (Cresson, 1878)	14-May, 27-Aug		8	8			*	*	*	*	
Megachile (Sayapis) inimica (Cresson, 1872)	20-Sep		1	1				*		*	
Megachile (Litomegachile) mendica (Cresson, 1878)	12-Jun, 15-Oct	2	45	47	*		*	*	*	*	
Megachile (Leptorachis) petulans (Cresson, 1878)	20-May, 15-Oct	8	52	60	*	*	*	*	*		
Megachile (Litomegachile) pseudobrevis (Mitchell, 1936)	26-Apr, 27-Jun	3	1	4	*			*			
Megachile (Megachiloides) rubi (Mitchell, 1924)	23-Apr		1	1			*				*
Megachile (Litomegachile) texana (Cresson, 1878)	14-May, 8-Oct	5	10	15	*	*	*	*	*		
Osmia (Melanosmia) atriventris (Cresson, 1864)	11-Apr	1	1	2		*					

species	*early and late date of collection*	*pan traps*	*netting*	*total*	*CRP*	*FEX*	*LLP*	*REF*	*SLR*	*above-ground nesting*	*oligolectic*
Osmia (Helicosmia) chalybea (Smith, 1853)	1-Apr, 30-Apr	2	2	4	*	*		*			
Osmia (Melanosmia) inspergens (Lovell and Cockerell, 1907)	22-Apr	1		1					*		
Osmia (Melanosmia) sandhouseae (Mitchell, 1927)	5-May		1	1				*			
Paranthidium (Paranthidium) jugatorium (Say, 1824)	5-Oct		1	1				*			*
Stelis (Heterostelis) australis (Cresson, 1878)	7-Sep, 11-Sep	1	2	3				*			
Stelis (Dolichostelis) costalis (Cresson, 1872)	13-Jun		1	1				*			
Trachusa (Heteranthidium) dorsalis (Lepeletier, 1841)	7-Oct		1	1				*			
MELITTIDAE											
Melitta (Cilissa) americana (Smith, 1853)	7-May, 21-May		2	2		*					*