Pollinator Diversity on a Shortleaf Pine-Oak-Hickory Restoration Site

Taygan Kohlman

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POLLINATOR DIVERSITY ON A SHORTLEAF PINE-OAK-HICKORY RESTORATION SITE

by

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be accepted in partial fulfillment of the requirements for the degree of

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ABSTRACT

Land use and cover are increasingly changing due to climate change and anthropogenic activities with many of these changes negatively impacting biodiversity. However, ecosystem restoration may help reverse these patterns. Northern Louisiana was historically dominated by shortleaf pine-oak-hickory forests but has been converted largely to loblolly pine forests with little-to-no herbaceous ground cover. The objective of this study is to determine how different practices on a shortleaf pine-oak-hickory forest restoration site affect pollinator diversity. I sampled pollinators from locations within areas under three different management regimes: 1) prescribed burning with active herbicide treatments for non-desirable plant species, 2) burning only, and 3) no active management. I predicted prescribed burning would increase pollinator species diversity and abundance and increases will be highest when combined with herbicide treatments. For data collection I set out pan traps containing ~200 ml of water with soap. Pan traps were placed 5m apart in an “X” shape and left out for 24 hrs. Three plots in each of the three management regimes (n=9) were sampled once a month for 6 months. I found greater diversity, evenness, and richness where burning occurred compared to no management. Specifically, Burn+Herbicide sites had ~1.5 times higher diversity than no management sites, and Burn+Herbicide sites were similar to burn only sites. These results are useful for future restoration efforts and management practices of shortleaf pine-
hardwood forests because they show prescribed fire alone has positive impacts on pollinator diversity.
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Author _____________________________

Date _____________________________
DEDICATION

To my friends and family that supported and encouraged me throughout this process.
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CHAPTER 1
INTRODUCTION

Land use and cover are increasingly changing due to climate change and anthropogenic activities (Haines-Young, 2009). Many of these changes negatively impact biodiversity via increased habitat fragmentation and degradation, decreased habitat heterogeneity and structural complexity, and facilitating invasive species establishment (Vitousek et al., 1997; Sala et al., 2000; Tilman et al., 2001; Potts et al., 2010; Guldin and Black, 2018; Beckmann et al., 2019; Baidya and Bagchi, 2022). Changes to land use and cover include increased conversion of natural habitats to agricultural and commercial land, which often results in decreased forestland (Meyer and Turner, 1992). Moreover, removal of native vegetation and changes in abiotic habitat characteristics that accompany land use change may reduce competition and open or create niches that enable establishment of fast-growing and invasive species (Guldin and Black, 2018). Together, these changes may decrease ecosystem stability and biodiversity causing habitats to become less resistant and resilient to disturbance and shift ecosystems to alternate stable states (Schmid et al., 1999; Scheffer et al., 2001; Suding et al., 2004; Beckage and Ellingwood, 2008; Hanberry, 2021). Reversion to a prior stable state is sometimes possible by manipulating the presence or absence of governing abiotic and biotic factors such as fire, nutrients, and fishing and hunting pressure (Aronson et al., 1993; Hobbs and Norton 1996; Fahrig, 2003; Suding et al., 2004; Guldin and Black,
However, assessment is necessary to determine the status of current ecosystems states that have experienced or are undergoing change.

Habitat restoration ecologically restores an area to provide suitable habitat for one or more species via maintenance of soil and water quality, as well as the establishment and management of native plant communities (Miller and Hobbs, 2007). Often the goal of habitat restoration is to shift ecosystems back to their historical states, which can subsequently improve air quality, decrease rates of biodiversity loss, and improve human-nature relationships in altered, degraded, damaged, or destroyed ecosystems (Perring et al., 2015; Martin, 2017). Restoration efforts differ depending on habitat size, geographic region, and overall goal of the restoration (Miller and Hobbs, 2007). Forest restoration is increasing because forest ecosystems may support a large diversity of plant and animal species, they positively impact carbon cycling through sequestration and storage of carbon, and they have undergone lots of change and degradation in the recent past (Allen et al., 2010; Bremer and Farley, 2010; Hicke et al., 2012). In particular, wildfires, logging, and urbanization have strongly contributed to the loss of native and old growth forest habitat (Hanula et al., 2015; Souza-Alonso et al., 2022). Forest restoration may include reintroduction of native plant species, implementation of disturbance techniques based on desired habitat goals (i.e., prescribed burning, thinning, etc.), and an evaluation plan to habitat development (Guldin, 2019; Stanturf et al., 2014; Turley et al., 2017). Assessment of focal animal and plant species are often used to evaluate efficacy of restoration, particularly when species are indicative of a specific forest type or condition (McGeoch, 1998; De Cáceres et al., 2010). For example, insect pollinators like bees may indicate efficacy of restoration efforts when habitats include a large proportion of insect-
pollinated plant species, but these are not common practices (Schultz et al., 2008; Williams, 2011). However, monitoring ecological restoration progress and efficacy is not typically studied after restoration is conducted, due to time or money constraints (Bried et al., 2014), and thus many restoration efforts lack data to measure success.

Many insect species are effective bioindicators that may help determine the efficacy of different management techniques because of their importance to ecosystems as pollinators, decomposers, herbivores, and prey for other animals (Didham et al., 1996; Kevan and Campos, 1983; McCullough et al., 1998; McGeoch, 1998; Toth, 2003; Holt and Miller, 2011; Mason et al., 2021). Moreover, insect populations are declining globally (Bartomeus et al., 2013; Lebuhn et al., 2013), and in part due to habitat loss (Burkle et al., 2013; Hanula et al., 2015; Hristov et al., 2020; Wagner et al., 2021). Insect pollinators, such as bees and wasps (Hymenoptera), flies (Diptera), butterflies and moths (Lepidoptera), and beetles (Coleoptera) are similarly experiencing global declines (Halvorson et al., 2021) and are often used to assess habitats (Mason et al., 2021; Zaric et al., 2021; Hristov et al., 2020). Insect pollinators facilitate the success of more than 80% of flowering plants and about 75% of agricultural crops through increased out-crossing success (Hristov et al., 2020; Ghisbain et al., 2021). Additionally, pollinators indirectly contribute to a variety of ecosystem functions, such as increased water and air quality via increased plant fitness leading to soil stabilization through increased root biomass and increased CO₂ uptake via photosynthesis (Wagner et al., 2021). Given pollinators support the health of natural ecosystems and they may serve as effective monitors of restoration progress and efficacy, understanding how habitat restoration impacts pollinator diversity
is increasingly important (Mortimer et al., 1998; Morse and Calderone, 2000; Williams 2011; Bried et al., 2014; Mason et al., 2021).

Bees (Hymenoptera: Apoidea) have some of the largest impacts on plants of all insect pollinators (Klein et al., 2007; Winfree, 2010; Hanula et al., 2015; Affek et al., 2021). Unlike many other pollinators (e.g., flies, beetles, etc.), bees are dependent on flowers across their life cycle as immature bees are fed from honey derived from flowers and adults are sustained from nectar (Winfree, 2010). Native bees play an important role in the management of plant communities, which in turn affects soil health, water quality, vegetative food and cover for wildlife, and trophic levels higher in the food web (Tepedino, 1979; Potts et al., 2003; Potts et al., 2010). Thus, bees are often effective bioindicators and may help restoration ecologists determine if their management practices are having a positive impact in a restored ecosystem (Barganska et al., 2016; Zaric et al., 2021).

In the southeastern United States, much of the historical land cover was forest, which included shortleaf pine (*Pinus echinata*)-hardwood forests (Mattoon, 1915; Ramankutty and Foley, 1999; Guldin and Black, 2018). Shortleaf pine-hardwood forests covered an estimated 70 to 80 million acres and had a range covering most of the southern United States (Guldin and Black, 2018; Hanberry, 2021). Shortleaf pine-hardwood forests are characterized by a relatively open canopy and the increased sunlight results in a prominent herbaceous ground cover of grasses and forbs (Hedrick et al., 2007). Fire maintains shortleaf pine-hardwood forests via increased shortleaf pine seedling success, herbaceous ground cover dominated by native vegetation, and canopy openness (Lester, 2005; Tauer et al., 2012; Hanula et al., 2015; Guldin, 2019). Today
only about 6.1 million acres of these forests remain, which is a decline of more than 90% (Anderson et al., 2016; Guldin and Back, 2018). About 52% of the shortleaf pine-hardwood forest declines that have occurred happened after 1980 (Anderson et al., 2016). This loss has been attributed to historic fire suppression which often facilitated the success of other species like loblolly pine (*Pinus taeda*), resulting in dense canopies prohibiting herbaceous ground cover and shortleaf pine seedling success (Lawson, 1990; Tauer et al., 2012; Hanula et al., 2016; Simmons and Bossart, 2020). This recent decline is of great concern because shortleaf pine-hardwood forests provide habitat for a large number of wildlife species throughout all stages of succession (Masters, 2007). Additionally, shortleaf pine-hardwood forests provide a greater food supply due to more vegetative ground cover resulting from the decreased canopy cover (Masters, 2007).

Of particular importance in shortleaf pine-hardwood forests is the prominence of herbaceous ground cover that leads to increased plant diversity, habitat heterogeneity, and often animal diversity from increased plant resource and habitat structure (Haines-Young, 2009; Burrow et al., 2021). Shortleaf pine-hardwood restoration efforts often combine promoting the establishment of historically dominant and important woody and herbaceous plant species with removing undesirable species that may outcompete them, such as loblolly pine, sweetgum (*Liquidambar styraciflua*), and Chinese tallow (*Triadica sebifera*) via prescribed burning, targeted herbicide applications, and seeding of target species (Edwards-Burke et al., 1997; Wagner et al., 2004; Iglay et al., 2010). Shortleaf pine-hardwood forest restoration may result in increased pollinator diversity and abundance because of the increased herbaceous ground cover and forbs, but there is a
paucity of research on shortleaf pine-hardwood forest restoration impacts on pollinator diversity.

Here I examine insect pollinator diversity in a shortleaf pine-hardwood restoration forest and compare pollinator communities among sites subject to three different management regimes: prescribed burning only, both prescribed burning and targeted herbicide treatments, and unmanaged. I predict sites undergoing regular implementation of prescribed burning and targeted herbicide will have higher pollinating insect diversity, and bee species diversity in particular, followed by sites receiving only prescribed burning, with the lowest pollinating insect and bee diversity on sites that undergo no management. I predict the sites undergoing regular prescribed burning and targeted herbicide will have higher pollinating insect diversity and bee species diversity because of the increased canopy openness that allows for more herbaceous ground cover. More herbaceous ground cover usually results in more flowering plants, which should attract a higher diversity of pollinators. This study will increase our understanding of how current habitat restoration efforts and management practices impact pollinator diversity in a shortleaf pine-hardwood restoration site.
CHAPTER 2

METHODS

2.1 Study Site

This study was conducted at Wafer Creek Ranch (hereafter WCR), near Ruston, Louisiana, USA (32.57614°N, -92.72049°W), and on privately-owned property adjacent to WCR, from April through September 2022. WCR is a 500-acre area enrolled as a Nature Conservancy easement. WCR comprises two main soil types: the Cockfield sediments present in higher elevations, which originate from non-marine deposits, and the older Cook Mountain sediments found at lower elevations originating from marine deposits. Soils on the property are fine sandy loams that are low in nutrients, acidic, highly weathered, and highly leached (USDA, NRCS, Soil Survey of Lincoln Parish, Louisiana, April 1996).

In the early to mid-1800s, land cover at WCR was shortleaf pine-oak (*Quercus spp.*)-hickory (*Carya spp.*), mixed hardwood-loblolly pine, and small-stream forest, a rare type of forest ecosystem that occurs along small rivers and large creeks (J. Armstrong, personal communication; Jones, 2005). After 1850, however, the land was partially cleared and used for agricultural purposes, most notably cotton production (J. Armstrong, personal communication). Due to these agricultural practices, portions of the land underwent frequent disturbance that removed most of the native vegetation, such as native grasses and forbs, from these areas.
Currently, WCR is undergoing restoration to the native shortleaf pine-oak-hickory forest of northern Louisiana. This restoration began in early 2007 and is taking place because fragments of the original, untouched shortleaf pine-hardwood forest still remain, despite the land being partially cleared for agriculture. Botanists from The Nature Conservancy of Louisiana estimated that some of the shortleaf pine trees present on WCR are ≥ 100 years old. Shortleaf pine trees in the area may have also naturally reestablished themselves in areas where agriculture was abandoned in the 1800s (J. Armstrong, personal communication).

Current management techniques at WCR include seeding areas with little bluestem (*Schizachyrium scoparium*) to restore the native herbaceous ground cover that characterizes shortleaf pine-hardwood forests. Additionally, about 300 acres of the property receives prescribed burns approximately every other year in the winter. The first burn was conducted in March of 2009 and the most recent burn conducted was in the winter of 2021, with the prescribed burn sites and the prescribed burn sites with targeted herbicide treatments being burned at the same time. Targeted glyphosate herbicide application is used within upland areas and in areas with evidence of invasive or dominance of undesirable vegetation, this began sometime in 2009. Herbicide is applied via the hack-and-paint method or spot treating unwanted trees and ground vegetation. The hack-and-paint method of treatment is usually done in the winter, and it is done by ringing a tree with an axe and then painting on 41% glyphosate herbicide with a paint brush. This method is used to remove larger trees. To remove herbaceous ground cover, the spot treatment method is used during the vegetative growing season (spring-summer) when everything is in bloom and at optimal growth. It involves directly spraying a
glyphosate herbicide on unwanted vegetation, like Chinese privet (*Ligustrum sinense*). This spot herbicide treatment is done as needed during the growing season to prevent new growth of invasive species. The sites that receive no management have not undergone any restoration to a shortleaf pine-hardwood forest and all have a similar land use history. Ultimately, this creates a mosaic of three different management practices in the restoration site: 1) sites subjected to regular prescribed burns hereafter referred to as Burn sites, 2) sites that are burned and receive targeted herbicide applications, hereafter referred to as Burn+Herbicide sites, and 3) sites that have not undergone any restoration, hereafter referred to as No Management sites (Figure 2-1).
2.2 Experimental Design

Three plots in each management treatment were randomly selected using ArcGIS® (n = 9; Figure 2-1). Distance between plots ranged from 230 m (B1 to B2) to 1,680 m (B1 to B3). At each plot, I set out pan traps to collect pollinators following the standard monitoring protocol of the Food and Agriculture Organization of the United Nations (LeBuhn et al., 2016). I set out 20 pan traps per plot in an ‘X’ formation, ~ 5 m

![Sampling Points for Wafer Creek Ranch Pollinators](image)

**Figure 2-1:** Satellite image of Wafer Creek Ranch, Ruston, Louisiana with pollinator collection points (dots), and associated disturbance treatments in the shortleaf pine-hardwood forest: B (yellow polygons) represent sites receiving prescribed burns; BH (red polygons) represent sites receiving prescribed burns and herbicide treatment; C (green polygons) represent sites not undergoing restoration management. Areas on Wafer Creek Ranch not within a polygon represent lower elevation riparian forest not undergoing restoration.
apart, and they were left out for 24 hours over a period of good weather with no rain and minimal wind (Figure 2-2). I placed pan traps at all nine plots on the same day on the ground, making sure they were not placed directly in dense clumps of tall vegetation where they would be obscured (Cane et al., 2000; Williams, 2011; LeBuhn et al. 2016). Pan traps are used because they mimic floral resources, which draws pollinators to them (Cane et al., 2000; Wallen, 2010). I used three different fluorescent colors of pan traps commonly used to collect pollinating insects (yellow, blue, and pink), and included a clear trap as a control (n = 5 of each pan trap color per plot). Pan traps consisted of clear plastic deli dishes 14.8 cm in diameter and 7.1 cm in height (~355 ml), painted with acrylic fluorescent paint on the outside (color pan traps) or not painted at all (clear control). I used these colors to determine if specific pan trap colors attract different pollinators because different groups of pollinators have different visual systems causing them to, potentially, have different color preferences. I used control pan traps to determine if insects collected represent pollinators or accidental bycatch (e.g., Collembolans). I placed pan trap colors randomly along the X-shaped transect and filled pan traps with enough soapy water to cover the bottom to prevent insects from escaping. I sampled pollinators from the nine plots monthly from April through September 2022, totaling six sampling dates. During pan trap collection, I pooled insects collected in pan traps at each site by color of the pan traps (i.e., all insects collected in a single color of pan trap were combined at a given site), yielding four pollinator collection samples per site. I collected insects in pan traps by filtering them from the soapy water with a piece of chiffon fabric to ensure even the smallest of insects were collected (e.g., parasitoid wasps and gnats). I placed the chiffon containing filtered insects into a labeled plastic bag and
brought them back to the lab. I then sprayed each of the samples with 70% ethanol to preserve the insects, and samples were stored in a freezer (-20 °C) until processed.

Upon collection of pan traps, I quantified habitat characteristics to determine if they accounted for variation in pollinator diversity patterns and management practice differences. Specifically, I measured the following at the center of the ‘X’ formation at each collection site on each sampling date in each of the four cardinal directions: percent canopy cover, percent ground cover, and vegetation height. Percent canopy cover was measured using a densiometer. Using the densiometer, the number of open quadrats in each of the 24 equal-sized concave mirror squares (meaning, they do not have any vegetation filling in that space of the canopy) was recorded. The four measurements were averaged at each sampling point and date. In addition, percent ground cover was measured by placing a 0.5 m x 0.5 m quadrat on the forest floor in each of the cardinal directions at each of the plots and taking a level picture at approximately 1.4 m from the ground. Each of these images was analyzed for percent ground cover using ImageJ. Specifically, in ImageJ I selected the area within each quadrat and manipulated the hues, saturation, and brightness of each ground cover photo to isolate all of the pixels that make up the green vegetation. I then turned the selected pixels black and the unselected pixels white, creating a masked image only showing the selected black pixels from which I calculated percentage of the quadrat area with green vegetation (Crawley, 2011; Xiong et
al., 2019). The percent ground cover from the four images per site was averaged for each sampling time. Vegetation height was measured using visual obstruction methods with a 1 m Robel pole with markings every 5 cm (Robel et al. 1970). Standing 4.5 m from the pole, I crouched to eye level with the vegetation and estimated the average height of the vegetation by noting the lowest visible marking on the Robel pole not covered in vegetation. Vegetation height measurements were averaged for each site at each sampling time.

2.3 **Insect Identification**

Insects were identified to family using Bland (2010) and Borror and DeLong (1971) and either pinned or preserved in 70% ethanol. To verify the identification of

![Figure 2-2: Schematic of the ‘X’ pan trap placement for collecting pollinating insects at each plot and sampling time. The different color circles indicate pan trap color, with black circles representing clear, with random color placement.](image)
specimens in the order Diptera, I used Marshall (2012) and Skevington (2019). To verify
the identification of specimens in the order Lepidoptera, I used Marks (2018). To verify
the identification of specimens in the order Hymenoptera, I used Carril and Wilson
(2021), Holm (2021), and Holm (2017). To verify the identification of the specimens in
the order Coleoptera, I used White (1998) and Evans (2014). To help determine if a
specimen is a pollinator or not, I used Holm (2014).

After all insects were identified to family level, the insects classified as bees were
separated. Once all bees were separated, they were further identified down to genus and
then separated into morphospecies. Each morphospecies was then identified to species
using Carril and Wilson (2021), Holm (2017), Holm (2014), and Borrer and DeLong
(1971).

2.4 Statistical Analysis

To test the null hypothesis that species diversity and community structure does
not differ among management strategies, I used Permanova (Anderson et al. 2001). I
quantified species diversity using Shannon’s Diversity Index,

$$H' = \sum_{i=1}^{s} \rho_i \times \ln \rho_i$$

Eq. 2-1

and evenness using Shannon’s Equity Index,

$$E_H = H' / \ln S$$

Eq. 2-2

where $H'$ is diversity, $E_H$ is equity, $S$ is the number of species, and $\rho_i$ is the proportion of
individuals the $i$th species in the sample. I calculated diversity indices for ‘all pollinators’
(family level) and ‘just bees’ (species level). All analyses were performed at both of these levels. To analyze bee data, pan traps with an abundance of zero were removed from the data set. This lowered the degrees of freedom to 16 due to the removal of one yellow pan trap, nine clear pan traps, and nine pink pan traps. To determine if pollinator diversity differed among different forest restoration management strategies and pan trap colors, I tested the null hypothesis that species diversity, evenness, richness, and abundance do not differ among the three management strategies, by pan trap color, or their interaction using adonis in the vegan package in R with 9999 permutations using Euclidean dissimilarity measures (Oksanen et al., 2022). Data were tested for normality and variables that were not normal were transformed to meet the assumption of normality. Specifically, abundance and evenness were not normal and were inverse (1/(x)) transformed.

Similarly, I tested the null hypothesis that community structure did not differ among management strategies, pan trap colors, and their interaction using the adonis function but with Bray-Curtis dissimilarity measures on square-root transformed abundances. Significant differences (α = 0.05) in analyses among management strategies and pan trap colors were followed by post-hoc analyses with adjusted p-values using the pairwise.adonis wrapper function (Martinez-Arbizu 2020). Significant differences in community structure analyses among management strategies and pan trap colors were followed by Indicator Species Analysis using the labdsv package in R (Roberts, 2019) to determine if any species are indicative of a specific forest management type or pan trap color. Separate tests were run for ‘all pollinators’ and ‘just bees.’ Community results were visualized using NMDS using the MetaMDS function in the vegan package.
To determine sampling effort efficacy, I performed rarefaction analyses, and to predict total species diversity, I used Chao estimation methods using iNEXT (Chao et al., 2014; Chao et al., 2016). iNEXT has non-asymptotic and asymptotic analyses for species diversity based on Hill numbers and computes predicted diversities based on sample completeness or a common sample size (Chao et al., 2014). Chao estimation methods use the collected data to estimate species diversity and extrapolate the data to predict species diversity if the sample size was doubled.

To determine which variables best accounted for variation in pollinator diversity, I used multiple regression analysis testing pollinator diversity, evenness, richness, and abundance relationships with canopy openness, vegetation height, and ground cover. Variables used in the multiple regression were tested for normality using SPSS (Version 28.0.1.0(142)). Variables that were not normal were transformed to help fit the assumptions of normality. Specifically, abundance, evenness, and canopy openness were log_{10} (x) transformed and vegetation height was log_{10} (x + 1) transformed due to some zero measurements. Lastly, ground cover was arcsine transformed as it was a proportion. In-text values are reported as mean ± standard deviation unless otherwise noted. I tested for differences among sites in vegetation characteristics using ANOVA in SPSS.
CHAPTER 3
RESULTS

3.1 Pollinator Data

3.1.1 Pollinator Data by Treatment

Across 6 months of sampling, I collected a total of 6028 insects, of which 3565 were classified as pollinators. Insect families collected but not classified as pollinators are listed in Table A-1. Pollinators were collected from 85 families of 5 orders. The number of pollinators collected over the entire study period varied among sites: a total of 1665 pollinators from 54 families were collected in the plots with No Management (555 ± 270), 988 pollinators from 60 families in the Burn sites (329 ± 134), and 912 pollinators from 58 families in the Burn+Herbicide sites (304 ± 99; Figure 3-1). When comparing

![Figure 3-1: Mean abundance of pollinators collected over 6 months averaged by each of the 3 plots across each management regime. Error bars are standard deviation.](image-url)
families caught among treatment, I found that 70.59% of the total families caught were found in the Burn sites and 68.24% and 64.71% of the total families caught were found in the Burn+Herbicide and No Management sites, respectively. Of the 60 families found in the Burn sites, 41 were found in the Burn+Herbicide sites (68.33% shared) and 44 of those families were found in the No Management sites (73.33% shared). Of the 58 families found in the Burn+Herbicide sites, 41 were found in the No Management sites (70.69% shared).

Community composition differences were measured for each plot by pan trap color giving us 36 sampling points (9 sites x 4 colors). Community composition differed among sites (pseudo$F_{2,24}=1.449$, $p=0.020$; Figure 3-2a) and pan trap colors (pseudo$F_{3,24}=3.413$, $p=0.0001$; Figure 3-2b; Figure 3-3), and there was no interaction (pseudo$F_{6,24}=1.110$, $p=0.161$). Despite finding a difference in community composition, we only found a slight difference between Burn+Herbicide sites and No Management sites in post-hoc tests (Pseudo$F_{1,22}=1.600$, $p=0.093$). Additionally, there was no difference between Burn sites and No Management sites (pseudo$F_{1,22}=0.943$, $p=0.453$), and between Burn sites and Burn+Herbicide sites (pseudo$F_{1,22}=1.341$, $p=0.188$). Chalcididea (Hymenoptera) was an indicator taxa for the Burn sites and was on average 16-fold and 4-fold more abundant on Burn sites than Burn+Herbicide and No Management sites, respectively. Elateridae and Scarabaeidae (Coleoptera) were indicators of the No Management sites and were at least 4-fold and 3-fold more abundant on No Management sites than Burn sites and at least 3-fold and 9-fold more abundant than on Burn+Herbicide sites, respectively. There were no indicator taxa for the Burn+Herbicide sites (Figure 3-4; Table A-2).
Figure 3-2: The data points are the pollinator communities measured at each site. The polygons encompass sites within each management regime to facilitate visualization of the differences and similarities among communities and management regimes. The centroids represent what the average community would be within each of the polygons. The stress from this NMDS is 0.15. (a) Community composition of the three treatments, Burn (yellow), Burn+Herbicide (red), and No Management (green). (b) Community composition of the four pan trap colors, Blue, Clear (black), Pink, Yellow.
Pollinator diversity differed among management regimes (pseudo-$F_{2,24}=26.519$, $p=0.0001$). Burn+Herbicide sites had the highest diversity ($2.19 \pm 0.38$) and No Management sites had the lowest ($1.48 \pm 0.33$). Similarly, pollinator diversity was at least 1.5-fold higher in Burn+Herbicide sites than No Management sites. Richness did not
differ across management regimes (pseudoF\(_{2,24}=1.271, p=0.296\)). Burn sites had the highest richness (15.75 ± 11.78) and No Management sites had the lowest richness (10.67 ± 4.6). Pollinator richness was at least 1.48-fold higher in Burn sites than No Management sites. Evenness differed among management regimes (pseudoF\(_{2,24}=23.788, p=0.0001\)). Burn+Herbicide sites had the highest evenness (0.88 ± 0.07) and No Management sites had the lowest (0.68 ± 0.2). Similarly, pollinator evenness was at least 1.29-fold higher in Burn+Herbicide sites than No Management sites. Abundance did not differ among management regimes (pseudoF\(_{2,24}=1.241, p=0.30\); **Figure 3-5**). 

![Figure 3-5: Diversity, Abundance, Richness, and Evenness measurements by management regime. Different letters indicate differences between sites. Bars represent standard deviation.](image)

3.1.2 **Pollinator Treatment by Pan Trap**

Pollinators were most abundant in yellow pan traps with 2950 insects across 70 families (328 ± 179; 82.35% of the total families collected) followed by blue with 252
insects across 45 families (28 ± 8; 52.94% of the total families collected), then clear with 199 insects across 29 families (22 ± 12; 34.12% of the total families collected), and lastly pink with 163 insects across 34 families (18 ± 8; 40.0% of the total families collected; Figure 3-3).

Pollinator composition differed among pan trap colors (pseudoF\(_{3,24}=3.413\), p=0.0001; Figure 3-2b). Pollinator composition in yellow pan traps differed from clear pan traps (pseudoF\(_{1,16}=9.175\), p=0.001), blue pan traps (pseudoF\(_{1,16}=10.384\), p=0.001), and pink pan traps (pseudoF\(_{1,16}=9.603\), p=0.001). Only 26 families were shared between yellow and clear pan traps (37.14% shared), 36 between yellow and blue (51.43% shared), and 30 between yellow and pink (42.86% shared). Blue pan traps also differed from pink (pseudoF\(_{1,16}=2.623\), p=0.001) and clear pan traps (pseudoF\(_{1,16}=3.568\), p=0.001). Only 26 families were shared between blue and pink pan traps (57.78% shared), and 22 between blue and clear pan traps (48.89% shared). Clear pan traps did not differ from pink pan traps (pseudoF\(_{1,16}=1.343\), p=0.169) and shared 64.7% of their families (22 families shared; Figure 3-2b).

There were 26 indicator species found for pan trap colors; 25 were indicators for yellow pan traps and one, Apidae, was an indicator for blue pan traps (Figure 3-6; Table A-3).
Diversity differed by pan trap color (pseudoF\(_{3,24}=7.260, p=0.0015\)). I found yellow pan traps had the highest diversity (2.16 ± 0.81), and clear pan traps had the lowest (1.61 ± 0.26). Similarly, pollinator diversity was at least 1.34-fold higher in yellow pan traps than clear pan traps. I found richness differed by pan trap color (pseudoF\(_{3,24}=15.913, p=0.0001\)). Yellow pan traps had the highest richness (26.33 ± 8.93) and clear pan traps had the lowest richness (7.89 ± 2.2). Pollinator richness was at least 3.34-fold higher in yellow pan traps than clear pan traps. Evenness also differed between pan trap colors (pseudoF\(_{3,24}=18.408, p=0.0001\)). I found pink pan traps had the highest

**Figure 3-6**: Mean abundance of pan trap color indicator species for Coleoptera, Diptera, Hymenoptera, and Lepidoptera families. Indicator Values (IV) are labeled on the bar representing the pan trap color for which this family was an indicator. Bars represent standard deviation.
evenness (0.9 ± 0.05) and yellow pan traps had the lowest (0.66 ± 0.2). Similarly, pollinator evenness was at least 1.36-fold higher in pink pan traps than yellow pan traps. I found a difference in abundance between pan trap colors (pseudoF,3,24=16.2136, p=0.0001). Yellow pan traps had the highest abundance (328 ± 179) and pink pan traps had the lowest (18 ± 8). Pollinator abundance was at least 18-fold higher in yellow pan traps than pink pan traps (Figure 3-7).

![Diversity, Abundance, Richness, and Evenness measurements by pan trap color. Different letters indicate differences between colors. Bars represent standard deviation.](image)

**Figure 3-7:** Diversity, Abundance, Richness, and Evenness measurements by pan trap color. Different letters indicate differences between colors. Bars represent standard deviation.

Sample-based rarefaction of all 36 sampling points (9 sites x 4 colors) combined estimated 143 pollinating-insect families (99–186; 95% CI; Chao 2 estimate) for the entire study area. Individual-based rarefaction curves demonstrated Burn and Burn+Herbicide sites had the highest and similar family richness (Figure 3-8). Sample-based rarefaction curves estimated similar family diversities for all management regimes,
but family diversity was highest in Burn sites, then Burn+Herbicide sites, and lastly No Management sites (Figure 3-8). The Chao 2 estimate of the asymptotic family richness was 118 (42–195; 95% CI) for Burn, 100 (69–131; 95% CI) for Burn+Herbicide, and 98 (64–133; 95% CI) for No Management.
Average vegetation height differed among sites and was highest on Burn+Herbicide sites, which was 1.2-fold and 2.8-fold higher than on Burn and No Management sites, respectively (pseudoF$_{2,36}=5.529$, p=0.0004; Figure 3-9). Average percent ground cover differed among sites and was highest for the Burn+Herbicide sites.
which was 1.5-fold and 4.8-fold greater than Burn and No Management sites, respectively (pseudoF_{2,36}=11.519, p=0.0001; **Figure 3-9**). Similarly, average canopy openness was different among sites with the greatest canopy openness in Burn+Herbicide sites, with ~4-fold less canopy openness in the Burn sites, and 8-fold less canopy openness in the No Management sites (pseudoF_{2,36}=15.491, p=0.0001). Vegetation measurements accounted for a total of 18.4% of the variation in pollinator family richness, but only canopy openness was a significant predictor in the model.

**Figure 3-9**: Different site characteristics by treatment. Different letters represent differences between management regimes. Bars represent standard deviation.
(pseudoF_{2,6}=4.548, p=0.005; **Table 3-1; Figure 3-10**). Similarly, 29.8% of the variation in pollinator abundance was accounted for by vegetation height, which was the only statistically significant variable (pseudoF_{2,6}=8.314, p=0.005; **Table 3-1; Figure 3-10**). None of the vegetation measurements accounted for significant variation in either diversity or evenness.

![Figure 3-10: Multiple regression models showing the relationships between predictor variables and outcome variables. The solid lines indicate significance (p<0.05) and dotted lines indicate no significant relationship. The original vegetation height measures were in centimeters, but data were log x+1 transformed to meet normality assumptions. Ground cover was arcsine transformed and canopy openness was log transformed to meet normality assumptions.]

3.2 **Bee Data**

3.2.1 **Bee Data by Treatment**

Of the 3565 insect pollinators collected, 127 of them were bees. The 127 bees collected comprised 12 species of 9 genera in 4 families. I captured 11 bees of 4 species
in No Management sites (4 ± 3), 25 bees of 9 species in Burn sites (8 ± 3), and 91 bees of 8 species in Burn+Herbicide sites (21 ± 8; **Figure 3-11**).

**Table 3-1:** Multiple regression results showing the b-values, F-values, p-values, R squared and adjusted R squared for all of the variables in the multiple regression models.

<table>
<thead>
<tr>
<th>Outcome Variable</th>
<th>Predictor Variable</th>
<th>b</th>
<th>F</th>
<th>p</th>
<th>R²</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diversity</td>
<td>Vegetation Height</td>
<td>-0.093</td>
<td>1.49</td>
<td>0.729</td>
<td>0.082</td>
<td>0.027</td>
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<td></td>
<td>Ground Cover</td>
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<td></td>
<td></td>
<td>0.139</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Canopy Openness</td>
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<td></td>
<td>0.074</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abundance</td>
<td>Vegetation Height</td>
<td>-0.446</td>
<td>8.314</td>
<td>0.005</td>
<td>0.333</td>
<td>0.293</td>
</tr>
<tr>
<td></td>
<td>Ground Cover</td>
<td>0.199</td>
<td></td>
<td>0.148</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Canopy Openness</td>
<td>-0.136</td>
<td></td>
<td>0.295</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Richness</td>
<td>Vegetation Height</td>
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<td>4.548</td>
<td>0.399</td>
<td>0.214</td>
<td>0.167</td>
</tr>
<tr>
<td></td>
<td>Ground Cover</td>
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<td></td>
<td>0.172</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Canopy Openness</td>
<td>-4.307</td>
<td></td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evenness</td>
<td>Vegetation Height</td>
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<td>1.645</td>
<td>0.331</td>
<td>0.09</td>
<td>0.035</td>
</tr>
<tr>
<td></td>
<td>Ground Cover</td>
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<td></td>
<td>0.944</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Canopy Openness</td>
<td>-0.003</td>
<td></td>
<td>0.598</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When comparing the families shared between management regimes, I found that 75% of the total bee species caught were found in Burn sites, 67% in Burn+Herbicide sites, and 33% in No Management sites. Of the 9 species found in Burn sites, 6 species were found in Burn+Herbicide sites (66.67% shared) and 3 were in No Management sites.
(33.33% shared). Of the 8 bee families found in Burn+Herbicide sites, 3 of them were found in the No Management sites (37.5% shared).

![Figure 3-11: Mean abundance of bees collected over 6 months across management regimes. Error bars are standard deviation.](image)

Community composition of bees differed among sites (pseudo$F_{2,11}=2.231$, $p=0.0018$; Figure 3-12a) and pan trap colors (pseudo$F_{1,11}=2.050$, $p=0.02$; Figure 3-12b), and there was no interaction (pseudo$F_{2,11}=1.115$, $p=0.321$). Specifically, Burn+Herbicide sites differed from No Management sites (pseudo$F_{1,10}=4.498$, $p=0.004$) and from Burn sites (pseudo$F_{1,11}=3.535$, $p=0.003$). There was no difference between Burn sites and No Management sites (pseudo$F_{1,10}=0.820$, $p=0.557$). *Lasioglossum tegulare* (Halictidae) was an indicator species for Burn+Herbicide sites and was on average 50-fold more abundant on Burn+Herbicide sites than Burn sites. No comparisons of abundance could be made with the No Management sites because no *Lasioglossum tegulare* were found on the No Management sites. There were no indicator species for Burn sites or No Management sites (Figure 3-13). I found differences in diversity (pseudo$F_{2,11}=4.574$, $p=0.038$),
richness (pseudoF\(_{2,11}\)=7.163, p=0.0122), and abundance (pseudoF\(_{2,11}\)=29.415, p=0.0001), but no difference in evenness (pseudoF\(_{2,11}\)=1.069, p=0.364) of bees among treatments (Figure 3-14).

![Figure 3-12:](image)

**Figure 3-12:** The data points are the bee communities measured at each site. The polygons encompass sites within each management regime to facilitate visualization of the differences and similarities among communities and management regimes. The centroids represent what the average community would be within each of the polygons. The stress from this NMDS is 0.036. (a) Community composition of the three treatments, Burn (yellow), Burn+Herbicide (red), and No Management (green). (b) Community composition of the pan trap colors that collected bees, Blue and Yellow.

3.2.2 **Bee Data by Pan Trap**

Bees were most abundant in yellow pan traps with 67 bees of 9 species (7 ± 8; 75% of the species collected), followed by blue traps with 60 bees of 8 species (7 ± 5; 66.67% of the species collected). No bees were collected in the clear or pink pan traps (Figure 3-16).
Figure 3-13: Mean abundance of indicator species across each management regime. Indicator Value (IV) for *Lasioglossum tegulare* (Halictidae) is labeled on the bar representing the management regime for which this species was an indicator. Bars represent standard deviation.

Bee composition differed by pan trap color (pseudoF_{1,11}=2.050, p=0.019; Figure 3-12b). Specifically, bee composition in yellow pan traps differed from blue pan traps (pseudoF_{1,15}=2.122, p=0.047). Only 5 bee species in yellow pan traps were shared with blue pan traps (55.55% shared; Figure 3-12b).

There was only one indicator species found for pan trap colors. *Melissodes bimaculata* (Apidae) was an indicator for the blue pan traps and was, on average, 2.11-fold more abundant in blue than in yellow traps (Figure 3-15). There were no indicator species for yellow pan traps. I found no differences in diversity (pseudoF_{1,11}=1.684, p=0.215), evenness (pseudoF_{1,11}=0.432, p=0.522), abundance (pseudoF_{1,11}=0.530, p=0.484), and richness (pseudoF_{1,11}=1.769, p=0.208) of bees among the two pan trap colors that collected bees.
Figure 3-14: Measures of Diversity, Abundance, Richness, and Evenness of only the bee data by management regime. Different letters indicate differences between colors. Bars represent standard deviation.

Sample-based rarefaction of all 18 sampling points for bees (9 sites x 2 pan colors that caught bees) estimated 29 bee species (9–49; 95% CI; Chao 2 estimate) for the entire study area. Individual-based rarefaction curves demonstrated Burn sites had the highest species richness (Figure 3-17a). Sample-based rarefaction curves estimated species richness was highest in Burn sites, followed by Burn+Herbicide sites, and lastly No Management sites (Figure 3-17b). The Chao 2 estimate of the asymptotic species richness was 16 (0–34; 95% CI) for Burn sites, 11 (0–22; 95% CI) for Burn+Herbicide sites, 9 (2–16; 95% CI) for No Management sites.
**Figure 3-15:** Mean abundance of indicator species across pan trap colors. Indicator Value (IV) for *Melissodes bimaculata* (Apidae) is labeled on the bar representing the management regime for which this species was an indicator. Bars represent standard deviation.

**Figure 3-16:** Mean abundance of bees collected by pan trap color from all sites and collection times. Bars represent standard deviation.
Figure 3.17: Rarefaction curves of the sampling effort across all three treatments over the 6-month sampling period. The rarefaction analysis shows estimates of species richness. (a) Individual based rarefaction curves of bees captured in three different treatments. (b) Sample based rarefraction curves of bees captured in three different treatments over the 6 sampling periods. The shaded areas represent the 95% confidence intervals. The solid line represents the rarefaction based on the data. The solid shapes at the end of each of the solid lines represent highest values collected during the study. The dashed lines represent the extrapolation of the data up to double the sample size.
CHAPTER 4
DISCUSSION

Global biodiversity loss is in part driven by land use change and habitat loss (Vitousek et al., 1997; Sala et al., 2000). Shortleaf pine-hardwood forests have been declining rapidly over the past 50 years and are often replaced with habitats that support lower plant and animal diversity (Anderson et al., 2016). Ecological restoration can mitigate changes, but tests of restoration efficacy are often lacking (Sala et al., 2000; Williams, 2011). This study compared insect pollinator communities across two restoration management regimes (prescribed burns or prescribed burns with targeted herbicide application) on a shortleaf pine-hardwood study area undergoing restoration since 2007 with adjacent unmanaged forest. Areas undergoing active restoration supported higher estimated pollinator family richness and abundances compared to forest not undergoing restoration with 1.5-fold higher estimated pollinator family richness on Burn sites than No Management sites, and this was particularly pronounced for bees. Areas with different management regimes differed in their habitat characteristics, which likely led to distinct pollinator family compositions, particularly between Burn+Herbicide and No Management sites. Together, these results support that ecological restoration through prescribed burns increases pollinator diversity.

I estimated a total 143 families of insect pollinators over the entire study area with the highest number of families on Burn sites and similar estimates for Burn+Herbicide.
and No Management sites. In support of these results, prescribed fire and practices that increase canopy openness (e.g., thinning or logging) are often associated with increased pollinator diversity (Campbell et al. 2007; Campbell et al. 2018; Ulyshen et al. 2021; Gelles et al. 2021; Glenny et al. 2022; Brokaw et al. 2023). Campbell et al. (2007) found prescribed burns that followed mechanical thinning had higher insect pollinator abundance and richness than controls. Glenny et al. (2022) found logging helps reduce canopy cover and this reduction had been shown to cause increased pollinator diversity when compared to a site that was not logged. Brokaw et al. (2023) found a higher abundance of ground-nesting bees in stands burned early in the year of sampling when compared to an unburned control.

Fire may impact pollinators by decreasing vegetation density, which increases canopy openness and forb diversity (Ulyshen et al., 2021). Open canopies allow more sunlight to reach the forest understory so forbs may grow to support pollinators (Pacala et al., 1994; Russavage et al., 2021). Surprisingly, I found pollinator family richness was negatively correlated with canopy openness and vegetation height, which is not consistent with many other studies (Bremer and Farley, 2010; Hanula et al., 2015; Glenny et al. 2022). Hanula et al. (2015) found the best predictor of bee abundance was canopy openness. Bremer and Farley (2010) found closed canopies prohibit shade intolerant species from growing which could cause a decline in the ecosystem’s biodiversity. However, most other studies only examined bee diversity, and other pollinators like flies, beetles, and wasps may differ in habitat factors that support their populations. Nol et al. (2006) found click beetles (Elateridae) preferred old, logged habitat (old meaning last logged 15–20 years prior to data collection) with 25–35% canopy cover over a wilderness
area (last logged 40 years prior) with a 60–70% canopy cover or a recently logged area (6 months to 3 years prior) with about 25–35% canopy cover. Considering Burn and Burn+Herbicide sites in this study are frequently disturbed and Nol et al. (2006) found beetles preferred sites that had not recently undergone disturbance, it would support my results of a higher abundance of beetles in the areas that do not experience frequent disturbance, like the No Management site. However, a negative correlation could also result if pollinators like beetles, which are not as effective fliers, have reduced foraging efficacy in tall vegetation due to dispersal limitation or if pollinators were less able to see pan traps (Bach, 1988; Nol et al., 2006; Tuell and Isaacs, 2009).

Burn+Herbicide sites had distinct pollinator communities and vegetation characteristics from Burn and No Management sites (Figure 3-2). Specifically, canopy openness, ground cover, and vegetation height were greatest on Burn+Herbicide sites and lead to distinct communities. Burn+Herbicide sites had 2.6-fold and 5.3-fold higher abundances of bees and 1.3-fold and 2.4-fold higher richness of bee species than Burn and No Management sites, respectively, but other pollinators were more abundant in Burn sites. Increased bee and butterfly pollinator abundance and richness in habitat that has received prescribed burns is commonly reported (Fettig et al., 2022). Hanula et al. (2015) found the highest bee abundances were in areas with the highest percentage of canopy openness. Similarly, the targeted reduction of invasive species through herbicide in this study may have facilitated increased bee-pollinated plant species. For example, Hanula et al. (2011) found removing Chinese Privet (Ligustrum sinense), an exotic invasive, from a riparian forest by mulching and using herbicide increased native bee abundance 20-fold and richness 4.8-fold when compared to a control site. However, non-
bee pollinators showed slightly different trends in my study. Burn+Herbicide sites shared fewer pollinators with or had significantly lower pollinator abundances of some families than No Management sites (e.g., Coleoptera: Elateridae and Scarabaeidae) and Burn sites (Hymenoptera: Chalcididae; Figure 3-4). Lautenschlager and Sullivan (2002) found insect populations that rely on foliage are negatively impacted by herbicide treatments initially but re-stabilize themselves over time. Cobb et al. (2007) found combining fire with other kinds of disturbance can reduce variability and increase homogeneity between sites that also undergo the same combination of disturbances. These findings coupled with the fact that the Burn+Herbicide site was treated with herbicide during our sampling period could provide an explanation for the lower diversity in the Burn+Herbicide site. These results suggest sites that have undergone prescribed burns with targeted herbicide treatments have a more specific community composition, which could limit the number of pollinators that are able to live on these sites.

Each management regime had unique indicator species of pollinators and bees and results suggest management practices that increase habitat heterogeneity may be the most effective for supporting the highest diversity of pollinators (Cobb et al., 2007). I estimated a total 29 species of bees over the 18 sampling sites with the highest estimated number of species on Burn sites with 16 species, 11 estimated species for Burn+Herbicide sites, and 9 estimated species for No Management sites. Hanula et al. (2015) estimated 132 species across 35 sampling sites that covered 7 different forest types (dense young pine, thinned young pine, mature pine with shrub understory, mature pine with herbaceous understory, mature upland hardwood, mature riparian hardwood, and cleared forest), which is a much higher estimate than what I found in a shortleaf pine-
hardwood forest. Winfree et al. (2007) estimated about 75 species across 14 sampling sites in a pitch pine (*Pinus rigida*)-oak forested area, which is also a much higher estimate than this study. These higher species estimates could be the result of using multiple sampling methods (e.g., Malaise traps or observational surveys) when sampling bees. Surprisingly, the highest abundances (but lowest family richness) of pollinators were collected on No Management sites. No Management sites had little to no ground cover (Figure 3-9), which could facilitate ground nesting or largely walking pollinators (e.g., Beetles) that are dispersal-limited relative to non-ground nesting pollinators (Gathmann and Tscharntke, 2002; Nyoka, 2010; Antoine and Forrest, 2021). Pan traps in No Management sites may have also been more visible due to lack of ground cover. Even though No Management sites had a higher abundance of pollinators, they have a lower diversity of pollinators. Thus, increased ground cover leads to an increase in pollinator diversity (Hanula et al., 2015). Despite lower pollinator family richness, maintaining and including forest patches not undergoing management could maximize pollinator diversity through increased habitat heterogeneity that may support additional pollinator resources like increased nesting sites (Antoine and Forrest, 2021).

Pan traps are meant to mimic floral resources for pollinators and may provide insights into floral preferences across sites (Cane et al., 2000; Wallen, 2010). Yellow pan traps were most effective in collecting pollinators in this study, which is consistent with other studies (Vrdoljak and Samways, 2012; Acharya et al., 2021; Buffington et al., 2021). However, bees had similar abundances in blue and yellow pan traps (Figure 3-16). Bee pan trap color preference has been shown to differ among sampling location, habitat characteristics, and elevation of pan traps (Tuell and Isaacs, 2009; Buffington et
When bees were sampled near an agricultural field, bees preferred blue to yellow pan trap colors (Grundel et al., 2011), but in a forest clearing bordered by deciduous forest, bees preferred yellow to blue pan traps (Buffington et al., 2021). This suggests the most effective pan trap color for collecting bees could differ depending on the habitat where the study is taking place (Buffington et al., 2021). Pink pan traps did not differ from clear pan traps in their pollinator composition and had no indicator families; neither color caught any bees. Therefore, the use of blue and yellow pan traps is likely to yield the highest pollinator capture in this system.

There were some limitations to my study. Other studies often combine pan trapping with additional pollinator collection or survey methods such as observational surveys or netting (Campbell et al., 2007; Wilson et al., 2008; Westphal et al., 2008; Grundel et al., 2011). This may limit my ability to discuss absolutes about bee and pollinator communities and make comparisons with other studies. However, it still enables a general indication and valid comparison of pollinator communities at our site and among the management regimes. Increased sampling efforts and types used in combination with pan traps would have allowed a wider range of pollinators to be collected, including Lepidopterans, which may differ in which habitat characteristics drive their diversity patterns. Additionally, all pan traps were placed on the ground similar to Williams (2011). However, placing pan traps in trees (Tuell and Isaacs, 2009), or elevating pan traps (Campbell et al., 2007; McCune et al., 2020) may capture a different set of the pollinator community that was missed here. Lastly, although vegetation characteristics were measured, other more pollinator-specific measurements like abundance of flowering plants and forbs were not measured. These data may explain
more of the variation in pollinator data (Williams et al., 2011; Hanula et al., 2015; Affek et al., 2021; Campbell et al., 2018).
CHAPTER 5

CONCLUSION

Ecological restoration may mitigate or reverse biodiversity loss from land use change, resulting in alternate stable states of ecosystems (Schmid et al., 1999; Benayas et al., 2009). Ecological restoration often requires disturbance, and the effects of disturbance are different depending on the ecosystem. This disturbance alters the ecosystem in a way that meets the goals of restoration, but it does not guarantee it will provide the expected result. Long-term effects of restoration are unknown in some ecosystems, but more research is being conducted on the impacts. When conducting tests of restoration efficacy, however, most focus on a single group of pollinators. My study is one of only a few to examine restoration impacts on a broad range of pollinators (Rader et al., 2009; Christmann, 2019). Interestingly, bees and non-bee insect pollinators differed in their response to restoration efforts, indicating examination of only one focal pollinator class may not fully capture impacts of restoration efforts on pollinators as a whole (Rader et al., 2016; Requier et al., 2022). My results suggest restoration of shortleaf pine-hardwood forests using prescribed burns and potentially other management regimes, like herbicide treatments, leads to distinct site characteristics and pollinator communities. The results of this study may inform future restoration and management practices of shortleaf pine-hardwood forest. Tests of restoration efficacy are scarce; future research should continue to evaluate restoration efforts across a wide range of habitats and taxa.
APPENDIX A

SUPPLEMENTARY INFORMATION
This section is made up of information that is not necessary to understand the results but it can be helpful to further understand the data. **Table A-1** provides more details on the orders and families of insects that were removed from the data. **Table A-2** provides more data for the families that were indicators for all pollinators by treatment. **Table A-3** provides more data for the families that were indicators for all pollinators by pan trap color.

**Table A-1:** The classifications of insects that were removed from the data because they do not provide pollination services.

<table>
<thead>
<tr>
<th>Order (or higher taxonomic classification)</th>
<th>Family</th>
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</thead>
<tbody>
<tr>
<td>Acari</td>
<td></td>
</tr>
<tr>
<td>Arachnida</td>
<td></td>
</tr>
<tr>
<td>Collembola</td>
<td>Isotomidae</td>
</tr>
<tr>
<td>Collembola</td>
<td>Sminthuridae</td>
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<tr>
<td>Dictyoptera</td>
<td>Blattidae</td>
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</tr>
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<td>Order</td>
</tr>
<tr>
<td>-------------</td>
<td>----------------</td>
</tr>
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<td>Issidae</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>Lygaeidae</td>
</tr>
<tr>
<td>Hemiptera</td>
<td>Membracidae</td>
</tr>
<tr>
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<td>Nabidae</td>
</tr>
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<td>Hemiptera</td>
<td>Reduviidae</td>
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<tr>
<td>Orthoptera</td>
<td>Gryllidae</td>
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<td>Phasmida</td>
<td>Phasmatidae</td>
</tr>
<tr>
<td>Plecoptera</td>
<td>Perlidae</td>
</tr>
<tr>
<td>Protura</td>
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<td>Thysanoptera</td>
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Table A-2: The indicator values (IV) for each significant (p<0.05) taxon and the specific trap color they were associated with.

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Trap Color</th>
<th>IV</th>
<th>Probability</th>
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<td>Hymenoptera</td>
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<td>No Management</td>
<td>0.368</td>
<td>0.0001</td>
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<tr>
<td>Coleoptera</td>
<td>Scarabaeida</td>
<td>No Management</td>
<td>0.346</td>
<td>0.040</td>
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</table>

Table A-3: The indicator values (IV) for each significant (p<0.05) taxon and the specific treatment they were associated with.

<table>
<thead>
<tr>
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<th>Family</th>
<th>Treatment</th>
<th>IV</th>
<th>Probability</th>
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<td>Burn</td>
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<td>0.037</td>
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<td>0.037</td>
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<td>Scarabaeida</td>
<td>No Management</td>
<td>0.346</td>
<td>0.040</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Order</th>
<th>Family</th>
<th>Trap Color</th>
<th>IV</th>
<th>Probability</th>
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<tr>
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<td>0.0001</td>
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<tr>
<td>Diptera</td>
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<td>0.900</td>
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BIBLIOGRAPHY


understory and midstory of a temperate forest in North Carolina. Forest Science, 64(3), 299-306.


http://chao.stat.nthu.edu.tw/wordpress/software_download/inext-online/.


Miller, J. R., & Hobbs, R. J. (2007). Habitat Restoration-Do We Know What We’re Doing?


https://doi.org/10.1890/ES15-00121.1


https://doi.org/10.1656/058.019.0316


https://doi.org/10.1111/1365-2664.12903


