

**Community composition and pollination network structure in a fire managed
Canadian tall grass prairie.**

by

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ABSTRACT

Pollination networks summarize interactions between plants and pollinators, providing insight into ecosystem stability. An unplanned fire provided the opportunity to assess network structure following disturbance in the Tall Grass Prairie Preserve in southern Manitoba. I established transects in sites burned <1 year, 5-6 years, or 10+ years ago. I assessed species richness, diversity, abundance, and phenology of insects and flowering plants. I created interaction matrices by recording plant-insect interactions, and sampled pollen loads from insects. Network structure was assessed by connectance, nestedness, and interaction strength. Flowers were more abundant and bloomed two weeks earlier in newly burned sites in 2010. Bees showed responses to fire based on nesting habitat, however visits by syrphids were related to precipitation. Network structure showed that tall grass prairie pollination networks were resilient to disturbance and variable environmental conditions, and management of prairie by fire did not negatively impact plant-pollinator interactions within the community overall.

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1. INTRODUCTION AND GENERAL BACKGROUND

1.1 Pollination networks

Ecological networks summarize the interactions occurring between members of a community and how those members contribute to the stability of the system (Ings et al. 2009). Pollination networks summarize relationships between flowering plants and their insect pollinators specifically. Pollination generally occurs through the transfer of pollen from anther to stigma by anthophilous (flower visiting) insects (Kevan 1999). Anthophiles are not always pollinators, as some flower-visiting insects consume the nectar, pollen, or oils of the flower without contributing to reproductive success (Kevan 1999). Surveys of plant-insect interactions identify and categorize the interactions taking place, leading to an understanding of network structure. Interest in this area of ecology has greatly increased over the last decade, and multiple recent reviews explain the network approach (see Blüthgen 2010, Ings et al. 2009, Vázquez et al. 2009).

Flower visiting insects and flowering plants that require insect pollination are intimately linked, each relying on the other for nutrition and reproductive success. Not surprisingly, the loss of pollinators has negative effects on the flowering species that rely on them. Analysis of one million records of bee and hoverfly observations from Britain and the Netherlands showed that flowering plants requiring insect pollination decline in abundance with the loss of pollinators (Biesmeijer et al. 2006). As a result, the structural integrity of these communities is likely at risk. Assessing network structure can help prevent or predict the effects of species losses on network stability and ecosystem functionality (Memmott et al. 2004).

Pollination network studies are used to address a variety of ecological questions. Observations of plant-insect interactions provide insight into coevolutionary relationships

(Bascompte et al. 2006, Dupont and Olesen 2008), physical determinants of interactions (Stang et al. 2009, Stang et al. 2006), and how interactions work to contribute to biodiversity (Bastolla et al. 2009). Perhaps the most useful application of network studies is assessing the structural integrity of ecosystems, particularly in light of disruptions such as habitat loss or other human caused disturbances. Network studies have revealed how ecosystems may respond to the extinctions of rare or highly connected species (Memmott et al. 2004, Solé and Montoya 2001) and how disturbance or fragmentation can alter pollinator abundance, plant communities, and plant-pollinator relationships (Taki et al. 2007, Potts et al. 2003). Network studies also provide important assessments of habitat maintenance, as they offer information regarding the success of habitat restoration (Forup and Memmott 2005), and how pollinator diversity contributes to pollen transport (Alarcón 2010) and flowering plant reproductive success (Fontaine et al. 2006).

1.2 Network metrics

a. Qualitative analysis

In their simplest form, pollination networks are described using unweighted or binary (qualitative) metrics (Blüthgen et al. 2008), which are based on the presence or absence of interactions, or links, between each species of plant and insect (Vázquez et al. 2009, Jordano 1987). Each link is assumed to be of equal importance in the network, regardless of quality (Vázquez et al. 2005). Three commonly used descriptions of network structure are connectance, nestedness, and modularity.

Connectance is the percentage of potential links among species that actually occur (Olesen and Jordano 2002, Jordano 1987), and can be determined by expressing the interactions as a proportion I/AP , where I represents the realized links and AP represents the number of

potential links between the animal and plant species (Dupont et al. 2009). Connectance may be useful for determining specialization or generalization in a network, with high generalization usually being reflected through high connectance (Blüthgen 2010). Connectance may also provide insight regarding coevolution between connected partners (Jordano 1987).

Nestedness refers to asymmetrical plant-insect interactions (Blüthgen 2010, Dupont et al. 2009), where specialists interact with generalist partners (Vázquez et al. 2009, Bascompte et al. 2003). More specifically, specialist plant species will interact with one or a few more generalized insect species and *vice versa* (Lewinsohn et al. 2006) (Figure 1.1). A strongly interacting group of generalist plant and insect species comprise the core of the nested structure (Lewinsohn et al. 2006, Bascompte et al. 2003). Departures from nestedness are expressed by temperature (T), where 0° represents perfect nestedness and 100° represents a random network (Alarcón et al. 2008). Nested structures are common in mutualistic networks, and likely offer structural stability in the face of disturbance through alternate linkages (Bascompte et al. 2003) or pollinator redundancy (Memmott et al. 2004).

Modules are composed of densely linked species that have few links with species in other modules (Dupont and Olesen 2008, Olesen et al. 2007). Within the module, one or more hub plant species may act as the nuclei for the module, drawing in the greatest activity (Dupont and Olesen 2008). While nestedness only relies on the presence or absence of links to determine structure (Fortuna et al. 2010), modularity considers link density, essentially high connectance, between species (Fortuna et al. 2010, Dupont and Olesen 2008, Krause et al. 2003). A modular structure is commonly found in large pollination networks with greater than 150 species (Olesen et al. 2007) and can be present within a nested network (Fortuna et al. 2010). Modularity is expected to facilitate co-evolution, as tightly linked species are more likely to experience

reciprocal selection (Dupont and Olesen 2008). A modular network may also be more resistant to species losses or disturbance, as the effects are confined to modules rather than the entire network (Krause et al. 2003).

To summarize, connectance describes the proportion of realized links, nestedness reveals network structure based on specialist-generalist interactions, and modularity describes the presence of modules by linkage density between species. However, all these metrics assume that each interaction is equally important (Vázquez et al. 2005). As a result, conclusions about the impact of specific links at the community level are limited (Vázquez et al. 2005).

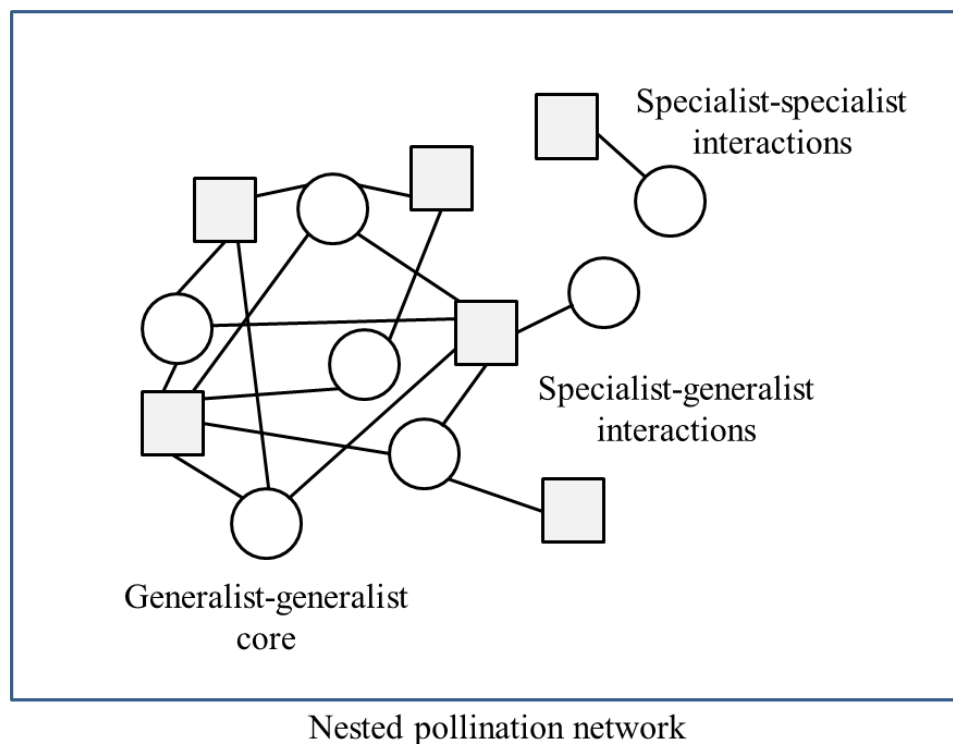


Figure 1.1 Graphical representation of a nested pollination network. Squares represent flowers, circles represent insects, and lines indicate an interaction.

b. Quantitative analysis

Weighted network metrics are quantitative, in that they take into account the interaction strength of links between species (Blüthgen 2010, Blüthgen et al. 2008). Interaction strength is the dependence of one interacting species on another (Jordano 1987). It is determined by calculating the proportion of the total visits received by a focal plant species that are from a given insect species. This value is compared with the proportion of the total visits by the same insect species that are to the focal plant species (Fig. 1.1) (Bascompte et al. 2006). This usually results in asymmetrical dependence between the species, where the effects of one species on another do not equal that of the reciprocal comparison (Vázquez et al. 2007). Interaction strength still assumes that all pollinators contribute equally to plant reproduction, but acts as a transition from binary to quantitative analysis when interaction frequency is considered (Vázquez et al. 2009, Vázquez et al. 2005).

Interaction frequencies are important when assessing interaction strengths (Vázquez et al. 2005). Links that occur in high frequency are considered strong, while links in low frequency are considered weak (Blüthgen 2010). A large community may have many weak links, while smaller communities have stronger links between interacting partners (Jordano 1987). Interaction frequency becomes an important tool when examining the relative impact of certain linkages on ecosystem functioning (Blüthgen 2010, Vázquez et al. 2005).

The effectiveness of pollinating species can vary in the community. Interaction frequencies have been correlated with pollen transportation, suggesting that the number of visits can be a good indicator of pollinator quality (Alarcón 2010, Vázquez et al. 2005). Pollinator importance or effectiveness can be assessed by quantifying pollen deposition (Sahli and Conner 2006). Linkages that appear to result in small contributions to the community, such as inefficient

pollen deposition, may actually be important due to the frequency of the interactions (Vázquez et al. 2005). However, some frequently visiting species have been found to carry very little pollen between flowering plants while insects considered to be infrequent visitors transported substantial amounts of pollen (Alarcón 2010). Further investigation into the amount and types of pollen grains transported by insects could provide insight into the potential reproductive benefits offered to flowering plants and the importance of certain insect groups to mutualistic networks (Alarcón 2010).

1.3 Determinants of network structure

Interaction neutrality and trait matching are two processes that contribute in tandem to network structure (Vázquez et al. 2009). Interaction neutrality assumes an equal probability of interactions and equal interaction quality between individual members of a network (Vázquez et al. 2009, Krishna et al. 2008). Species that are more abundant will interact more frequently, meaning that abundance increases the probability of interactions whereas rarity has the reverse effect (Vázquez et al. 2009, Krishna et al. 2008). However, more studies that involve the collection of abundance data beyond visitor observations are needed to offer the best insight into the relationship between abundance and linkage (Olesen et al. 2008).

Interactions require overlap in phenophases, defined as either the time between the first and last open flower, or the first observed and last observed pollinating visit by an individual pollinator (Olesen et al. 2008). Long phenophases, in combination with high species abundance, greatly increase the likelihood of species acquiring many links (Olesen et al. 2008).

Phenophases can also constrain interactions regardless of abundance (Olesen et al. 2008).

Phenology determines links that cannot exist, because overlapping life histories do not occur for

all species present in the community (Jordano et al. 2003). There is evidence that disturbance can alter floral phenology (Pemble et al. 1981), and there is interest in climate related changes in phenology (see Dupont et al. 2009, Olesen et al. 2008).

Trait matching occurs when visitation patterns are determined by the matching of floral and insect morphologies, or by pollinator preference for floral rewards (Vázquez et al. 2009, Stang et al. 2006). While interaction neutrality assumes random interactions through abundance, trait matching offers rules or restrictions to the kinds of interactions that can occur (Vázquez et al. 2009, Stang et al. 2006). Trait matching restricts interactions when the morphologies of insects and plants are antagonistic (Stang et al. 2006). Long, narrow corollas can act as a barrier against visiting insects attempting to access floral rewards (Stang et al. 2006), resulting in decreased interaction strength between the species (Blüthgen et al. 2008). Conversely, trait matching can increase interaction strength when interspecies preferences are complementary (Blüthgen et al. 2008). Flowers that offer nectar and flowers that offer only pollen will attract visits from pollinators based on their preferred reward (Olesen et al. 2008, Dicks et al. 2002). Floral colour influences the potential for links, because bees (Hymenoptera: Apidae) exhibit preferences for blues or violets (Raine and Chittka 2007, Dicks et al. 2002, Giurfa et al. 1995) while some syrphids (Diptera: Syrphidae) prefer yellows or whites (Dicks et al. 2002, Haslett 1989). While floral traits play a large role in the likelihood of interaction, it has been suggested that phenology may be the greater determinant of interaction probability (Herrera 1988).

I propose to apply current understandings of network dynamics to plant-pollinator interactions in the tall grass prairie habitat in Manitoba. I will assess general insect and flowering plant diversity, determine relationships between abundance and visitation frequency, determine how phenology influences interactions, and whether certain insect groups prefer flowering plants

with particular traits. I will also examine pollinator quality by sampling pollen loads from visiting insects, then relating the amount of pollen carried with interaction strength.

1.4 Disturbance and plant-insect interactions in tall grass prairie

Tall grass prairie throughout North America has suffered substantial fragmentation and habitat loss as a result of human activity, particularly due to the conversion of prairie to agricultural land (Sampson and Knopf 1994). Less than 1% of the original tall grass prairie remains in Canada (Reaume 1993), so careful management of remnant prairie is vital.

Prescribed fires are accepted as an important management tool for the maintenance of tall grass prairie vegetation because fires are a natural part of the ecosystem (Vogl 1974).

Historically, fires are believed to have been caused by lightning strikes or set by aboriginal peoples to influence the movement of bison herds (Higgins 1986, Hulbert 1986, Moen 1998). Fires would have burned quickly over large areas (Vogl 1974), varied in intensity, and left areas of unburned refugia (Higgins 1986, Moen 1998).

Fires typically occur in either spring or late summer into fall (Higgins 1986). Wildfires occurring in spring are the result of a high litter to live vegetation ratio left from the previous growing season (Bragg 1982, Engle and Bidwell 2001). As the season progresses into fall, the litter layer accumulates and, in combination with warm, dry conditions, can result in intense late season fires (Bragg 1982, Engle and Bidwell 2001).

Historical evidence of the frequency of fire in tall grass prairie is difficult to obtain. The frequency of fires might be determined based on fire scars inflicted on forest stands. However, prairie lands are often sparsely treed, leaving little evidence of tree scarring by fire for study (Higgins 1986). The available evidence of scarring has revealed varied estimates ranging from 2

to 11 year intervals (Henderson 1982 as cited in Abrams 1985, Reinking 2006). Due to this difficulty in detection, most information regarding historical fires is drawn from records by early settlers or the histories of aboriginal peoples (Higgins 1986). This has made it difficult to determine a managed fire regime that may be the most beneficial for the maintenance of natural tall grass prairie. Currently, managers in North America use a variety of fire intervals based on management goals, varying from 2 year to 10 year cycles (DeBano et al. 1998).

Responses to fires vary with the plant species studied, but in general fires remove dead litter allowing for an increase in grasses and forbs (Gibson and Hulbert 1987). Much research has been done on the varied responses of vegetation to fire (see Engle and Bidwell 2001), although insects, which make up a large component of the prairie community, have often been overlooked (Panzer and Schwartz 1998).

There is some concern over the effects of prescribed burns on the mortality of resident insect species, particularly in fragmented habitat (Panzer 2002). Some insect species have the ability to rapidly recolonize burned habitat and do not show strong negative effects after fire (Panzer 2002). However, insects that are obligates of prairie fragments, or nest or overwinter in the litter layer are particularly susceptible to mortality by fire (Swengel et al. 2010, Williams et al. 2010, Panzer 2002). Insect responses will vary with disturbance, species, and life-history traits (Williams et al. 2010), and this emphasizes the need for specific studies of insect responses to fire in the tall grass prairie in Manitoba.

Disturbances such as fire can affect flowering phenology and abundance, altering the potential for interactions between plants and insects. Recent fires have resulted in bloom times two weeks earlier than flowers of the same species in unburned areas, and can increase floral abundance (Pemble et al. 1981). This can influence insect abundance, with areas of increased

floral density attracting greater numbers of pollinators (van Amburg et al. 1981). At the same time, the floral species that bloom after a fire may differ from the pre-fire community (Howe 1994). Insects, who generally visit a small number of flowering species compared to flowering plants who receive a large number of visitors, would be vulnerable to this change in flowering community (Taki and Kevan 2007).

How fire affects pollination network structure in tall grass prairie is currently unknown. Indeed, very few studies have attempted to assess plant-pollinator interactions in this habitat (for an exception see Robson 2008). The Tall Grass Prairie Preserve (TGPP) in Manitoba contains the largest remaining fragment (~3000 ha) of tall grass prairie habitat in Canada. Habitat within the preserve is primarily maintained through managed fire events to attempt to mimic natural disturbance. In fall 2009, a 420.7 ha unplanned fire created the opportunity to study the recovery of tall grass prairie insects and plants. I plan to construct pollination networks for habitats of differing burn ages. My research will include surveys of insect and flowering plant diversity and phenology, pollinator services to flowering plants, and observations of insect visitation patterns. The construction of this network and assessment of visitation qualities will increase our understanding of community dynamics in response to fire in tall grass prairie. My research goals complement those of the Canadian Pollination Initiative, an NSERC strategic network responsible for the assessment of pollinator diversity across Canada.

1.5 NSERC-CANPOLIN

In 2009, Canadian researchers saw the need to survey pollinator communities to better understand pollinator decline across Canada. A national collaboration of researchers from 26 Canadian institutions established the Canadian Pollination Initiative (NSERC-CANPOLIN)

(CANPOLIN 2009). Their goals were to organize a nation- wide survey of pollinators, including their taxonomy, their associated plant species (native or agricultural), and the habitat and environmental factors that influence their interactions (Kevan et al. 2010). Manitoban researchers became involved with CANPOLIN in 2009, which initiated pollinator surveys of tall grass prairie as part of working group five (WG5). This group focused on the study of pollination networks at the ecosystem level.

My research objectives work to understand how fires influence pollination network structure in Manitoba tall grass prairie. At the same time, they satisfied the objectives for WG5, namely assessing connectance, generalization and specialization within networks, how phenology and habitat structure may influence pollination, and the extent to which seed set is limited by pollen availability. CANPOLIN was the primary source of funding for my research, and provided broad research goals and protocols for the standardization of studies across Canada.

1.6 Objectives and predictions

The purpose of this thesis is to assess plant-pollinator networks in tall grass prairie habitat. Tall grass prairie is a dynamic and complex ecosystem with many opportunities for interactions between insects and flowering plants. These opportunities for interactions will be influenced by abundance, phenology, morphology, and a variety of environmental factors such as climate and wildfire. The interactions will be best understood when assessing each contributor to network structure individually to reduce complexity. Chapter 2 and Chapter 3 examine the flowering community and pollinating insects respectively, with an emphasis on their response to fire and climate. Chapter 4 assesses plant-insect interactions and network structure based on the

structural elements, or building blocks, of pollination networks. Chapter 5 provides conclusions about pollination networks in tall grass prairie.

My first objective is to compare insect and plant phenology and diversity in recently burned habitats with phenology and diversity in habitats with older burns. In Chapter 2, I predict that sites with recent burns will exhibit earlier bloom times and a higher density of forbs, increasing the potential for differences in linkage between new sites and older sites. In Chapter 3, I predict that diversity of insects will be higher in the new burns where floral resources are abundant. I also expect that insects in the new burns will be in lower abundance because of fire mortality.

My second objective consists of two parts:

- 1) I assessed pollination networks using binary metrics. If newly burned sites experienced increased interactions due to greater floral resources over the length of the season, I expected that connectance would be lowest in those sites. I also expected that nestedness would be low in recently burned sites, as interactions could be uncoupled by changes in flowering phenology and the mortality of certain pollinator guilds. The frequency of visits from frequently visiting pollinating flies differed with annual precipitation, so I expected to see a difference in nestedness values between years.
- 2) I assessed the influence of the structural elements, or building-blocks, of plant-pollinator networks on network structure based on burn history. This was followed by assessing pollinator quality through pollen loads, as this would provide greater insight into the impact of particular interactions. In Chapter 4, I investigated the following assumptions:

- a. I expected that the most abundant flowers would receive the greatest number of visits. If recently burned sites have higher floral abundance, I should detect a greater number of visits in those sites.
- b. I expected that changes in flowering phenology by burn class would influence interacting partners through phenological uncoupling. This could be indicated by differences in interacting partners between burn classes and years.
- c. I expected that insects would exhibit preferences in floral colour. If consistent, preferences should be similar between counts in pan traps and observed visits and could determine the likelihood of interaction between species.
- d. I expected that pollen loads would differ by taxa, and that frequently visiting species would be some of the most high quality pollinators due to the amount of pollen transferred with each visit.

My third objective is to assess temporal variation in plant and pollinator diversity. Long term studies have indicated that visitation patterns and species compositions within pollination networks fluctuate widely (e.g., Alarcón et al. 2008, Olesen et al. 2008). This seems particularly likely in the tall grass prairie where the continental climate contributes to extreme variations in seasonal temperatures and precipitation. Flowering phenology and insect flight times will be compared for 2009, 2010 and 2011, as well as plant-insect interactions observed in 2010 and 2011. Pollination network structure is expected to exhibit stability across our sample seasons, while temporal differences in links between pollinator taxa related to the presence of particular flowering species are expected to vary with burn age.

2. FLOWERING PLANT COMMUNITY STRUCTURE FOLLOWING FIRE

2.0 Chapter Summary

Fire is a natural part of the tall grass prairie ecosystem. How fire influences the flowering community could be important for pollination network structure, as pollinating insects rely on the flowering community for food and shelter. The effects of fire were assessed by floral counts in six sites of differing burn ages. Measures of alpha and beta diversity were used to compare the species present, while repeated measures ANOVA and PCA were used to determine differences in composition. Burn classes were similar in floral diversity, and site-to-site variation in species was common. Bloom abundance was greater in the new burn class in 2010, but this effect was lost in 2011. Flowering plants also bloomed two weeks earlier in the new burn class in 2010 only. The similarity of species present in each site between years was independent of fire, likely due to the presence of fire adapted, perennial species that have evolved in tall grass prairie habitat. Understanding the response of the flowering community to fire will be important if changes in the flowering community influence interactions.

2.1 Introduction

The tall grass prairie is a complex ecosystem greatly influenced by natural disturbance, particularly fire. Fire in prairie is of great interest; fire consumes the plant community while at the same time replenishing it. As flowering plants are directly affected by this disturbance, it is important to understand their response before looking at the effect of fire on interactions.

The responses of prairie vegetation to fire are based on a combination of post fire effects (Hulbert 1988). An important effect of fire is the removal of the dead litter that accumulates

annually (Hulbert 1988). This decrease in litter allows for an increase in soil temperature and solar radiation, both of which have been shown to have strong influence on increased productivity following fire (Rice and Parenti 1978, Hulbert 1988). Increased growth in vegetation will also occur following high soil nitrogen levels that result from burning (Hulbert 1988). The combination of decreased litter, increased light and heat, and available nutrients can result in earlier phenology of flowering and potentially greater biomass during optimal growing conditions (Vogl 1974). These increases in growth of tall grass prairie species are juxtaposed with declines, with fire suppressing the trees and shrubs that would otherwise encroach upon tall grass prairie habitat (DeBano et al. 1998). Declines in overall biomass are common as the habitat matures and litter accumulates in the years following fire (Suding 2001).

The time of year a fire takes place has been shown to influence the pattern of regeneration in tall grass prairie, potentially changing the composition of the post fire community. Spring fires have been found to encourage the abundance of dominant tall grass prairie grasses such as big bluestem (*Andropogon gerardii* Vitman) (Smith et al. 2010), while late summer or fall fires can produce short term increases in forbs and decreases in grasses in the season following fire (Engle et al. 1998).

The responses of vegetation to fire may also be influenced by variation in precipitation between years. While burning generally has a stronger effect on productivity, these effects in combination with precipitation may dictate the structure of the post-fire community (Gibson and Hulbert 1987, Dhillon and Anderson 1994). In a previous study, low rainfall in burned sites decreased grass biomass in sandy prairie, though forb density in plants with taproots did not show similar declines (Dhillon and Anderson 1994). High rainfall may allow for the production

of more flowers within the typical flowering period of a given species (Kebart and Anderson 1987).

The diversity of the flowering community may or may not be influenced by fire. Research in mixed grass prairie showed differences in species between burned and unburned areas may be quite small, perhaps because prairie vegetation is well adapted to fire (Biondini et al. 1989). In other cases, diversity increased up to eight years following fire in upland tall grass prairie (Gibson and Hulbert 1987). Some differences in fire response by forbs are best assessed on a species by species basis, rather than at the level of the community to better understand the effects of fire (Biondini et al. 1989). It may be difficult to assume what the levels of diversity in historical prairie resembled, and there is the potential for managed disturbance to encourage the growth of common species over rare species if fire regimes are repetitive in terms of season and interval (Howe 1994b).

Though a variety of studies exist that assess the effects of fire on tall grass prairie vegetation, it does not appear that one fire regime fits all habitats, and not all habitats recover in a similar fashion following fire. Many factors influence the response of vegetation, examples being past management tactics such as grazing, the local climate, and past fire frequency (Biondini et al. 1989). Though there is good evidence that tall grass prairie is fire adapted, it is recommended that the response of vegetation to fire be assessed within each unique habitat to create a thorough understanding of fire effects on the plants in that particular community (Engle and Bidwell 2001).

Disturbances such as fire that influence flowering phenology, diversity, and abundance could be considered to alter the potential for interactions between plants and insects. Fires have resulted in bloom times up to two weeks earlier than flowers of the same species in unburned

areas (Pemble et al. 1981). Phenological shifts, in addition to increased floral abundance (Pemble et al. 1981) could in turn influence insect abundance, with areas of increased floral density attracting greater numbers of pollinators (van Amburg et al. 1981). At the same time, the flowering species that bloom after a fire may differ from the pre-fire community (Howe 1994). Insects, who generally visit a small number of flowering species compared to flowering plants who receive a large number of visitors, could be vulnerable to this change (Taki and Kevan 2007). Disturbances such as fire have the potential to cause shifts in floral phenology (Pemble et al. 1981), and phenology will impact the interactions that can occur in a community (Jordano et al. 2003, Olesen et al. 2008, Olesen et al. 2011).

My first thesis objective is to compare plant phenology and diversity in recently burned habitats with phenology and diversity in habitats of older burns. Following evidence that late season fires can increase flowering plant densities (Biondini et al. 1989, Engle et al. 1998, Engle and Bidwell 2001) and induce earlier flowering phenologies (Vogl 1974, Pemble et al. 1981), I predict that sites of recent burns will exhibit earlier bloom times and a higher density of forbs compared to mature sites. Assessments of alpha and beta diversity will show differences in post fire flowering communities should they exist. I predict that the increase in floral density will translate into increased species richness and diversity in sites that were most recently burned.

2.2 Materials and methods

2.2.1 Study sites

Research took place in the Tall Grass Prairie Preserve in Southern Manitoba, located in the Rural Municipality of Stuartburn near the Canada-US border (49° 08' N, 96° 40' W). The preserve consists of interconnected prairie remnants that are roughly grouped into northern and

southern blocks. The northern block is typically wetter with a dominance of sedge meadow habitat. The southern block consists of upland prairie habitats which include stands of aspen (*Populus* spp.) and oak (*Quercus* sp.). The preserve is surrounded by agricultural land involved in crop production and cattle grazing. Seasonal temperatures range from 19.8°C to -17.1°C, and the area receives an average of 579.1 mm of annual precipitation (Environment Canada 2004).

Habitat within the preserve is managed with a combination of prescribed burns, grazing, mowing and haying. In the fall of 2009, an unplanned wildfire burned approximately 420 ha of upland prairie in the south block (C. Hamel, Nature Conservancy Canada, *pers. comm.*), allowing for the assessment of freshly burned habitat beginning in the spring of 2010.

Study sites were established in habitats of differing burn ages. New 1 and New 2 were within areas burned in the fall of 2009 (Table 2.1). Mature 3 and Mature 4 were within areas burned in 2005 or 2006 (Table 2.1). New and mature burns were located in upland prairie habitat, and the four sites were sampled in both the 2010 and 2011 seasons. An additional two sites (Old 5, Old 6) located in sedge meadow habitat in the north block were established in areas that had not been burned for greater than 10 years (Table 2.1). Sampling in 2010 revealed substantial differences in community composition between sedge meadow and upland sites, potentially confounding comparisons. Sampling of the ‘old burns’ was discontinued in 2011 in order to focus on upland prairie habitat.

Table 2.1. Descriptions, locations, and burn histories of study sites, 2010-2011.

Site #	Site name	GPS location	Habitat	Year of burn ¹	Class of burn
1	Dead Chicken (DC)	49° 5'15.5" N 096° 45'20.7" W	Upland	Fall 2009	New
2	Doyle	49° 5'16.17" N 96°44'3.75" W	Upland	Fall 2009	New
3	Antonyshyn (Ant)	49° 5'24.87" N 96°44'9.31" W	Upland	Spring 2005	Mature
4	Prairie Shore Trail (Trail)	49° 4'23.3" N 096° 46'02.3" W	Upland	Spring 2006	Mature
5	Mimic	49° 9'01.6" N 096° 40'16.5" W	Sedge	Spring 2000	Old ²
6	Smook	49° 9'49.1" N 096° 40'29.1" W	Sedge	No record	Old ²

¹Burn information provided by C. Borkowsky, Biologist, Tall Grass Prairie Preserve, MB, Canada

²Sampling was discontinued in 2011

For each site, central transects of 90 m were established in an East-West orientation (Fig. 2.1). Parallel transects (90 m) were placed 3 m away from the central transect, and each were divided into five 18 m segments (Fig. 2.1). Transect placement was consistent between years. Sampling took place every 10-14 days from June to mid-September in both years, and each season included eight sample periods.

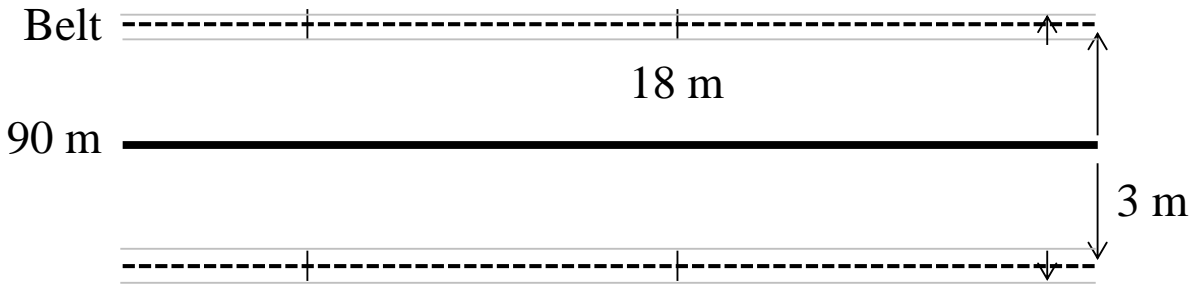


Figure. 2.1. Diagram showing transects for floral counts. Quadrats were placed at 18 m intervals, or counts were made along belt transects.

2.2.2 *Floral counts*

Vegetation was assessed through two methods: counts within 1 x 1 m quadrats and counts along 1 x 90 m belt transects (Fig. 2.1). Quadrats were placed along the 90 m parallel transects by random numbers table, with one quadrat per 18 m segment over 180 m of transect length. Each site had 80 sampled quadrats per sample season over both years (2010: 480 total, six sites; 2011: 320 total, four sites). Open flowers within quadrats were counted as ‘flowering units’, either as complete flowers, or flower heads or individual umbels in species with dense floral arrangements (Alarcón 2010, Memmott 1999). Parallel belt transects were used for counts of open flowers in each site, covering 1 x 180 m per site per sample period. In 2010, belt transect counts were not initiated until 15 July (mid-season), but transect counts were completed for the entire 2011 season.

Flowering plants that were difficult to identify in the field were collected as vouchers for identification, or photographed when the specimen was potentially rare. Vouchers were deposited in the University of Manitoba herbarium.

2.2.3. Assessments of flowering species composition and diversity

Rarefaction was used to compare the number of flowering species present within sites. Rarefaction is a process of sampling without replacement that results in a decreasing number of new species encountered with each sampling event (Gotelli and Colwell 2001). This method allows for the comparison of species richness from data with uneven sample sizes (MacIntosh 1967, Tipper 1979). Rarefaction was conducted using the total number of open blooms per species over the sample season. Sites were combined using averaged floral counts for comparisons of burn classes. Significant difference was determined by comparing the confidence intervals at the level of the site with the fewest individuals. If the intervals overlapped, the curves were similar. If the overlap was small, the diversity score was used: Curves were similar when the diversity score fell within the confidence interval. Rarefaction was conducted using EcoSim v.7 (Gotelli and Entsminger 2001).

The relative abundance of the top three most abundant species in each site was compared to better understand community composition.

Diversity was assessed using selected tests of alpha and beta diversity. Alpha (α) diversity measures diversity within a defined sample area such as a specific habitat or community (Whittaker 1972, Southwood and Henderson 2000, Magurran 2004), and beta (β) diversity measures species turnover, or the change in species composition along a gradient or between habitats (Whittaker 1972, Southwood and Henderson 2000, Koleff et al. 2003, Magurran 2004). The diversity indices selected were based on recommendations within Magurran (2004) and the usage of these indices in ecological literature. Diversity was calculated

using a diversity calculator constructed in Microsoft Excel (Danoff-Burg and Chen 2005) based on Magurran (1988), or calculated using equations provided in Magurran (2004).

Simpson's diversity (D) determines the probability that two individuals randomly drawn from a specified community will belong to the same species (Simpson 1949, Magurran 2004). Results were expressed as the reciprocal $1/D$, or as the complement $1-D$, considered by some as the more suitable representation of the index (Lande 1996, Magurran 2004). In both cases, increasing scores indicated an increase in diversity (Magurran 2004) (Glossary).

Berger-Parker dominance (B-P) was used to assess the dominance of the most abundant species by calculating its relative abundance within the sample (Magurran 2004). Higher scores indicated a higher proportional abundance of the most dominant species on a 0-1 scale (Magurran 2004) (Glossary).

The Sørensen quantitative index considered the number of individuals within a species, allowing for the comparison of the relative abundance of shared species between sites (Southwood and Henderson 2000, Magurran 2004). On a 0 to 1 scale, results closer to 1 indicated greater similarity (Magurran 2004) (Glossary).

The association between site and species abundance between years was assessed through Principal Component Analysis (PCA). Tests like PCA are forms of ordination, which take complex data and display them in a more simplified dimensional space (Kenkel et al. 2002, Gotelli and Ellison 2004). Floral counts from quadrats were assessed as sampling including both early and late species between years. Old sites were excluded to better compare the interactions of upland sites. Flowering species were variables and sites were individuals. Floral abundance was calculated as the total observed open blooms for each species over the sample season by site.

Data were $\log(n+1)$ transformed, and analysis was carried out using ORDIN (Podani, 1998) (Appendix 2.1).

2.2.4 Assessments of floral abundance and phenology

Floral phenology was graphed to compare bloom times in different burn classes for each species. Bar graphs were constructed using both flowers counted in quadrats and involved in insect visitations. Site data were combined by burn class to see if burn age influenced flowering phenology.

Overall effects of recent burns on flowering phenology and abundance were assessed using 16 species and repeated measures ANOVA. Peak dates of flowering or abundances for a species were repeated across burn classes. Thus, burn class was the within subject effect, and flowering time (early or late season) was the between subject effect. This test is appropriate for habitat that has experienced a disturbance, and when the rebounding habitat is sampled at intervals following the treatment or event (Gotelli and Ellison 2004). Flowering species that were present in quadrats in at least three of the four new and mature sites were selected for the ANOVA and peak dates were averaged for each burn class. These 16 species were all relatively common as determined from counts in quadrats. Peaks were designated as either early season (early June to mid July) or late season (early August to early September) and were compared by Julian date. For abundance, the number of open blooms by site was averaged for burn class and square root transformed. Analyses were completed using SAS v.9.1.3.

2.3 Results

2.3.1 *Assessments of species composition and diversity*

The quadrat and belt transect sampling methods yielded similar trends in results. In order to clearly define certain trends, results of quadrat counts will be shown for tests in which the identity of species was important, and belt transect counts will be used when the distribution of species was more important than species identity. However, it should be noted that belt transect counts were not initiated until mid season in 2010.

Rarefaction showed similarities in species richness for the new sites and differences for the mature sites. Floral counts from belt transects showed that New 1 and 2 were similar in species richness in both 2010 (23 and 24 species) and 2011 (41 species) (Fig. 2.2, 2.3). Mature 3 was significantly different with the highest richness (2010: 32; 2011: 46) in both years. Mature 4 was significantly different from all sites in both years (2010: 28; 2011: 30).

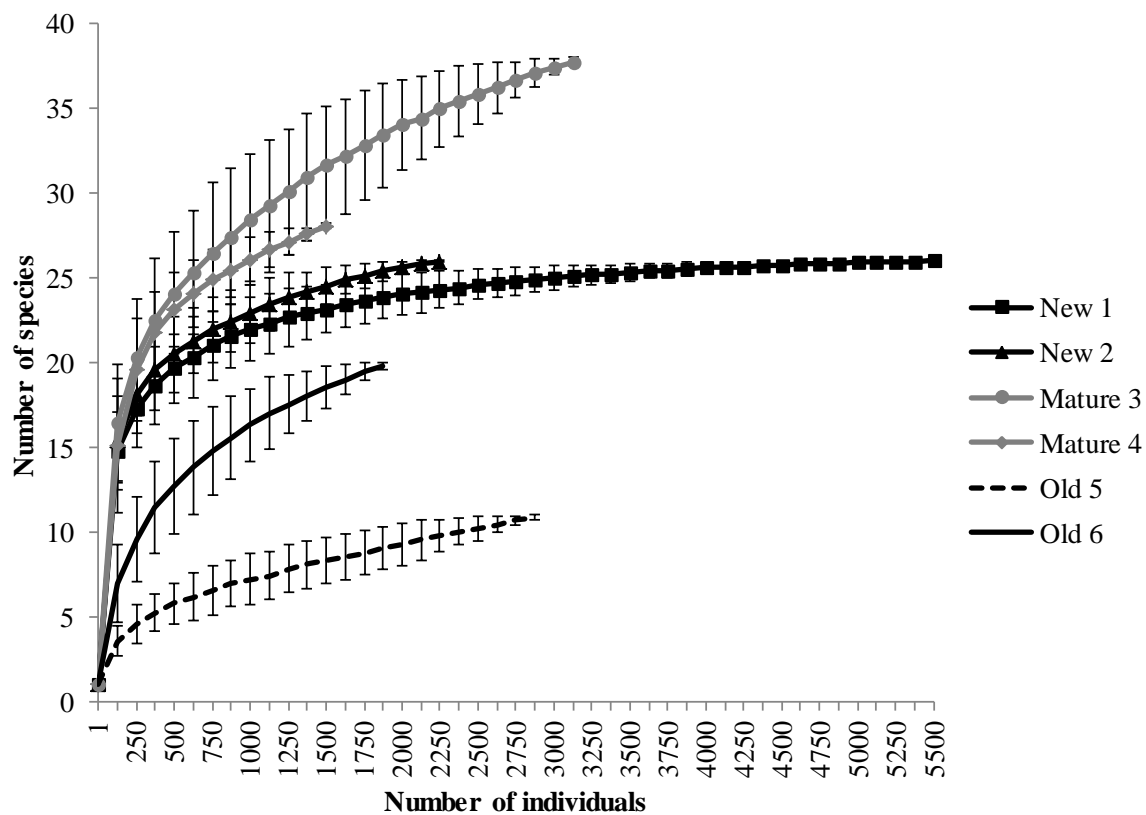


Figure 2.2. Rarefaction of flowering species by site for 2010. Species richness was compared at 1500 individuals.

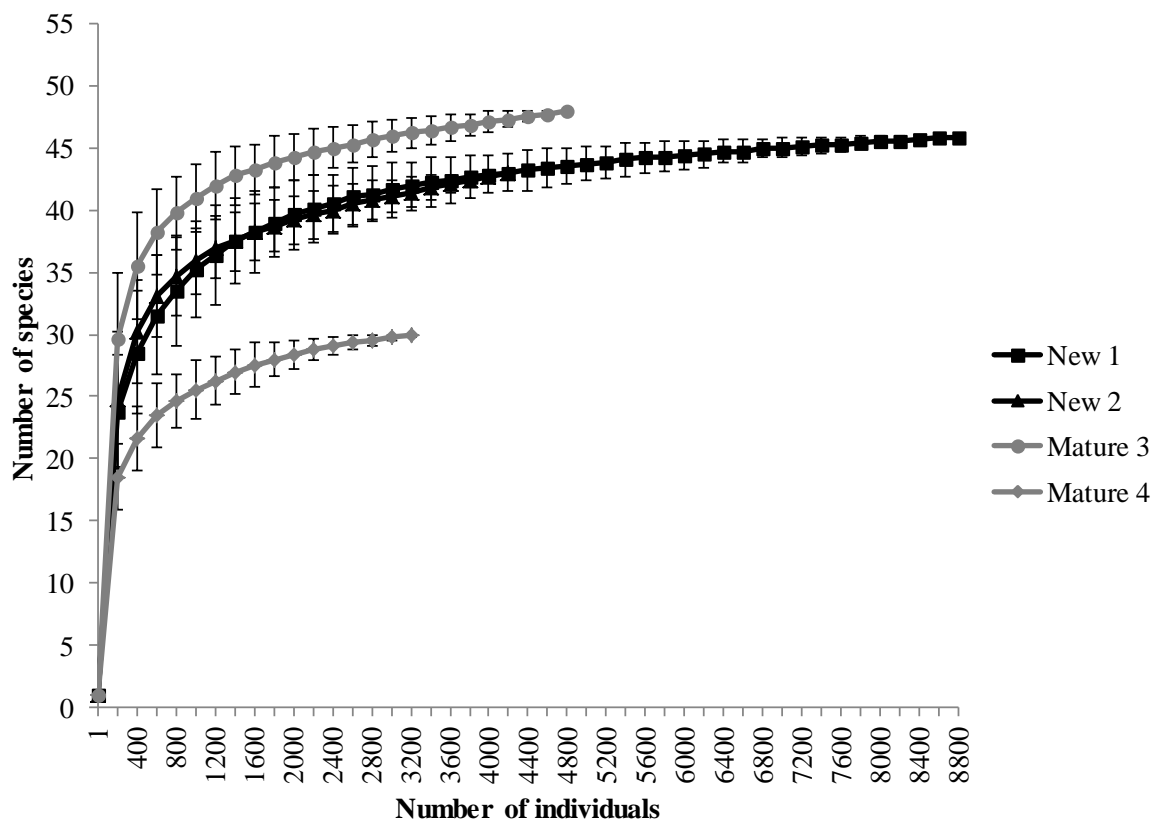


Figure 2.3. Rarefaction of flowering species by site for 2011. Species richness was compared at 3200 individuals.

Sites were typically dominated by one to three flowering species, with remaining species present in lower abundance over the season. Dominance of the 2010 top 3 species from quadrats was highest in New 1 (~61%), similar in New 2 and Mature 4 (~57%), and slightly lower in Mature 3 (~51%) (Fig. 2.4). Site pairs New 1 and Mature 3 and New 2 and Mature 4 showed similar dominance of the top three species (1: ~53%; 3: ~48%; 2, 4: ~70%) in 2011, again with Mature 3 showing the lowest dominance of a few species (Fig. 2.5).

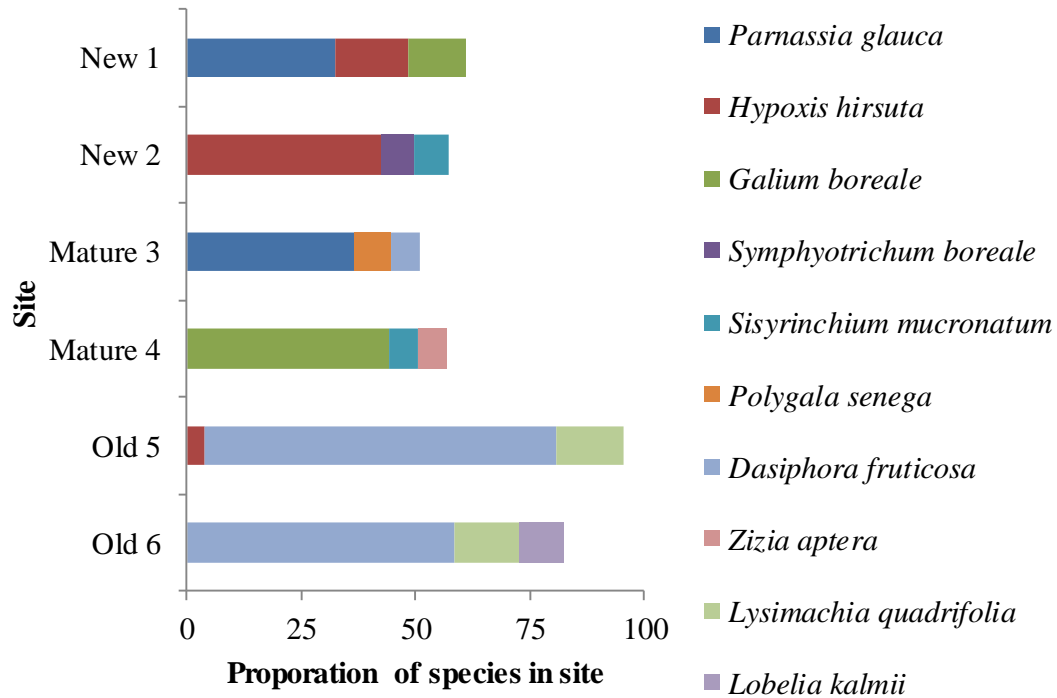


Figure 2.4. Bar graph showing the proportion of the top three most abundant flowering species by site for 2010. Flowers are arranged by site for improved interpretation of colour key.

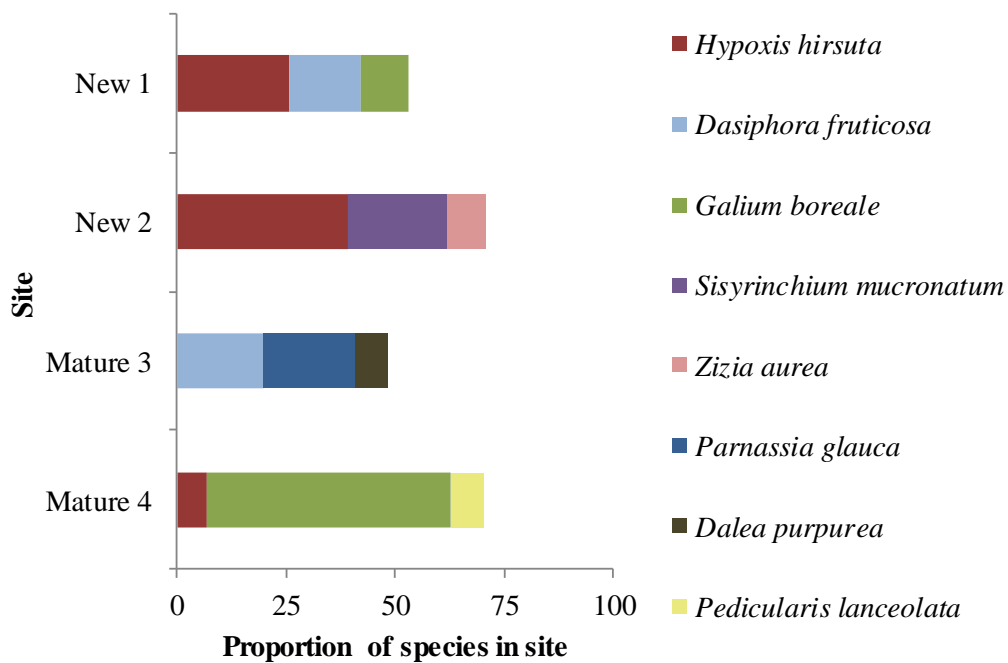


Figure 2.5. Bar graph showing the proportion of the top three most abundant flowering species by site for 2011. Flowers are arranged by site for improved interpretation of colour key.

Diversity measures showed that diversity scores could vary by site within burn class. In 2010, Simpson's diversity for belt transects was most similar between New 1 and Mature 4, while Mature 3 had the lowest diversity and New 2 the highest (Table 2.2). In 2011, New 1 and Mature 3 had the highest and most similar diversity, Mature 4 was intermediate, and New 2 was the low. Berger-Parker dominance was most similar between New 2 and Mature 4 in 2010, with New 1 intermediate and Mature 3 the highest. In 2011, New 1 and Mature 3 were lowest and most similar, with Mature 4 intermediate and New 2 the highest. Old sites had comparatively low diversity and very high dominance.

When diversity measures were assessed for quadrats, the results showed similar trends in variation between sites. However, the trend in diversity values for upland sites in 2010 were reversed, with New 1 being somewhat higher than New 2. This was also the case for Mature 3 and Mature 4. The 2011 scores had an identical trend to the belt data. The alpha diversity table for quadrats was included in Appendix 2.2.

Overall, comparisons of the new and mature burn classes showed similar results across sample methods, and assessments of species composition and alpha diversity did not suggest an effect of burn class. An exception was the old burn class, which consistently showed low species richness and diversity, high dominance, and high within burn class similarities when compared to new and mature upland sites.

Table 2.2. Alpha diversity calculations using bloom counts from 1 x 90 m belt transects. Bold numbers represent burn class.

Burn class	Site	Number of individuals	Number of species	Simpson's diversity ($1-D$)	Simpson's diversity ($1/D$)	Berger-Parker dominance (d)
2010*						
New	1	5505	26	0.8137	5.3667	0.3448
	2	2265	26	0.8312	5.9237	0.272
		3893	34	0.8537	6.8365	0.2975
Mature	3	3219	38	0.7472	3.9557	0.4464
	4	1505	28	0.8169	5.4617	0.2671
		2374	45	0.8317	5.9411	0.3029
Old	5	2948	11	0.1218	1.1387	0.9359
	6	1963	20	0.5241	2.1013	0.6062
		2462	23	0.3311	1.4949	0.8022
2011						
New	1	8998	46	0.8534	6.8234	0.2831
	2	4214	43	0.7216	3.5918	0.5047
		6620	56	0.8335	6.0045	0.3530
Mature	3	4868	48	0.8474	6.5532	0.2732
	4	3357	30	0.8113	5.2981	0.3667
		4127	53	0.8881	8.9351	0.2210

*Belt transect counts initiated halfway through sample season (mid July).

Comparisons of the Sørensen index for floral counts from quadrats showed low similarities in species composition between sites in both years (Table 2.3). New 1 shared the most species with other upland sites, particularly New 2 and Mature 3. New 2 and Mature 4 had comparatively few species in common with other sites. Old sites had the greatest similarity.

Table 2.3. Calculations of the Sørensen Index using counts of open blooms in 1 x 1 m quadrats for 2010 (a) and 2011 (b).

2011		Site					
		New		Mature		Old	
A		1	2	3	4	5	6
	1		0.4301	0.4323	0.3279	0.1200	0.1403
	2			0.3228	0.2213	0.0448	0.0774
	3				0.2753	0.1449	0.2138
	4					0.1039	0.1140
	5						0.6222
	6						

2011		Site			
		1	2	3	4
B					
	1		0.5271	0.4993	0.2640
	2			0.2619	0.2417
	3				0.1460
	4				

The relationship between site and species composition remained relatively consistent between years when compared by PCA (Fig. 2.6). Mature 3 and 4 changed little between years, whereas New 1 and 2 showed evidence of shifts in floral abundance. *Dasifora fruticosa* [(L.) Rydb.], *Dalea purpurea* Vent, *Polygala senega* (L.), and *Parnassia glauca* (Raf.) were most associated with New 1 and Mature 3. *Solidago* spp. and *Euthamia graminifolia* [(L.) Nutt.] were associated with New 2. Mature 4 was most different from all sites, particularly from New 1. Overall, New 1 and Mature 3 appeared to be associated with the greatest variety of flowering species between years. The results of PCA did not indicate an effect of burn class on sites and floral associations.

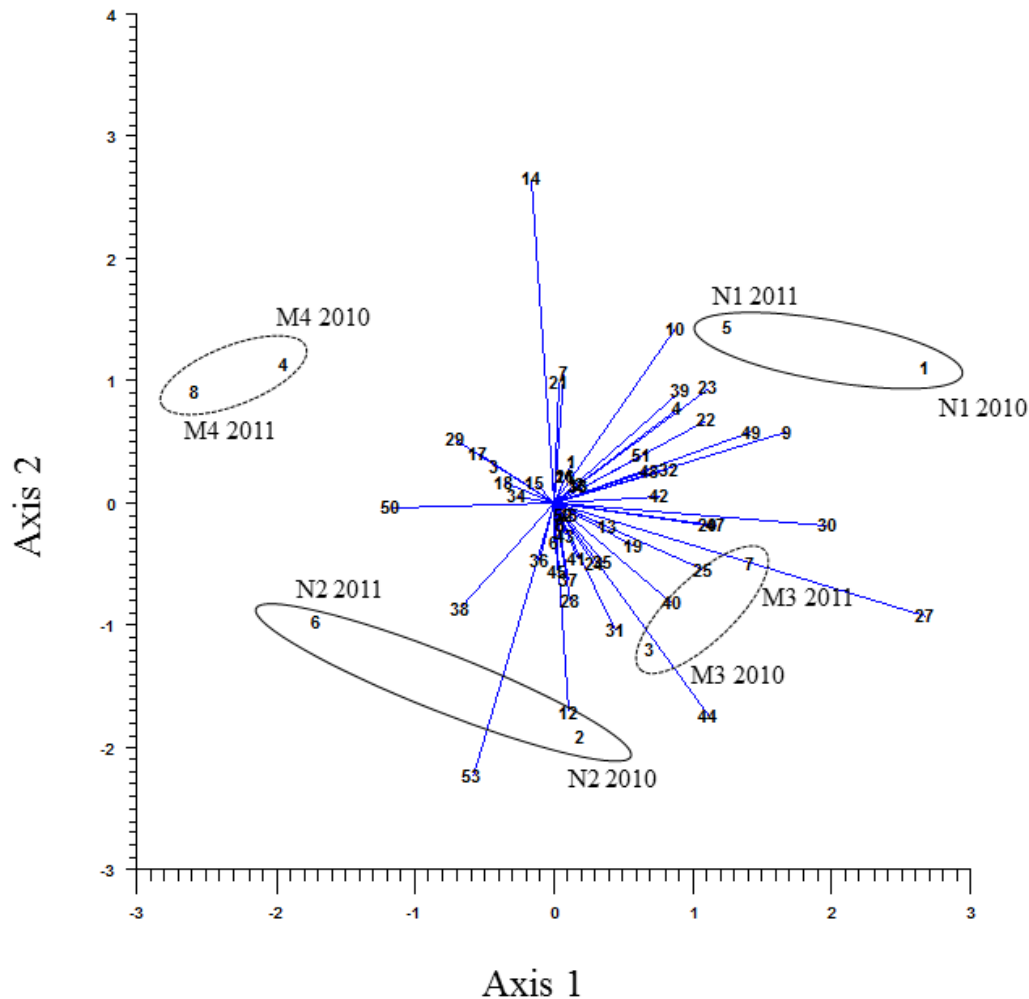


Figure 2.6. PCA of flowering species sampled by quadrat in 2010 and 2011. Solid circles include sites in the new burn class and broken circles the sites in the mature burn class (1: New 1 2010; 5: New 1 2011; 2: New 2 2010; 6: New 2 2011...). Axis 1 39.63%, Axis 2 18.96%. The identity of the species can be found in Appendix 2.1

2.3.2 Assessments of floral abundance and phenology

Floral counts from upland quadrats totaled 1754 open blooms, with 360 counted in the old burns. In 2011, 1171 open blooms were counted (Fig. 2.7). For upland belt transects, 12 494 open blooms were counted in the half season, with 4911 counted in the old burns. The corresponding half season in 2011 totaled 9728 open blooms, with 21 437 for the full season.

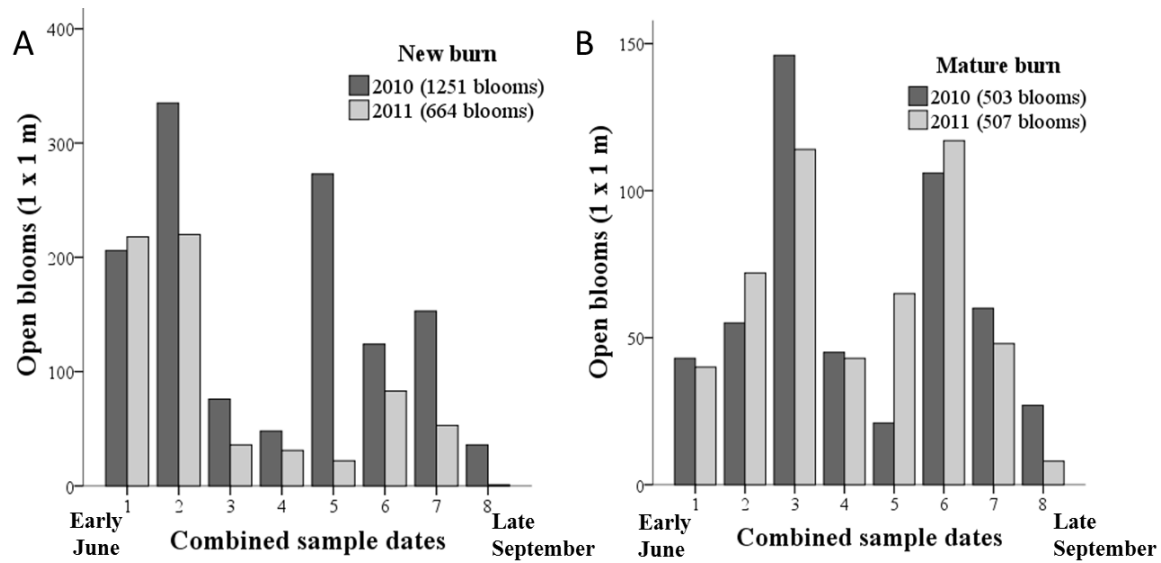


Figure 2.7. Floral abundance by burn class from bloom counts in quadrats by sample date. Counts in each site were pooled by burn class.

Repeated measures ANOVA of floral peaks in 2010 showed that peak date of flowering was earlier by two to three weeks in the new burn class, compared to the mature class ($F_{1,14} = 6.06, p < 0.03$) for the 16 species tested, and the floral abundance was greater in the new burn class ($F_{1,14} = 5.67, p < 0.04$) in 2010. Results were non-significant in 2011.

Phenology bars graphs also showed earlier flowering in the new class. In 2010, 12 flowering species were in bloom on the first sample day (1 June), and occurred in both the new and mature burn classes. Of these 12 species, 10 flowered for one to two additional sample periods longer in the mature class (Fig. 2.8), while only four species bloomed longer in the mature class in 2011 (Fig 2.9). From the second sample date (15 June) onwards, 17 of 27 species that occurred in both burn classes were encountered one to two sample periods earlier in the new burn class in 2010 (Fig. 2.8). When comparing those 17 flowering species in 2011, only six of those species showed earlier flowering in the new burn class (Fig. 2.9).

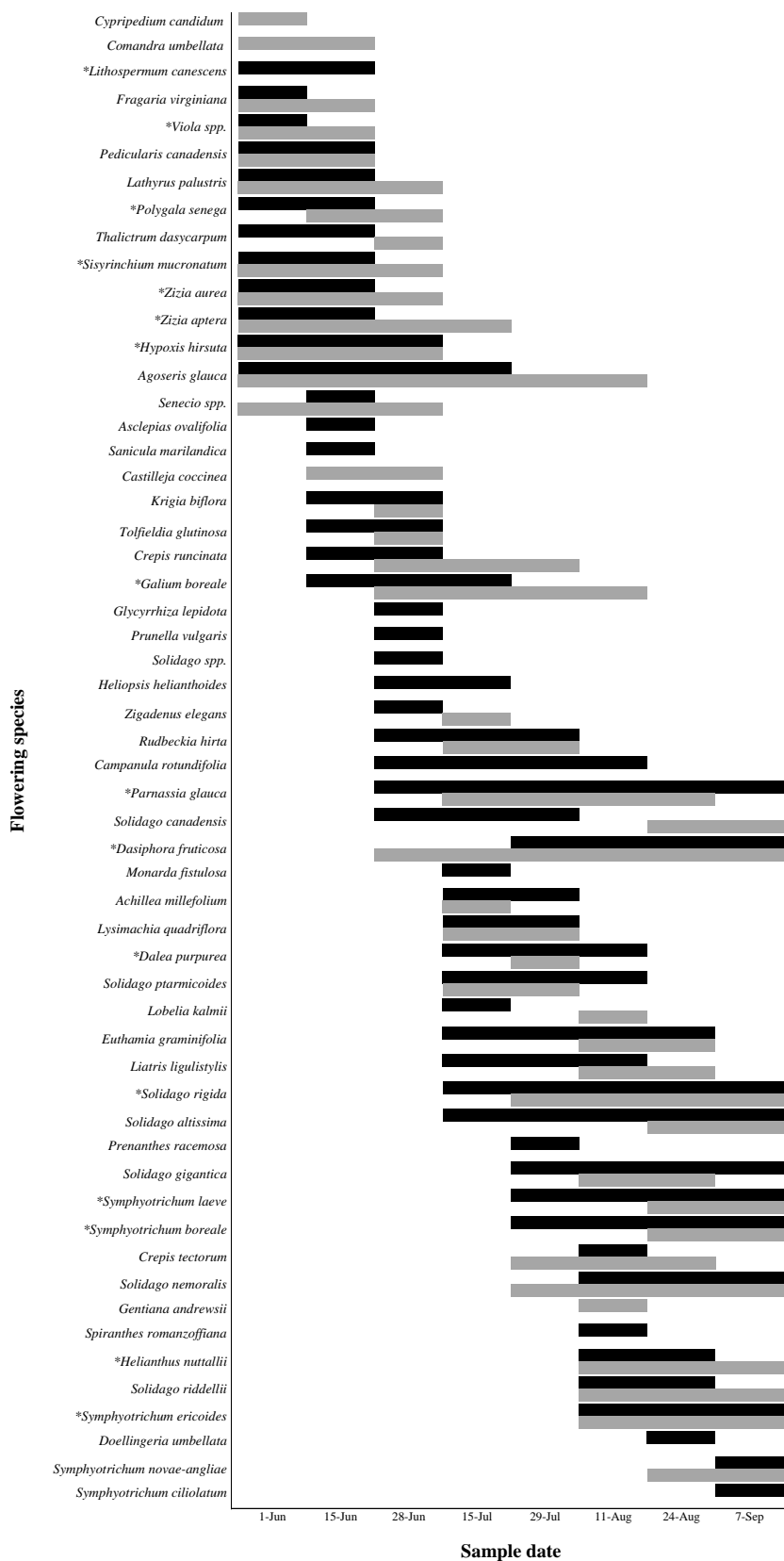


Figure 2.8. Phenology of flowering plants for new and mature burn classes, 2010. Black bars represent New 1 and 2 pooled, grey bars represent Mature 3 and 4 pooled. Open flowers were present in 1 x 1 m quadrats or along transects during insect observations. Stars (*) indicate species used in the repeated measures ANOVA.

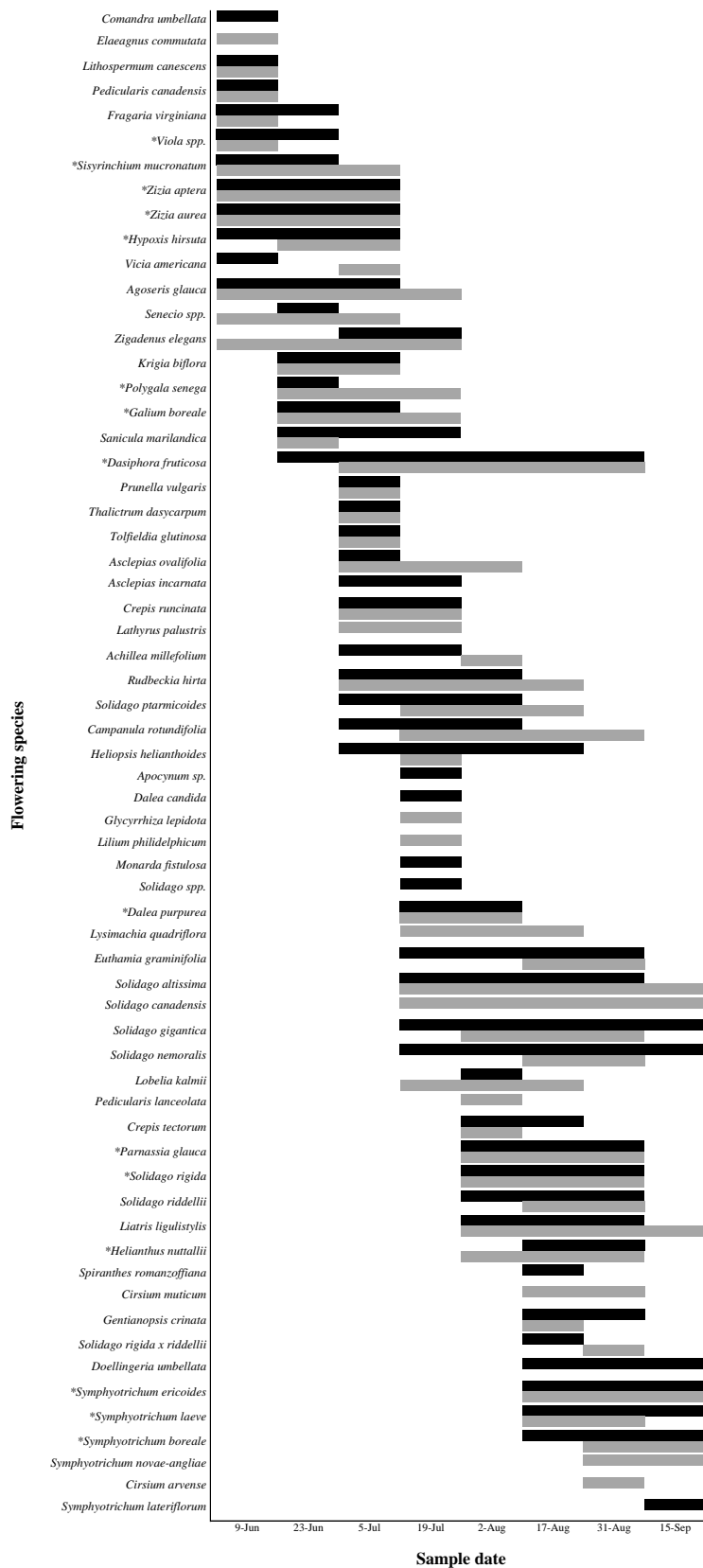


Figure 2.9. Phenology of flowering plants for the new and mature burn classes, 2011. Black bars represent New 1 and 2 pooled, grey bars represent Mature 3 and 4 pooled. Open flowers were present in 1 x 1 m quadrats or along transects during insect observations. Stars (*) indicate species used in the repeated measures ANOVA.

2.4 Discussion

Long years without fire (10+) appeared to restrict the growth of many flowering species, as was shown in the old burn class. It has been argued that a lack of disturbance in fire adapted habitats is a form of disturbance in itself, with fire being necessary to induce increases in floral growth and species richness (Biondini et al. 1989). Long fire free intervals result in deep litter that limits sunlight to growing plants (Knapp 1984). In addition, the presence of litter can reduce convective cooling by wind to growing shoots, increasing leaf temperatures and water loss (Knapp 1984). These difficult growing conditions likely contributed to the low species richness and diversity, high dominance, and the high number of shared species between older sites. The old sites also differed in environmental conditions such as higher soil moisture leading to a sedge and rush dominated community, markedly different from upland prairie sites. So while the absence of fire likely contributed to the uniformity of species, the habitat characteristics themselves would also limit the flowering species that could thrive. These combined effects created difficulties when comparing the flowering community of the old sites with that of comparatively recently burned upland prairie, resulting in discontinued sampling in 2011. Despite this, the old sites showed important effects of both the lack of disturbance and the influence of habitat characteristics.

Variation in the composition of the flowering plant community existed between sites, but there wasn't a strong effect of burn class in upland sites. The stability of species composition within sites with burning may be evidence of a fire-adapted perennial community. Perennial species have the ability to regenerate from roots or tubers that persist below the soil, resulting in low species turnover and low regeneration by seeds in frequently burned habitat (Morgan 1999). In an examination of the effects of fire in mixed-grass prairie, Biondini et al. (1989) showed that

community structure was only mildly influenced by fire, creating increases in floral abundance rather than dramatic changes in species composition, as was supported by this study. Engle et al. (2000) also showed that frequent fires had little influence on species richness.

It was difficult to determine if there was a difference in diversity between burn classes. The old sites were not a suitable comparison for sites in upland habitat, thus statistical tests were may not have been as useful with only two classes. Instead, interpretation of the alpha and beta diversity was more qualitative. By comparing the diversity scores with the raw data, and by including knowledge of the sites obtained from observing them for two seasons, site-to-site variation appeared to be more prominent than differences in burn class. There was a high and a low diversity site for each burn class, but highs and lows were subjective, and when averaged by burn class, did not appear to differ. Berger-Parker dominance helped with interpretation, as greater dominance indicated lower diversity, although sites were also fairly similar in dominance. The general explanation for all three burn classes was that diversity was similar, and that upland sites showed less dominance and greater diversity than old sites.

Site-by-site differences in floral abundance and species composition were perhaps best illustrated by the comparison of sample years by PCA. Mature 3 and 4 showed little change in position on the axes between years, suggesting that despite some differences in species between these sites, the species present were generally similar within each site from year to year. New 1 and 2 also differed in species composition between sites, but increased floral abundance following fire resulted in stronger variation between 2010 and 2011 when compared to mature sites. This increased abundance of blooms was consistent with evidence that occasional late-season fires increase flowering plant abundance in the following season (Ehrenreich and Aikman 1963, Howe 1994, Engle et al. 2000). Site-to-site differences were also supported by the

Sørensen index, which showed a low number of shared species between most sites. The ability of perennial species to survive and regenerate in burned habitat seems to have a strong influence on floral community structure following fire.

The lack of a full early season for belt data did cause some discrepancies between belt and quadrat data in 2010 even though many of the trends were similar. For example, New 1 had the lowest species richness in 2010, but had similar richness to New 2 with quadrat sampling. The assessments of Simpson's diversity and Berger-Parker dominance by quadrats showed New 1 and Mature 3 to be the most diverse in 2010, but belt data showed similar scores for New 1 and New 2, and Mature 3 was low. Differences in results were observed because belt transect sampling was not initiated until the fourth sample period in 2010. Belt transect counts were added to supplement data in the event that quadrat sampling was biased towards common species. This meant that the early flowering period was not detected with belt data, and this period provided substantial information on the effect of fire on growth. However, when quadrat and belt data were compared for 2011, the trends were similar. Discussing one sampling method over the other to avoid confusion, depending on whether the results were focused on species identity (quadrats) or distribution (belt), better clarified the results. The general trends in the data were still consistent, with no effect of burn class, but strong site-to-site differences.

While fire may not have a strong influence on flowering species composition, previous land use should be considered when comparing site differences. The Tall Grass Prairie Preserve is located in an area of Manitoba that is both currently and historically agricultural, and properties acquired for the preserve have varying land use histories. Mature 3, one of the more consistently diverse sites, was both plowed (40+ years ago) and sporadically grazed by cattle since the land was purchased by the NCC in the 1990s (C. Borkowsky, Tall Grass Prairie

Preserve pers. comm). New 1, which also scored high in diversity and floral counts, lacked evidence of plowing but was likely grazed over 30 years ago. Moderate grazing has been shown to increase forb cover while decreasing grasses in tall grass prairie, as well as increasing soil nutrients and organic matter (Walters and Martin 2003). Tall grass prairie was historically grazed by bison, and increased perennial forb densities following bison grazing, as well as patchy fire islands that result from bison grazing lawns, suggest that prairie dynamics are strongly influenced by grazing (Towne et al. 2005). New 2, a low diversity site, was partly used for oat production in the 1940s but was allowed to regenerate through the seed bank and surrounding vegetation after only a few years of cultivation (C. Borkowsky, Tall Grass Prairie Preserve, pers. comm). Relying on the seed bank for re-establishing native prairie may have created some of the differences in diversity observed in New 2. Regenerating communities may not reflect the proportions of species in the seed bank or surrounding vegetation, as the seeds in the soil will have accumulated from a variety of previous habitat conditions (Davies and Waite 1998). Mature 4, which often differed in species composition from the other three upland sites, did not show evidence of plowing and may have been grazed in the past (C. Borkowsky, Tall Grass Prairie Preserve pers. comm). A greater influence in this site was soil moisture, as preserve staff had reported wetter soils in this site over the last 12 years, possibly due to beaver activity in nearby waterways (C. Borkowsky, Tall Grass Prairie Preserve pers. comm). High soil moisture appeared to influence species composition in the old sites, and this may be exerting similar influence in Mature 4.

There was evidence of short term changes in the floral abundance and phenology between the two years of study when burn classes were compared. Floral abundance was highest in the new burn class, and phenology of flowering was also increased up to three weeks earlier

for some species in the new burn class in 2010 when compared by repeated measures ANOVA. There were three effects of fire that could have caused these changes in the community, primarily related to the removal of the litter layer by fire (see Hulbert 1988). First, soils in the new burns were exposed to greater sunlight. Dead litter would normally accumulate annually and act as a shade layer over the soil. Shading can considerably reduce production, and unburned shaded soils, as well as burned soil with shade treatments, have been shown to have reduced productivity compared to sites with ample sunlight (Hulbert 1988). The second effect was the increase in soil temperature due to increased sunlight. Higher soil temperatures are known to increase the rate of growth in flowering plants, accelerating the first date of flowering (Ehrenreich and Aikman 1963, Vogl 1974). Temperature increases can be quite pronounced, with soil in unburned sites requiring an extra month to match those of newly burned sites in the early growing season (Rice and Parenti 1978). Thirdly, the new burns would have lacked the physical barrier created by dead litter that slowed the growth of new shoots (Vogl 1974). Bloom times in older burns were likely delayed because emphasis would have been placed on elongation rather than flowering. For example, small yet abundant early flowering species like *Hypoxis hirsuta* [(L.) Coville] and *Sisyrinchium mucronatum* (Michx.), which were responsible for the majority of flowers encountered in the early season, were lower in comparative abundance in the mature and old burns. Robust, tall mid season species such as *Galium boreale* (L.) or *Dasiphora fruticosa* were in greater abundance in the mature and old burn classes compared to earlier flowering species.

The flowering community is a foundation of the prairie ecosystem, as all animal life within this habitat is reliant on plants to some degree. Understanding the structure of the flowering community will be important for interpreting insect responses to disturbance. The

plant community has the strongest influence on the trophic level that relies on it as a primary source of energy, that being herbivores (Shaffers et al. 2008). Pollinating insects act as herbivores by consuming floral resources at some point during development, whether that be as larvae or adults. The composition of the insect community can be predicted based on assessments of the flowering community due to this close relationship (Shaffers et al. 2008). However little is known about the interactions between plant and insect species in the Tall Grass Prairie Preserve at the community level, and no studies of pollination networks have been undertaken in the preserve.

2.5 Conclusions

The flowering plant community was influenced by disturbance, but only in the short term. Increased bloom abundance and date of peak flowering in the first year following fire was significant in 2010 but did not continue in 2011. It was expected that diversity might also be affected by fire, but there were few notable differences between burn classes. Instead, site-to-site variation in species composition was evident, but was obscured when the diversity scores for burn class were averaged. It appeared that species composition was similar within site between years, despite disturbance, and that abundance was most influenced by fire. This was likely evidence of the flowering community being fire adapted in tall grass prairie. Flowering plants were perennial and regenerated from the roots, offering a number of open blooms for visiting insects.

The influence of flowering phenology and abundance of blooms on insect communities is not well studied in this habitat. Assessments of the insect community will be required to better

understand their response to floral increases, changes in phenology, and fire mortality. As plant-insect interaction define the structure of pollination networks, these changes in plant community could influence the stability of network structure.

GLOSSARY

Simpson's diversity (D): The probability D , for a finite community, is defined as:

$$D = \frac{\sum n_i(n_i - 1)}{N(N - 1)} \quad (\text{Simpson 1949})$$

where “ n_i = the number of individuals in the i th species; and N = the total number of individuals” (Magurran 2004).

Berger-Parker dominance (B-P): The equation is expressed as:

$$d = N_{\max}/N \quad (\text{Berger and Parker 1970})$$

where N = the total number of individuals in the sample, and N_{\max} = the total individuals within the most abundant species (Magurran 2004). Dominance is scored from 0-1, where 1 = a population dominated by one species and 0 = a diverse population (Magurran 2004).

The Sørensen quantitative index (Bray-Curtis): The equation is expressed as:

$$C_N = \frac{2jN}{(N_a + N_b)} \quad (\text{Bray and Curtis 1957})$$

where N_a = total individuals encountered in site 1, N_b = total individuals encountered in site 2, and $2jN$ = the lower number of individuals for each species shared between site 1 and 2, summed (Magurran 2004).

APPENDICES

Appendix 2.1. Floral counts from quadrats used for PCA. Data was log(n+1) transformed.

Species	PCA#	2010				2011			
		New 1	New 2	Mature 3	Mature 4	New 1	New 2	Mature 3	Mature 4
<i>Achillea millefolium</i>	1	0	0	0	0	2	0	0	0
<i>Agoseris glauca</i>	2	1	0	0	0	0	0	0	0
<i>Asclepias ovalifolia</i>	3	0	0	0	0	0	0	0	4
<i>Campanula rotundifolia</i>	4	5	0	0	0	4	0	3	0
<i>Castilleja coccinea</i>	5	0	0	1	0	0	0	0	0
<i>Comandra umbellata</i>	6	0	0	6	1	0	0	0	0
<i>Crepis runcinata</i>	7	4	0	0	3	1	0	0	1
<i>Crepis tectorum</i>	8	0	0	1	0	0	0	0	0
<i>Dalea purpurea</i>	9	27	1	1	0	6	0	25	0
<i>Dasiphora fruticosa</i>	10	41	0	18	12	70	14	65	6
<i>Doellingeria umbellata</i>	11	0	0	0	0	1	0	0	0
<i>Euthamia graminifolia</i>	12	0	16	1	0	0	2	3	0
<i>Fragaria virginiana</i>	13	0	0	2	0	3	1	5	0
<i>Galium boreale</i>	14	100	12	2	108	46	1	4	102
<i>Gentiana andrewsii</i>	15	0	0	0	1	0	0	0	0
<i>Gentianopsis crinita</i>	16	0	0	0	0	1	0	1	0
<i>Glycyrrhiza lepidota</i>	17	0	0	0	0	0	0	0	7
<i>Helianthus nuttallii</i>	18	0	0	4	11	0	0	3	1
<i>Hypoxis hirsuta</i>	19	128	192	16	12	108	94	9	12
<i>Krigia biflora</i>	20	4	4	6	0	10	0	3	0
<i>Lathyrus palustris</i>	21	5	0	0	9	0	0	0	0
<i>Liatris ligulistylis</i>	22	23	3	0	0	4	0	0	0
<i>Lithospermum canescens</i>	23	2	0	0	0	24	0	16	0
<i>Lobelia kalmii</i>	24	0	0	3	0	0	0	3	0
<i>Lysimachia quadriflora</i>	25	6	1	4	0	0	0	17	0
<i>Monarda fistulosa</i>	26	0	0	0	0	1	0	0	0
<i>Parnassia glauca</i>	27	259	20	102	0	16	2	67	0
<i>Pedicularis canadensis</i>	28	0	2	3	0	0	0	0	0
<i>Pedicularis lanceolata</i>	29	0	0	0	0	0	0	0	14
<i>Polygala senega</i>	30	31	6	22	0	17	0	15	0
<i>Prunella vulgaris</i>	31	0	5	0	0	0	0	17	0
<i>Rudbeckia hirta</i>	32	3	1	0	0	3	0	5	0
<i>Sanicula marilandica</i>	33	0	0	0	0	1	0	1	0
<i>Senecio sp.</i>	34	0	1	0	3	0	0	0	0
<i>Sisyrinchium mucronatum</i>	35	19	33	17	15	44	54	23	4
<i>Solidago altissima</i>	36	0	3	0	0	0	0	2	2
<i>Solidago canadensis</i>	37	0	1	3	0	0	0	0	0
<i>Solidago gigantea</i>	38	0	5	0	0	0	6	1	5
<i>Solidago nemoralis</i>	39	4	0	1	0	12	0	1	0
<i>Solidago ptarmicoides</i>	40	7	4	4	0	3	7	9	0
<i>Solidago riddellii</i>	41	1	0	4	1	0	2	3	0
<i>Solidago rigida</i>	42	11	22	2	4	7	0	0	0
<i>Spiranthes romanzoffiana</i>	43	1	1	0	0	0	1	0	0
<i>Symphyotrichum boreale</i>	44	8	33	10	0	0	0	6	0
<i>Symphyotrichum ciliolatum</i>	45	0	3	0	0	0	0	0	0
<i>Symphyotrichum ericoides</i>	46	35	27	11	13	8	14	5	11
<i>Symphyotrichum laeve</i>	47	42	17	5	3	4	1	2	0
<i>Thalictrum dasycarpum</i>	48	9	4	2	1	3	0	1	1
<i>Tolfieldia glutinosa</i>	49	15	0	11	0	9	0	3	0
<i>Viola sp.</i>	50	0	1	5	11	2	15	2	10
<i>Zigadenus elegans</i>	51	6	0	0	0	0	0	1	0
<i>Zizia aptera</i>	52	2	14	2	15	14	5	2	0
<i>Zizia aurea</i>	53	0	20	10	1	0	21	2	2

Appendix 2.2. Alpha diversity calculations using bloom counts from 1 x 1 m quadrats.

Burn class	Site	Number of individuals	Number of species	Simpson's diversity (1- <i>D</i>)	Simpson's diversity (1/ <i>D</i>)	Berger-Parker dominance (<i>d</i>)
2010						
New	1	799	28	0.8421	6.3351	0.3242
	2	452	28	0.7956	4.8932	0.4248
		636	38	0.8697	7.6756	0.2516
Mature	3	279	30	0.8432	6.3795	0.3656
	4	224	18	0.7454	3.9277	0.4821
		261	34	0.9007	10.0742	0.2107
Old	5	218	7	0.3843	1.6243	0.7706
	6	142	10	0.6243	2.6618	0.5845
		182	11	0.4986	1.9946	0.6923
2011						
New	1	424	28	0.8769	8.1228	0.2547
	2	240	16	0.7787	4.518	0.3917
		340	33	0.8654	7.4294	0.2971
Mature	3	325	33	0.8957	9.5902	0.2062
	4	182	15	0.6680	3.0123	0.5604
		265	37	0.9136	11.5713	0.2000

3. INSECT COMMUNITY RESPONSE TO FIRE AND CLIMATE

3.0 Chapter Summary

Insects are extremely important to healthy ecosystems, particularly due to their role as pollinators. If fire has a negative influence on pollinating insects, community structure might become unstable. Insect activity is also closely linked to environmental factors such as temperature and rainfall, thus annual fluctuations in climate might also impact the presence of certain insect species. I used measures of species richness and diversity to understand insect community composition. I compared peak insect activity and abundance by ANOVA, and determined site associations using PCA. Overall, there was variation in community composition between sites, but burn classes had no significant effect on differences in diversity. Sites were dominated by visits of a few common species that were shared between most sites. There were no differences in insect phenology by burn age. The most prominent trends were related to weather: Syrphid activity was linked to the presence of larval habitat, particularly those that benefitted from sites that received high precipitation in 2010. Bees showed some site associations based on burn age, with nest location being an important factor. A better understanding of insect community structure will aid in assessments of pollination networks: insect dynamics play a vital role in network structure and stability.

3.1 Introduction

Insects are abundant within and have adapted to a variety of terrestrial and aquatic habitats throughout the world (Schowalter 2000). Insects act as links between levels within

ecosystems, serving as predators, prey, nutrient cyclers, and very importantly, facilitators of floral reproduction (Schowalter 2000).

Pollinating insects have been defined as insects that transfer pollen to the stigma of a flower (Kearns and Inouye 1993). Both nectar and pollen are key sources of nutrition and so are important floral rewards for a variety of anthophilous, or flowering visiting, insects (Kevan and Baker 1983, Kevan 1999). Butterflies may transport pollen during their efforts to acquire nectar, while bees feed on nectar while actively collecting and transporting pollen (Schowalter 2000). Pollinators may be categorized based on their behavior and the presence or absence of floral preference. Non-specialists, or generalists, may be found visiting a variety of flowering taxa, while specialists may actively seek out specific floral resources or floral traits (Schowalter 2000).

A diverse assemblage of insects, with floral preferences and morphologies that allow them access to different floral structures, greatly impact the structure of the flowering community and so the community as a whole (Fontaine et al. 2006, Stang et al. 2006, Stang et al. 2009). In habitats where pollinator diversity is higher, more flowering species are present and floral reproduction is more successful (Fontaine et al. 2006). At the same time, sites with greater floral diversity attract more pollinators, increasing reproductive success (Ghazoul 2006). As insects are such an important component of the ecosystem, it is important to understand their response to environmental stressors.

Insect activity is linked to temperature, potentially influencing observed interactions. Insects are poikilothermic, meaning their internal temperature is largely regulated by the conditions of their surroundings (Triplehorn and Johnson 2005). The ability to move or fly is dependent on maximum and minimum temperatures, with insect flight being possible with air temperatures between 19°C - 38°C, depending on the particular species (Chapman 1982). Many

insects in cooler climates are still active outside of this range due to thermal regulation using a variety of strategies. For example, bumble bees and flower flies will warm their bodies with pre-flight vibrations of wing muscles (Chapman 1982). Bumble bees have been found to have flight muscle temperatures 20°C - 30°C higher than that of the surrounding air temperature (Triplehorn and Johnson 2005). Insects will also use behavioural strategies to regulate temperature, such as basking in the sun (Chapman 1982). For insects that can thermoregulate more efficiently than others, fluctuations in temperature between years may not have a strong effect on their presence in the community. But for insects that rely heavily on local temperature, interactions might be lost or gained depending on the local conditions.

Weather and climate will affect the abundance of certain insects between years, causing potential fluctuations in dominant visiting taxa. Years of high rainfall might be beneficial to some visiting insects; many flower flies (Syrphidae) require standing water or moist substrate to develop as larvae. Rainfall can also have indirect consequences, such as physical damage from storms, increased flowering or vegetative growth available to plant feeding/visiting insects, and when combined with wind, can move insect populations to new areas of habitat (Speight et al. 1999). Alternatively, years of low rainfall can decrease flowering, and interactions (Alarón et al. 2008), while also creating difficulties related to desiccation (Schowalter 2000) and the loss of short-term aquatic habitats (Speight et al. 1999).

Disturbances such as wildfire can influence insect populations in different ways. To predict sensitivities, one must have a general understanding of the habitat requirements and life cycles of the insects in focus. Panzer (2002) found that the reliance on prairie remnant habitat, low mobility, single generations per season, and prairie habitat type greatly influenced the level of susceptibility to fire. Multi-year surveys have shown that prairie specialist butterflies were

negatively affected by burning (Swengel et al. 2010), a trend that has been observed in Manitoba (Bates 2007, Semmler 2010). For a variety of bee species, nest location is a major predictor of fire sensitivity, with above ground nesters showing declines in burned habitat (Williams et al. 2010). These species level responses may be overlooked when considering insects at the community level, particularly if abundant, where fire tolerant species are heavily encountered during surveys (Panzer 2002, Williams et al. 2010). Indeed, focusing on individual species has greatly improved the understanding of insect responses to disturbance (see Moretti et al. 2009, Richards et al. 2011, Sheffield et al. 2013).

The Tall Grass Prairie Preserve supports a diverse population of pollinating insects that range from specialist bees (ex. *Dufourea* spp.) to generalist flower flies (ex. *Toxomerus* spp.). A variety of flowering species are assumed to rely on these pollinators for reproduction. Disturbances such as fire may alter the landscape and community, potentially changing the identity and/or abundance of interacting partners (Potts et al. 2003).

In the previous chapter, my first objective was to assess the flowering plant community following fire. I will continue with my first objective by assessing the impact of burning on insect community structure by examining the abundance, diversity, and phenology of flower visiting insects. I expect that insect diversity will be high in recent burns due to abundant floral resources. I also expect that insect species will respond differently to fire, as certain species might have higher mortality following fire than others.

3.2 Materials and methods

3.2.1 Study sites

Site locations were those of Chapter 2.

3.2.2 Observations of plant-insect interactions

Insect observations took place along the same transects used in Chapter 2, with modifications to width (Fig 3.1). Observations took place within two parallel belt transects (90 m) placed 4 m away from the central transect. Transect placement was consistent between years, and the size and placement of transects was intended to maximize the amount of activity that could be observed and recorded. Sampling took place every 10-14 days from June to mid-September in both years, and each season included eight sample periods. Sampling included pan traps as described in section 3.2.3.

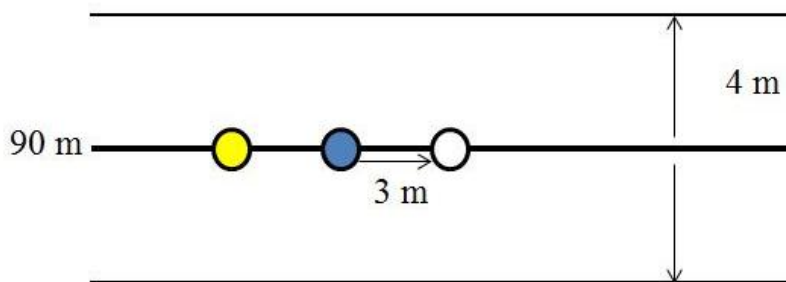


Figure 3.1. Schematic of transect design, showing two 4 x 90m belt transects for insect observations and a central transect for CanPolin pan traps.

Standardized observations of insect activity took place along belt transects in 2010 and 2011, but the number of sites, as well as the duration and timing of observation periods, differed between years. In 2010, observations took place in new, mature, and old sites for one hour blocks per sample day, totaling two hours per burn class each sample period (Table 3.1). In 2011, old habitat was not sampled leaving additional time for observations in upland habitat.

Observations in new and mature sites took place in one hour AM-PM blocks, totaling four hours per burn class each sample period (Table 3.2a). Site start times were rotated in the event that time of day influenced insect activity. The burn class rotation was adjusted partway through 2011 to avoid confounding time of day effects with burn class effects (Table 3.2b).

Table 3.1. An example of the sample schedule for the 2010 season. Burn class start times were rotated over the summer.

SAMPLE PERIOD		
Day 1	Day 2	Day 3
Set pan traps 07:30-09:30	New 2: observations 9:30-10:30	Vegetation surveys
New 1: observations 10:00-11:00	Mature 4: observations 11:00-12:00	
Mature 3: observations 12:00-13:00	Old 6: observations 14:00-15:00	
Old 5: observations 14:00-15:00	Vegetation surveys 15:30-16:30	
Collect pan traps 15:30-19:00		

Insect vouchers were collected with butterfly nets when identifications could not be made on the wing. These vouchers were assigned a number that was used for subsequent observations of the same insect. Insects that did not visit flowers, but were observed in the general area, were noted. Ants and insects under 3mm were not included.

3.2.3 Insect pan trapping

Pan traps were used as a method of sampling a subset of active insect species in each burn site, and the trap methods and supplies were provided by CanPolin. Pan trapping was used by all CanPolin participants in order to create comparable data between provinces, as well as pollinator research taking place in the United States (C. Sheffield, Royal Saskatchewan Museum, pers. comm.)

Pans were placed along the central transect at three meter intervals in alternating colour order (yellow, blue, white) (Fig. 3.1). Each pan was half filled with soapy water. The use of soap decreased surface tension in order to limit the number of potential escapes. Dawn dish soap had been selected by CanPolin to limit the possibility of different odours acting as an attractant rather than pan colour. Sampling in 2010 included 30 pans per transect, with old class sites sampled on alternate sample periods (Appendix 3.1). Concerns regarding oversampling reduced the number of pans to 15 on four sample periods, and 30 on three sample periods in 2011 (Appendix 3.1). Sampling of the old class was discontinued in 2011 due to the emphasis on upland habitat, as well as the difficulty in comparing sedge meadow with upland prairie.

Pan traps were set out between 09:00 and 16:00 on sunny days for one day during each sample period, with one exception. Trapping was suspended during the flight period of an endangered butterfly, the Poweshiek Skipperling, *Oarisma poweshiek* (Parker, 1870) at new 1 and mature 4 for one sample period in 2010. New 1 and mature 4 were suspended for one

sample period, and all sites were suspended for a second sample period in 2011 due to a lengthened flight period (Appendix 3.1).

Trapped insects were placed in 70% alcohol for transportation to the laboratory.

3.2.4 Insect processing and identification

Pinning required different techniques depending on taxa. Lepidoptera and Coleoptera were pinned following a rinse in distilled water. Solitary bees required a rinse in distilled water followed by drying and lifting of the pile with paper towel. Bumble bees were rinsed and then dried with paper towel, or they were blow-dried inside small containers so that the pile could be used for identification. Diptera and Hemiptera required baths in solutions of increasing concentrations of ethyl acetate so that the exoskeleton would not collapse upon drying. These insects were soaked in a bath of 50% ethyl acetate and 50% 70% alcohol for at least four hours, then were moved to a bath of 100% ethyl acetate containing a few drops of glycerol for at least another four hours.

Pinning was greatly assisted by summer students Marika Olynyk, Jessica Guezen, and Alex Hare. Many bumble bees from 2011 were blow-dried and pinned by Christa Rigney at the University of Winnipeg. She also assisted with soaking and pinning a number of Diptera and Lepidoptera.

Insects were pinned and identified to the lowest taxonomic level achievable. Additional assistance was provided by Dr. Cory Sheffield (Royal Saskatchewan Museum), Christa Rigney (University of Winnipeg) and students in the laboratory of Dr. Elizabeth Elle (Simon Fraser University). Voucher specimens are currently located in the laboratory of Dr. Anne Worley, and will eventually be stored in the Wallis Roughley Museum of Entomology, University of

Manitoba. Some specimens were retained by Dr. Sheffield for DNA barcoding, and some specimens in duplicate were retained by Dr. Elle.

3.2.5. Analysis of insect species composition and diversity

The insect community was assessed using the tests of species richness and diversity outlined in Chapter 2. The number of visits observed per insect species was used as a surrogate for insect abundance. Insect taxa included groups by family, genus, and morphospecies, as well as recognized species, and these groupings were consistent between sites. Observed visits in 2011 were halved to standardize for number of visits per hour where noted. Insects discussed below were common visitors and/or appeared to be influential in terms of pollination.

Rarefaction was used to compare the number of insect species between sites in order to detect an effect of burn class on species richness. Observations for 2011 were standardized by half. I compared the total number of visits per species over the sample season using EcoSim v.7 (Gotelli and Entsminger 2001).

Diversity was assessed to determine if fire influenced insect community composition. Simpson's diversity ($1-D$, $1/D$) and Berger-Parker dominance (B-P) were used to assess the diversity of insects by site and burn class. The Sørensen quantitative index was used to determine the proportion of shared species between sites within and between years. Insect visits were pooled for comparisons of burn classes. Standardizing to visits per hour for diversity data did little to change patterns between sites, so diversity scores from original data are shown.

I then compared visiting insects between sites by using the number of visits as a representation of abundance. I calculated relative abundance and compared the top 10 most

frequently visiting insects by site per season to determine if certain insects were consistently dominant in the community.

3.2.6 Assessing variation among sites for insect activity and abundance

Pan trapped insects were used to determine the ratios of different insect groups in each site. Emphasis was placed on insect guilds that were the most common visitors, such as syrphids and ground nesting bees. These ratios were compared with those of common insects observed visiting flowers.

Potential changes in insect phenology and frequency of visits between years and burn class were tested by repeated measures ANOVA. Burn age was treated as the within subjects (species) effect and season between subjects effect. Date of peak activity or frequency of visits by each species were the repeated measures. I selected common insects that were present in at least one new and one mature site. I analyzed 14 species in 2010 and 17 species in 2011. Peak date of activity was compared by designating insects as either early season visitors (peak in early June to mid July) or late season visitors (peak in early August to early September), and comparisons were made by Julian date. To determine differences in visitation frequency, I compared the total number of visits by the selected species within each site. Data were either square root transformed or log transformed as appropriate to stabilize variances. Analysis was conducted using proc GLM in SAS v.9.1.3.

In order to determine if insect life history influenced site-to-site or annual differences in site associations, I assessed insect activity within and between years using Principal Component Analysis (PCA). Insect species were arranged into guilds based on habitat requirements, body size, or Family where most appropriate (Appendix 3.2). Insect guilds were variables and sites

were individuals. The number of insect visits observed over each season was pooled for each site. Visits from 2010 were doubled for year to year comparisons (Appendix 3.3). Data were $\log(n+1)$ transformed, and analysis was carried out using ORDIN (Podani, 1998). I removed certain groups from the analysis to improve the accuracy of the results. For example, honey bees (*Apis mellifera* L., 1758), whose agricultural nest locations make them exempt from mortality by fire, would not assist in the detection of a fire effect. I also removed observations that I was only able to determine as “Diptera” in the field because classification by order was not sufficient to determine guild placement.

To account for annual changes in insect activity, climate data was acquired from the weather station located in the TGPP. Data were compiled by C. Borkowsky, Biologist, TGPP.

3.3 Results

3.3.1 Assessments of insect species diversity and composition

Rarefaction showed some small yet significant differences between sites. In 2010, species richness at 165 individuals was similar for the new sites and Mature 3 (30, 31, and 29 species, respectively), but Mature 4 significantly differed from those sites with 34 species (Fig. 3.2). Within the old burn sites, species richness was lower than other sites (Old 1: 21; Old 2: 26) (Fig. 3.2). In 2011, the new sites had similar numbers of species at 220 individuals (New 1: 50; New 2: 51), but Mature 3 and Mature 4 significantly differed from each other and the new burns (Mature 3: 59; Mature 4: 68.80) (Fig. 3.3).

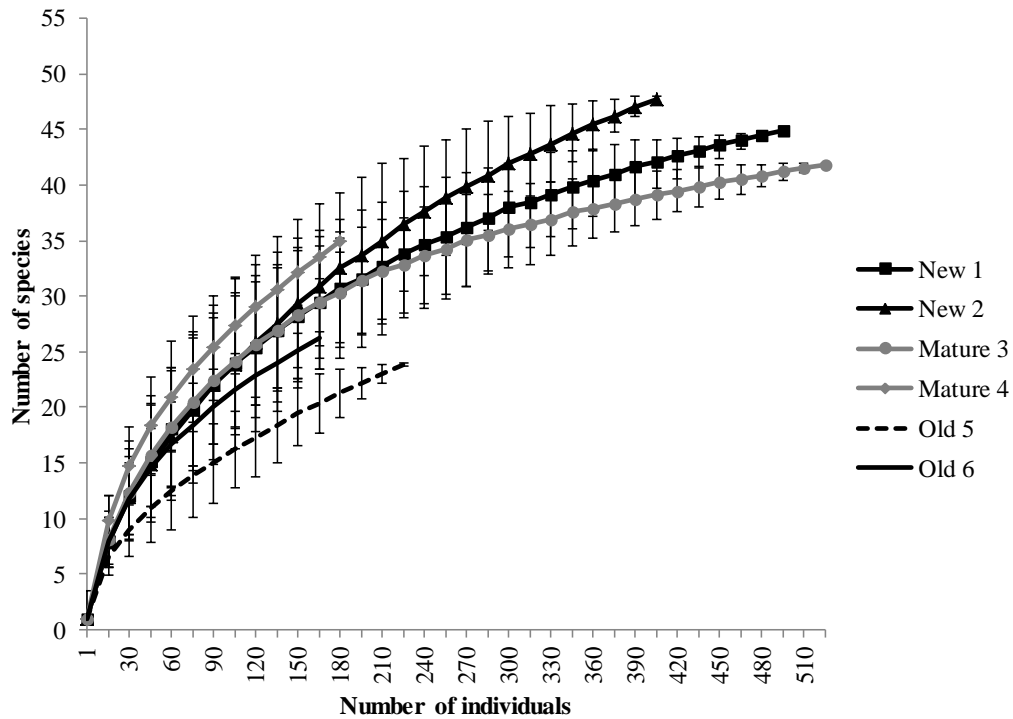


Figure 3.2. Rarefaction of insect visits by site for 2010. Species richness was compared at 165 individuals.

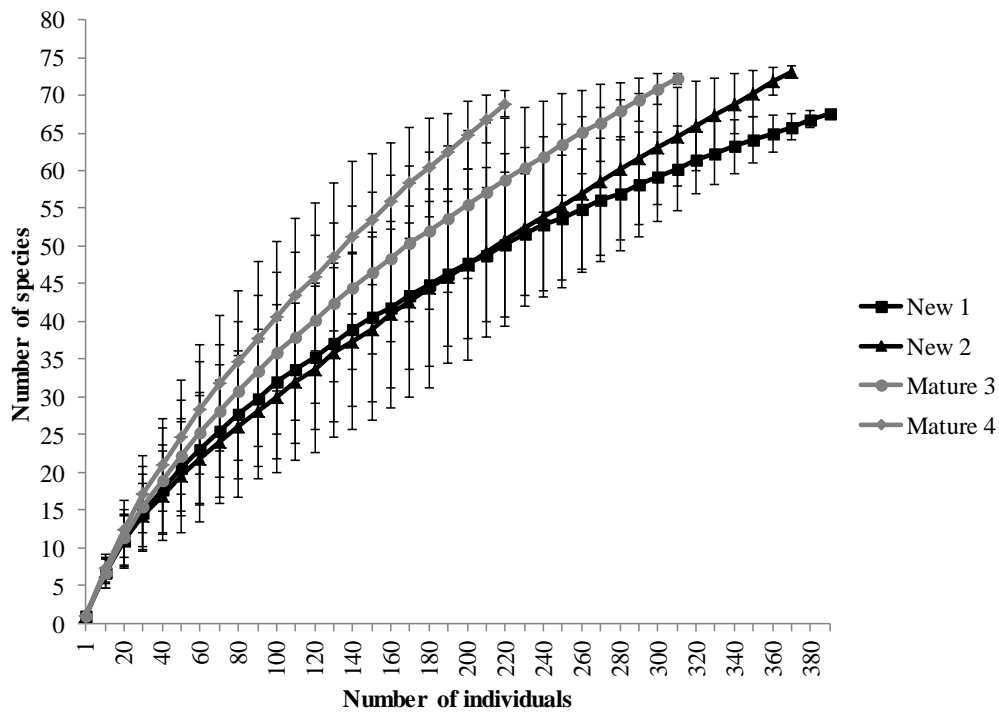


Figure 3.3. Rarefaction of insect visits by site for 2011. Species richness was compared at 220 individuals, and visits were halved for comparison with 2010.

Simpson's diversity showed that New 1, New 2 and Mature 3 had similar Simpson's diversity in 2010, and Mature 4 had comparatively high diversity (Table 3.3). Old 5 had lower diversity, yet Old 6 was comparable to upland sites (Table 3.3). In 2011, New 1 and New 2 were less similar than in the previous year, and Mature 3 was comparatively low while Mature 4 was comparatively high (Table 3.3). Values were very similar for each burn class between years despite variation within sites. Berger-Parker dominance varied by site and year, with the mature sites showing the greatest range in 2010 and the new sites showing the greatest range in 2011 (Table 3.3). The low dominance scores suggested that all sites had a few dominant species but that those species were not disproportionately abundant in terms of visits observed (compare with dominance of flowering plants in old sites, Chapter 2).

Table 3.3. Alpha diversity for insects by site and burn class. Bold numbers represent burn class.

Burn class	Site	Number of observed visits	Number of "species"	Simpson's diversity (1-D)	Simpson's diversity (1/D)	Berger-Parker dominance (d)
2010						
New	1	497	45	0.8604	7.1615	0.2596
	2	411	48	0.8601	7.1457	0.2968
		908	73	0.8602	7.1536	0.2782
Mature	3	533	42	0.8518	6.7484	0.3246
	4	193	36	0.9225	12.9115	0.1865
		726	60	0.8872	9.8300	0.2556
Old	5	228	24	0.8187	5.5165	0.2982
	6	177	27	0.8623	7.2615	0.2994
		405	37	0.8405	6.3890	0.2988
2011						
New	1	745	68	0.8406	6.2724	0.3664
	2	705	74	0.8811	8.4096	0.2667
		1450	109	0.8608	7.3410	0.3166
Mature	3	587	73	0.8316	5.9367	0.3850
	4	418	71	0.8736	7.9086	0.3206
		1005	105	0.8526	6.9226	0.3528

The Sørensen quantitative index showed over half of the insect species were shared between most upland sites within years (Table 3.4). Mature 4 shared few species with other upland sites in 2010 but increased in shared species in 2011 (Table 3.4). Old sites shared species within burn class but shared few species with upland sites (Table 3.4). When sites were compared between years, New 2 and Mature 3 maintained the most similar species, with mature 4 having the lowest similarity between years (Table 3.5).

Table 3.4. Sorensen quantitative index values for insects in 2010 (a) and 2011 (b). Bordered squares represent within year burn classes.

A		New		Mature		Old	
		1	2	3	4	5	6
New	1		0.6278	0.6718	0.3942	0.2124	0.2107
	2			0.5742	0.3775	0.3662	0.2551
Mature	3				0.3471	0.2181	0.2254
	4					0.2945	0.2973
Old	5						0.5778
	6						

B		New		Mature	
		1	2	3	4
New	1		0.6524	0.6877	0.5434
	2			0.5960	0.5272
Mature	3				0.6109
	4				

Table 3.5. Sorensen quantitative index comparing sites between years. Dark grey squares denote comparisons within the same site.

2011	2010			
	New 1	New 2	Mature 3	Mature 4
New 1	0.4081	0.4169	0.3944	0.3163
New 2	0.4719	0.5820	0.4642	0.4007
Mature 3	0.3592	0.3521	0.4523	0.2908
Mature 4	0.3769	0.4056	0.3250	0.3641

Dominance in sites was shown in greater detail when the top 10 visiting insects were investigated. The top 10 visiting insects showed that while the identity of species in different rankings changed, the patterns in relative abundance were very similar in upland prairie sites. Typically one generalist Dipteran species dominated the community and was responsible for 25-39% of observed visits, followed by <10% of visits made by the third or fourth ranked insect, and then close to 1% of visits made by each remaining insect species beyond the top 10 (Table 3.6). Similar species were present between upland and old sites, but the old sites had the majority of visits made by *Nemotelus* spp., a small genera of soldier fly (Stratiomyidae). In upland habitat, the dominant syrphid genera shifted between years. In 2010, *Eristalis* spp., *Helophilus* spp, and *Toxomerus* spp. made the majority of visits, while *Toxomerus* spp., *Odontomyia* spp. (Stratiomyidae) and *Sphaerophoria* spp. were some of the most common visitors in 2011. The most frequent bee visitors included *Bombus* spp. and halictid bees, and these only achieved a highest ranking of 4th (8% of visits) in New 1 in 2011.

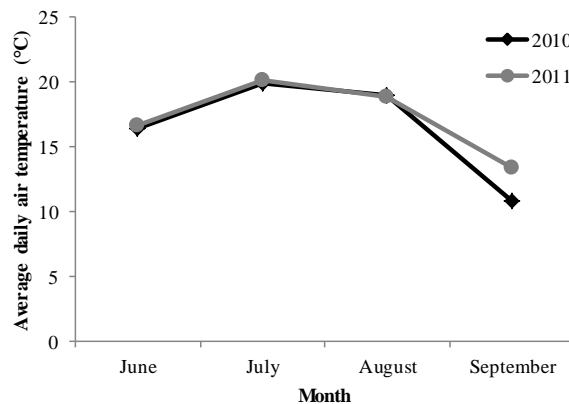
Table 3.6. Top 10 vising insect taxa showing the relative abundance (number of visits) for each site over the sample season.

2010			2011		
New 1			New 1		
Taxa	Total visits	Rel Abd	Taxa	Total visits	Rel Abd
<i>Eristalis (dimidiata/stipator)</i>	129	0.2596	<i>Toxomerus marginatus</i>	273	0.3664
<i>Helophilus (fasciatus/hybridus)</i>	110	0.2213	<i>Odontomyia pubescens</i>	78	0.1047
<i>Toxomerus marginatus</i>	62	0.1247	<i>Sphaerophoria (contigua/philanthus)</i>	54	0.0725
<i>Odontomyia pubescens</i>	29	0.0584	<i>Dialictus</i> spp.	46	0.0617
<i>Bombus rufocinctus</i>	23	0.0463	<i>Eristalis (stipator/dimidiata)</i>	25	0.0336
Mordellidae	13	0.0262	<i>Bombus rufocinctus</i>	24	0.0322
<i>Sphaerophoria (contigua/philanthus)</i>	12	0.0241	<i>Estheria</i> sp. 2	21	0.0282
<i>Bombus vagans</i>	11	0.0221	<i>Estheria</i> sp. 1	19	0.0255
<i>Lasioglossum (leucozonium/zonulum)</i>	10	0.0201	Mordellidae	17	0.0228
<i>Dialictus</i> spp.	9	0.0181	Muscidae sp. 1	17	0.0228
New 2			New 2		
<i>Eristalis (dimidiata/stipator)</i>	122	0.2968	<i>Toxomerus marginatus</i>	188	0.2667
<i>Toxomerus marginatus</i>	67	0.1630	<i>Odontomyia pubescens</i>	94	0.1333
Ulidiidae	43	0.1046	<i>Eristalis (stipator/dimidiata)</i>	85	0.1206
<i>Odontomyia pubescens</i>	30	0.0730	<i>Dialictus</i> spp.	50	0.0709
<i>Dialictus</i> spp.	28	0.0681	Ulidiidae	49	0.0695
<i>Helophilus (hybridus/latifrons)</i>	27	0.0657	<i>Sphaerophoria (contigua/philanthus)</i>	41	0.0582
Tephritidae	10	0.0243	<i>Helophilus (fasciatus/hybridus)</i>	23	0.0326
<i>Sphaerophoria (contigua/philanthus)</i>	8	0.0195	<i>Estheria</i> sp. 1	19	0.0270
<i>Andrena wilkella</i>	5	0.0122	Mordellidae	14	0.0199
<i>Toxomerus geminatus</i>	5	0.0122	Tenthredinidae sp. 1	13	0.0184
Mature 3			Mature 3		
<i>Eristalis (dimidiata/stipator)</i>	173	0.3246	<i>Toxomerus marginatus</i>	226	0.3850
<i>Toxomerus marginatus</i>	78	0.1463	<i>Estheria</i> sp. 1	57	0.0971
<i>Helophilus</i> spp.	58	0.1088	<i>Sphaerophoria (contigua/philanthus)</i>	43	0.0733
Mordellidae	35	0.0657	<i>Odontomyia pubescens</i>	22	0.0375
<i>Estheria</i> sp. 1	24	0.0450	<i>Dialictus</i> spp.	21	0.0358
<i>Toxomerus geminatus</i>	22	0.0413	Mordellidae	17	0.0290
<i>Odontomyia pubescens</i>	13	0.0244	<i>Bombus rufocinctus</i>	12	0.0204
<i>Bombus vagans</i>	11	0.0206	<i>Eristalis (stipator/dimidiata)</i>	11	0.0187
<i>Bombus ternarius</i>	9	0.0169	Muscidae sp. 1	10	0.0170
<i>Hylaeus</i> spp.	8	0.0150	<i>Eupeodes (americanus complex)</i>	9	0.0153
Mature 4			Mature 4		
<i>Eristalis (dimidiata/stipator)</i>	36	0.1865	<i>Toxomerus marginatus</i>	134	0.3206
<i>Helophilus (fasciatus/hybridus)</i>	23	0.1192	<i>Sphaerophoria (contigua/philanthus)</i>	44	0.1053
<i>Toxomerus marginatus</i>	18	0.0933	<i>Odontomyia pubescens</i>	32	0.0766
Muscidae sp. 1	15	0.0777	<i>Dialictus</i> spp.	23	0.0550
Mordellidae sp. 2	12	0.0622	Mordellidae sp. 2	14	0.0335
<i>Sphaerophoria (contigua/philanthus)</i>	12	0.0622	<i>Odontomyia virgo</i>	13	0.0311
<i>Estheria</i> sp. 2	12	0.0622	Muscidae sp. 1	11	0.0263
<i>Bombus borealis</i>	9	0.0466	<i>Lasioglossum (leucozonium/zonulum)</i>	7	0.0167
Culicidae	6	0.0311	<i>Augochlorella aurata</i>	7	0.0167
<i>Odontomyia pubescens</i>	6	0.0311	Coleoptera	7	0.0167
Old 5					
Ulidiidae	68	0.2982			
<i>Nemotelus</i> spp.	58	0.2544			
<i>Toxomerus marginatus</i>	30	0.1316			
<i>Helophilus (latifrons/fasciatus)</i>	18	0.0789			
<i>Eristalis (stipator/dimidiata)</i>	16	0.0702			
Tachinidae sp. 4	8	0.0351			
<i>Nabis</i> spp.	5	0.0219			
<i>Odontomyia virgo</i>	5	0.0219			
Miridae sp. 1	2	0.0088			
<i>Phymata</i> spp.	2	0.0088			
Old 6					
<i>Nemotelus</i> spp.	53	0.6795			
<i>Toxomerus marginatus</i>	28	0.3590			
<i>Estheria</i> sp. 1	17	0.2179			
<i>Sphaerophoria (contigua/philanthus)</i>	13	0.1667			
Ulidiidae	13	0.1667			
<i>Odontomyia virgo</i>	11	0.1410			
<i>Lycaena dorcias</i>	6	0.0769			
<i>Eristalis (stipator/dimidiata)</i>	5	0.0641			
<i>Dialictus</i> spp.	4	0.0513			
Diptera	3	0.0385			

3.3.2 Assessments of patterns of insect activity and abundance

Weather data from within the preserve showed variation between years. Average monthly temperatures were similar between sample years (Fig. 3.4a). The average rainfall from June to September was 83.57 mm in 2010, in contrast to the 8.97 mm measured in 2011 (Fig. 3.4b).

a.



b.

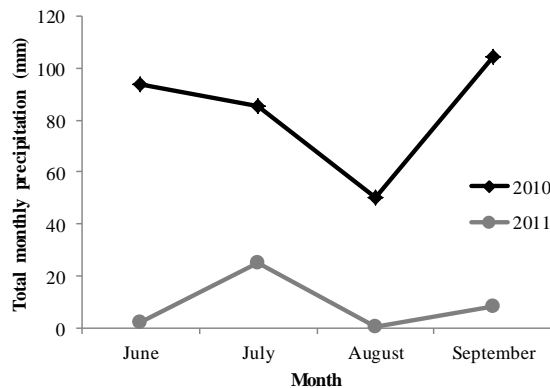


Figure 3.4. Annual climate data from the tall grass prairie preserve weather station, showing average air temperature (a) and precipitation (b).

Pan traps were a useful tool when attempting to determine the proportions of insect visitors. The proportions of syrphids in pan traps increased and decreased by taxa and year. Pan trapped syrphids totaled 387 in 2010 and 226 in 2011. Of these, 62% were in the genus

Toxomerus spp., 18.1% *Helophilus* spp., 6.7% *Eristalis* spp., and 0.8% *Sphaerophoria* spp. in 2010 (Table 3.7). In 2011, 69% were *Toxomerus* spp., with a decline in *Helophilus* spp. and *Eristalis* spp. to 5.8% and 5.3% respectively. *Sphaerophoria* spp. increased to 11.9%.

Bees in pan traps also showed changes in proportions between years. Pan trapped bees totaled 761 in 2010 and 664 in 2011. *Dialictus* spp. accounted for 47.8% of pinned bees, 18.8% *Lasioglossum* spp., 13.9% *Bombus* spp., (Table 3.7). In 2011, only 17.5% of pinned bees were *Dialictus* spp. *Lasioglossum* spp. dropped to 7.7% and *Bombus* spp. increased to 62.2%.

Table 3.7. Absolute values for frequently visiting pan trapped bees and syrphids for all sites. Note that in 2011 suspension of pan traps for the Powshiek Skipperling flight period was extended from one to two sample periods.

		Pan traps				Visits observed			
		# trapped		Proportion (%)		# observed		Proportion (%)	
Syrphidae (Diptera)		2010	2011	2010	2011	2010	2011	2010	2011
Aquatic larvae	<i>Eristalis</i> spp.	26	13	6.7	5.8	491	131	43.4	10.6
	<i>Helophilus</i> spp.	70	12	18.1	5.3	237	40	20.9	3.3
Predatory larvae	<i>Toxomerus</i> spp.	240	156	62	69	314	824	27.7	67.1
	<i>Sphaerophoria</i> spp.	3	27	0.8	11.9	53	182	4.7	14.8
Total		339	208			1095	1177		
Total Syrphidae		387	226			1133	1229		
Hymenoptera									
Underground	<i>Dialictus</i> spp.	364	116	47.8	17.5	51	140	25.1	37.7
	<i>Lasioglossum</i> spp.	143	51	18.8	7.7	24	27	11.8	7.3
Ground surface/ litter	<i>Bombus</i> spp.	106	413	13.9	62.2	90	115	44.3	31
	Total	613	580			165	282		
Total bees		761	664			203	371		

The proportions of insects from observed visits were different for certain taxa when compared to pan traps. Syrphids were typically observed in greater proportions than were captured in pan traps, but for bees the differences in proportions were more variable from year to year (Table 3.7).

The most commonly visiting insects had interesting patterns of visit frequency depending on burn class and year. The two most dominant visiting Orders were Diptera and Hymenoptera, with Diptera making up the most observed visits. A pronounced increase in Dipteran visit rates

was observed on the seventh sampling date (late August), 2010, in new and mature burn classes (Fig. 3.5a, b). On this date, approximately 93% of all visits in the new burn and approximately 77% in the mature burn were by *Eristalis* spp. and *Helophilus* spp. Comparisons by year and burn class showed that *Helophilus* spp. and *Eristalis* spp. averaged approximately 41% of the visits observed in 2010, while only about 7% in 2011 (Table 3.8). Conversely other common syrphid genera, *Toxomerus* spp. and *Sphaerophoria* spp., were responsible for 18% of visits in 2010 and an average of 42% in 2011 (Table 3.8).

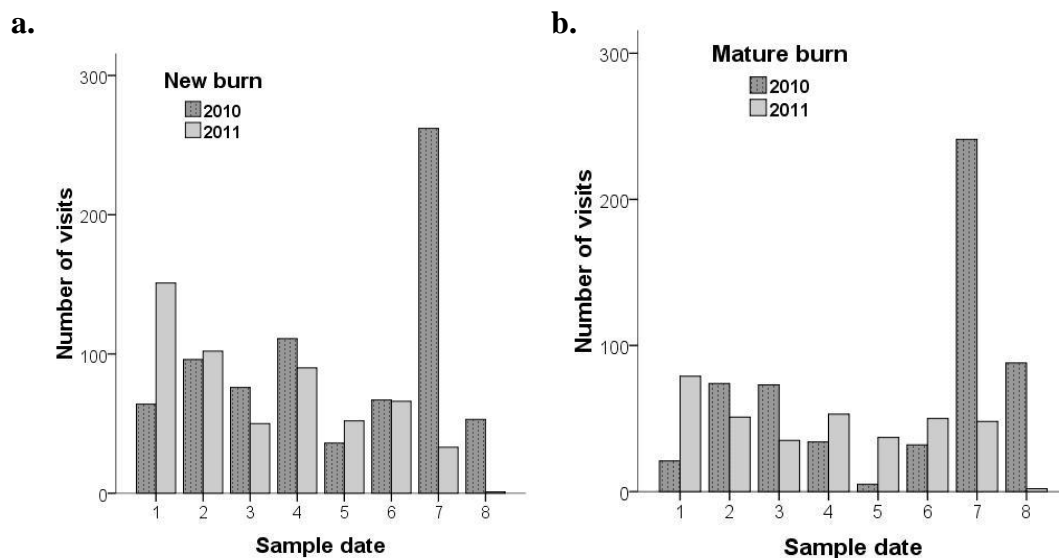


Figure 3.5. Frequency of visits for Diptera in the new (a) and mature (b) burn classes. Visits in 2011 were standardized to visits per hour. Note the peak in activity on Date 7 in 2010.

Table 3.8. Shifts in syrphid activity between years and burn classes. The proportion of visits by Syrphids with aquatic larvae declined in 2011, while syrphids with predatory larvae increased.

Larval guild	Fly genera	2010		2011	
		New	Mature	New	Mature
Aquatic	<i>Eristalis/Helophilus</i>	44%	38%	10%	3%
Predatory	<i>Toxomerus/Sphaerophoria</i>	18%	18%	39%	45%

A peak in Dipteran activity was also observed in the old burn class, but on the sixth sample date (10 Aug 2010). Of the total visits on this date, 95% were due to Diptera, with a small fly in the genus *Nemotelus* spp. (Stratiomyidae) responsible for approximately 64% of the observed visits (data not shown).

Patterns of activity in Hymenoptera were very similar within the new burn class in both years (Fig. 3.11a, b). Early season visits on the second sample date mainly consisted of visits from the Family Halictidae, with *Lasioglossum* spp. and subgenus *Dialictus* averaging 80% of visits on this sample date for both years overall (Table 3.9). On the sixth sampling date in the late season, *Bombus* spp. were responsible for an average of 84% of visits on this date between years overall while halictid visits declined. The shift in dominance by Family was less evident for halictids in the mature burn class in 2010. Early season visits were more comparable between Families (Halictidae: 24%; *Bombus* spp.: 35%). In 2011, visits by halictids declined in the late season (28 to 3%), while *Bombus* spp. increased (13 to 81%) (Table 3.9).

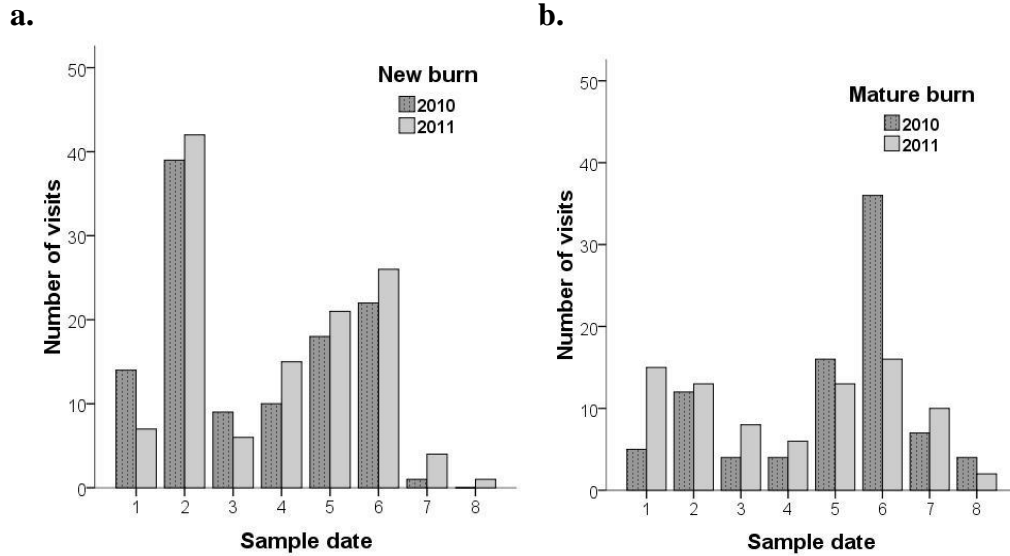


Figure 3.6. Frequency of visits for Hymenoptera in the new (a) and mature (b) burn classes. Visits in 2011 were standardized to visits per hour.

Table 3.9. Evidence of a shift in the proportion of bee visits by halictid bees and *Bombus* spp. within season and burn class. Shifts were observed between the early and late sample season.

		2010				2011			
		Early peak		Late peak		Early peak		Late peak	
New burn		by family		by family		by family		by family	
Halictidae	<i>Dialictus</i> spp.	49%	75%	No obs	No obs	64%	84%	8%	8%
	<i>Lasioglossum</i> spp.	26%		No obs		20%		No obs	
Apidae	<i>Bombus</i> spp.	3%		91%		5%		76%	
Mature burn		Early peak		Late peak		Early peak		Late peak	
Halictidae	<i>Dialictus</i> spp.	24%	24%	No obs	19%	25%	28%	3%	3%
	<i>Lasioglossum</i> spp.	No obs		19%		3%		No obs	
Apidae	<i>Bombus</i> spp.	35%		44%		13%		81%	

Repeated measures ANOVA showed no significant difference in peak dates of activity for the insects selected (2010: $F_{1,12}=0$, $p>0.95$; 2011: $F_{1,15}=1.23$, $p>0.28$). There was no

difference in the frequency of visits by these species between burns in 2010 ($F_{1,12}=1.69$, $p>0.21$), but frequency was significantly greater in the new burn in 2011 ($F_{1,15}=4.85$, $p<0.05$).

PCA showed that site and year associations varied between taxa. Bee groups that included stem nesters, cavity nesters, and *Bombus* spp. that nested just below or at the ground surface were more closely associated with mature sites in 2010, as were parasitoid flies (Fig. 3.7a). Small plant feeding flies in the families Ulidiidae and Tephritidae, a cleptoparasitic bee, and bees that nested deep below the ground surface were more associated with the new burn sites. In 2011, *Bombus* spp. was associated with New 1, particularly the bumble bees that nested close to the ground surface, while *Bombus* spp. that nested below ground did not have any noticeable site associations (Fig. 3.7b). Cavity and stem nesting bees remained as frequent visitors in the mature sites, and below ground bees (solitary bees) continued to be most prominent in new burns.

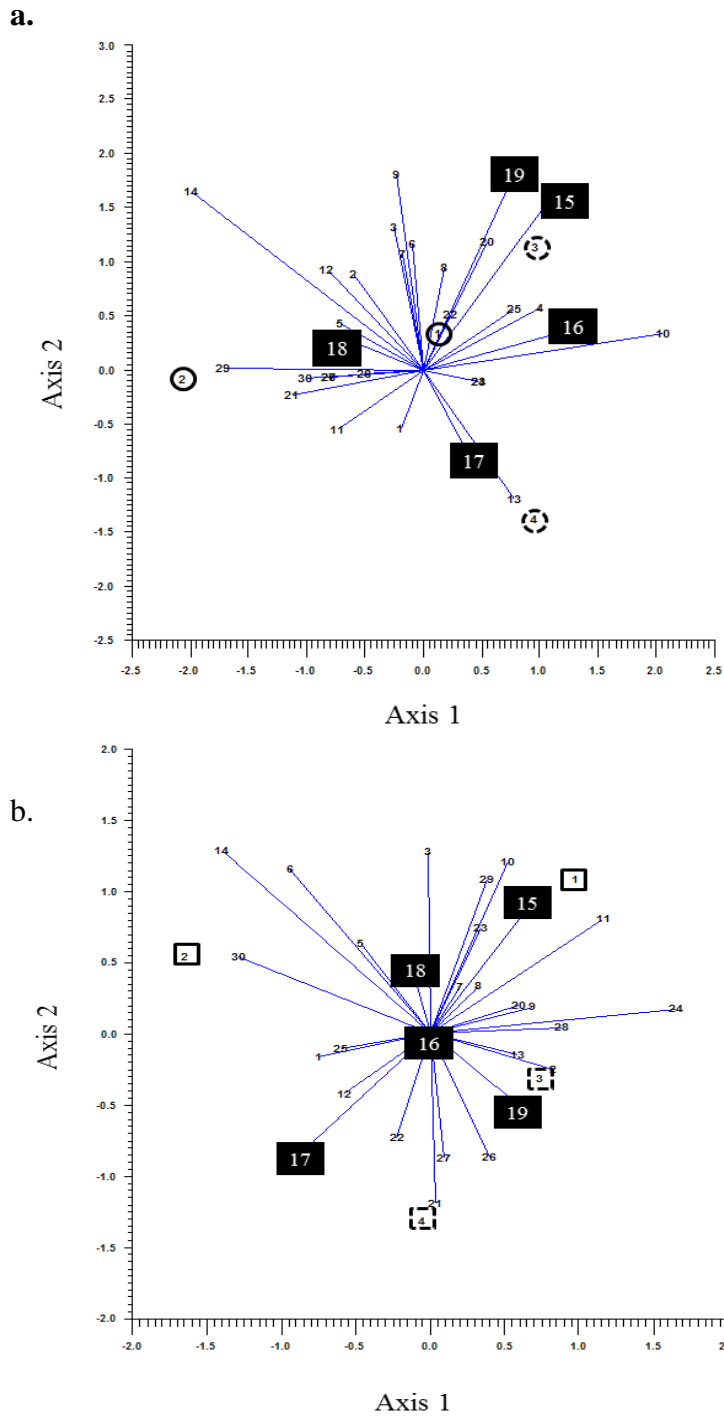


Figure 3.7. PCA of insects observed in 2010 **(a)** and 2011 **(b)**, with emphasis on bee guilds (Appendix 3.2). Solid borders contain sites from the new burn and broken borders contain sites from the mature burn. Guilds highlighted: *Bombus* spp., ground surface (15), *Bombus* spp., underground (16), bees and wasps, cavity nesting (17), solitary bees, underground (18), solitary bees, stems (19). 2010: Axis 1 54.45%, Axis 2 29.55%. 2011: Axis 1 45.54%, Axis 2 35.60%.

Site associations differed between years for certain insect taxa (Fig. 3.8). Flies with aquatic larvae, primarily Syrphidae and some Culicidae, were heavily observed in New 1 in 2010. Syrphidae with predatory larve were most associated with 2011 and New 2. Butterflies and moths were strongly associated with 2011.

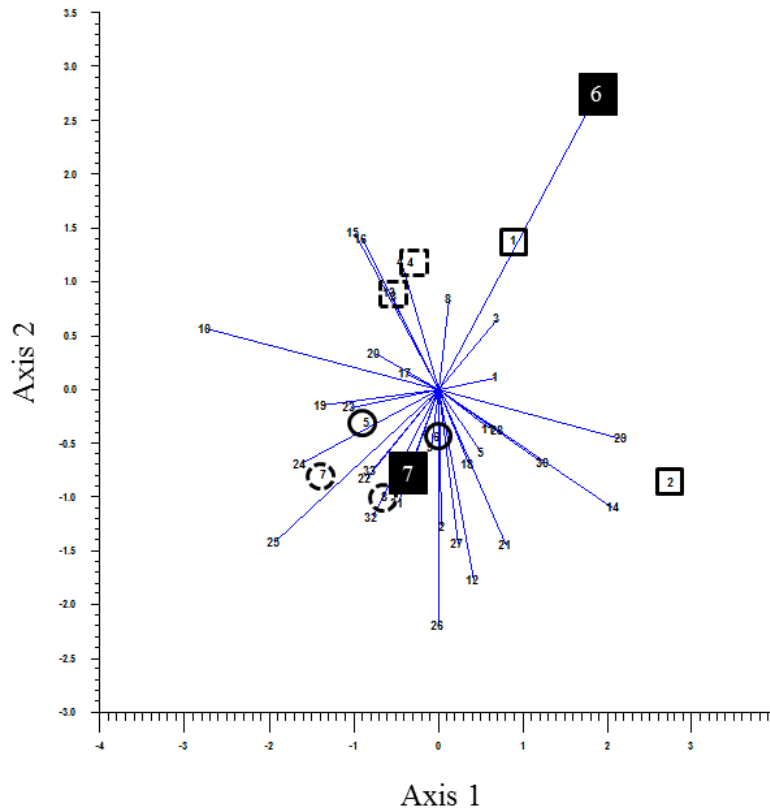


Figure 3.8. PCA of insect guilds for both years, with emphasis on the predatory and aquatic syrphids. Sites in squares were sampled in 2010 and sites in circles were sampled in 2011. Solid borders contain sites from the new burn and broken borders contain sites from the mature burn. Syrphids with aquatic larvae (6) and predatory larvae (7) are highlighted. Axis 1 34.04%, Axis 2 19.46%. Guilds are found in Appendix 3.3.

3.4 Discussion

3.4.1. Evidence for insect community resilience following disturbance

Rarefaction suggested that new burn sites had similar numbers of species in both sample years, with the mature sites being more variable in the number of insect species present annually. In both years, rarefaction curves did not reach an asymptote, suggesting that these sites would have increased in species detected with increased sampling. Mature 4 showed the greatest differences, with more species present with fewer individuals in both years. It may be surprising that mature 4 had the highest number of insect species for a given number of individuals, considering that this site was lower in open blooms and flowering plant diversity (see Chapter 2). However, higher species richness does not always dictate a high quality community. Sites that quickly rarify with fewer individuals can reveal low quality in terms of species richness: a community with few species will result in a more inclusive census of these species with less effort (Gotelli and Colwell 2001). This was likely the case with mature 4 and the old burns.

Simpson's Diversity and Berger-Parker dominance did not show an effect of burn class, and showed little difference in diversity between sites, even when upland prairie was compared with old sedge meadow habitat. These similarities in species composition and dominance were also evident when the top 10 visiting insect species were compared. The pattern of relative abundance (ie. the distribution of the most dominant species), was fairly consistent, and often included the same species in a slightly different ranking within years.

Previous studies of pollinator communities have shown declines in insect species with increasing site age following fire (Liow et al. 2001, Potts et al. 2003, Moretti et al. 2009), yet this trend was not obvious in this study. In forest habitats, for example, fire initially increased floral

resources, which declined with site age and succession (Liow et al. 2001, Potts et al. 2003, Moretti et al. 2009). Tall grass prairie may also see declines in floral resources, as accumulation of litter and dense grasses can outcompete forbs (see Chapter 2). However, fire frequency differs between forests and prairie. Historically, prairies may have experienced annual fires, far more frequent than decades-long fire cycles in some forests (Dickman and Cleland 2002). In habitats where fires are frequent, communities become adapted to disturbance through both resilience of species to fire and taking advantage of pollinator redundancies within ecosystems (Bascompte et al. 2003, Memmott et al. 2004, Moretti et al. 2009), meaning that changes in species composition might be difficult to detect in the short-term. Also, the fire particular to this study did not consume all available habitat within the preserve, so it is probable that surrounding unburned areas allowed for recolonization of species that were present in the pre-fire community.

Diversity and most common visitor species were similar between sites, but the insect species shared within burn classes changed between years. In 2010, sites in the new burn shared more species than sites in the mature burn, but in 2011 all upland sites were more comparable, as shown by the Sørensen index. This could also be an effect of sample effort, as observation times were doubled in 2011. While the number of visits could be standardized to visits per hour, the number of species encountered may be the result of an increased probability of observation with more time in the field. This could explain why Mature 4 shared few insect species with other sites in 2010: this site had low floral abundance, lowering the potential for observing visits with reduced observation time. The Sørensen index did, however, clearly show the differences in shared species between old sedge meadow sites and upland prairie.

The species shared in the same site between years were lower than those shared within year and burn class. Recall that the flowering community was similar between years due to the

dominance of perennial, fire adapted species (see Chapter 2). Insects, by comparison, are mobile and will exhibit variations in learned or species-specific behaviors related to floral preferences (Michener 2007). Therefore, while the plant community may be similar between years due to the presence of perennial species, the floral preferences and ranges of insects may differ between years. In addition, there was evidence of annual variation in insect visits due to environmental factors such as precipitation, meaning that the insects present within a given site may not be shared between years due to natural fluctuations in abundance.

The influence of climate on insect visits could be used to explain many of the trends in visit frequency. For example, Lepidopterans displayed annual differences in occurrence as shown by PCA. The majority of butterfly observations were made in the 2011 season, which had lower monthly precipitation. Butterflies and moths are sensitive to cool temperatures and rain which leads to decreased activity (R. Westwood, University of Winnipeg, pers. comm.). This likely explained the low number of observations of Lepidoptera in 2010, and this was supported by other butterfly surveys undertaken within the preserve during that year (R. Westwood, University of Winnipeg, pers. comm.). Annual differences in rainfall probably accounted for the changes in syrphid dominance between years. Syrphids such as *Eristalis* spp. and *Helophilus* spp. have aquatic larvae that would have benefited from increased soil moisture, pooling water, and the decaying material within pools as a food source (Vockeroth and Thompson 1987). The drier conditions in 2011 reduced the appropriate larval habitat for these genera, leading to lower rates of observation. The increased proportion of visits from *Toxomerus* spp. and *Sphaerophoria* spp. in 2011 may be due in part to the drier conditions influencing their prey. These two genera have predaceous larvae that feed on aphids (Vockeroth and Thompson 1987, Gilbert 1993). It was observed that aphids appeared to be in higher numbers in 2011, especially on *Solidago*

rigida L., though counts of aphids were not recorded. Drought stress can increase damage by mites and chewing insects (English-Leob 1990, Louda and Collinge 1992), though aphids often decline with low water availability (Sumner et al. 1986, Morris 1992). However, during seasons of drought greater numbers of aphids may take advantage of plants with weakened defenses (D. Gillespie, Agriculture Canada, pers. comm.). Surveyors have also observed aphid population booms in certain summers (B. Lamb, Agriculture and Agri-Foods Canada, pers. comm.). Annual fluctuations in climate, then, must play an important role in the structure of communities, especially where syrphid flies are key visiting species.

3.4.2 Evidence of species specific responses to disturbance

Fire did appear to have some short term effects on insect species, particularly for bees. The similarities in peak activity in the new burns between years were striking, with a dominance of halictid visits (the majority being from the subgenus *Dialictus* early in the season) and a shift to *Bombus* spp. activity later in the summer. The results of PCA also showed some associations between bees and burn age, with deep ground nesters (mainly halictids) having greater presence in newly burned sites, and ground surface bees (mainly bumble bees) more likely to be observed in mature sites. For both families of bees, spring started with an overwintered mated queen ready to work to produce that season's brood (O'Toole and Raw 2004). But the nesting behavior was different, and this could have contributed to the different associations with burn age.

Nest location was likely an important factor in determining bee visits in burned sites. In the case of halictids, nest depth can range from 6 to 70 cm below the ground (Roberts 1973, Packer 1994, O'Toole and Raw 2004), though this depth may vary by species, soil moisture, and the frost line in the winter (Packer 2010). In comparison, *Bombus* spp. will either take up

residence in abandoned rodent dens or will nest in the litter close to the ground surface, preferably in undisturbed habitat (Wilson 1971, O'Toole and Raw 2004). This would make *Bombus* spp. more susceptible to mortality from fire than other bee species protected deeper below ground. Prairie fires move quickly over the soil surface, burning the litter layer as well as elevating the soil surface (<5 cm) temperature to 50-80°C (DeBano et al. 1998). For *Bombus* spp. then, early season visits in the new burns could be low due to fire mortality and the slow re-colonization of the habitat with queens from surrounding areas. The early season peaks in *Dialictus* spp. may be the combination of the avoidance of mortality by fire due to nest depth, and possibly the abundance of bare ground in burned sites which would be enticing to queens looking for nesting areas in spring (Klein et al. 2002). Increased litter, as in the mature sites, might act as a deterrent for the establishment of underground colonies due to the lack of open ground, resulting in the reduced proportion of *Dialictus* in those sites. At the same time, the litter in mature burns would provide suitable habitat to surface nesting *Bombus* spp. The link between life history and mortality from disturbance has been investigated in the past with similar results: social bee species (such as bumble bees) that nested at the ground surface were more likely to suffer significant losses following agricultural disturbances (Williams et al. 2010).

The degree of sociality in bee species might be an indicator of how they will respond to disturbance. There is evidence that a local species of bee *Lasioglossum (Dialictus) tenax* is solitary rather than social. Packer (1994) found that this species peaked in activity in early summer, declined in July, had a foundress that raised a brood without the help of workers, and produced offspring with a 1:1 ratio of males to females. All of those traits were reminiscent of a solitary species (Packer 1994). If this was the case for some of the dialictid species encountered in the preserve, this would explain the early season peak and late season decline. It would be

important for these foundress bees to take advantage of early rewarding flowering species so that they could produce the most offspring in a limited timeframe. Since *Bombus* spp. is eusocial, colonies would be increasing through the season, which would account for the late season peak.

Nest location and fire sensitivity would also explain the site associations for cavity and stem nesting bees. Principle components analysis showed that cavity and stem nesting species were observed in mature sites in 2010 and in new sites in 2011. Habitat with extended fire free periods would have mature vegetation and deadfall that would be ideal for bees seeking pithy stems or wood. However, bees that nest in deadfall or require plant remains have been associated with newly burned habitat by Moretti et al. (2009), but this was only the case when fire was low-intensity, as opposed to locations where intense fires destroyed this material. This suggests that the fire considered in this study was of a high enough intensity to influence bee guilds based on nesting preference.

Although the explanations of site preference are plausible, the trends were not always strong. The abundance of visits for halictids and *Bombus* spp. were typically high, making assumptions about these insects more reliable. But, in the case of stem nesting bees, the number of bees observed in Mature 3 in 2010 (Appendix 3.5) could have been due to close proximity to a nest or preferred flowering plant, leading to the potentially false conclusion that time since burn was responsible. Observed visits of cavity nesters were absent from two of four sites in 2010, one from each burn class. This was likely more of an issue of rarity or low abundance than one of burn effect. There were also a large number of insects that were not discussed in this chapter that might offer more explanations with further investigation. For example, the parasitoid fly *Estheria* spp. was a common visitor in the mature sites in 2010 and most sites in 2011. Was this a response to host availability following fire? Picture-winged flies (Ulidiidae) were most

frequently observed in New 2 regardless of year. Was there an important floral association or larval habitat being overlooked? Due to the complexity of interactions in this community, not all could be explored in this study, but further investigation would surely offer more explanations for site associations.

There may also be year to year influences on insect dynamics that go beyond the short term effects of disturbance. Crone (2013) showed that the quantity of floral resources in the previous or current year affected bee abundance based on social behavior. Social bees produced colonies that increased in size over one season, so the abundance of social bees was greater in years with increased flowering (Crone 2013). Solitary bees that provisioned offspring for emergence in the following season, rather than forming an annual colony, increased in numbers in the year following a season of floral abundance (Crone 2013). Prior to the fire in 2009, New 1 had burned in 2002, and New 2 had burned in 2008. Perhaps the frequency of fire in these sites maintained a greater proportion of solitary bees as a result of the past abundance of floral resources. However, the mature sites in this study would share a similar history so should also show these trends. The mature sites also had differing land uses and traits, with Mature 3 being sporadically grazed by cattle and Mature 4 having higher soil moisture content (C. Borkowsky, Tall Grass Prairie Preserve, pers. comm.). Grazing by cattle increases soil compaction and decreases soil moisture due to erosion and runoff (Fleischner 1993). For solitary bees that dig below ground, dry, compressed soil may be a difficult nesting medium (Potts and Willmer 2003). At the same time, overly moist soil could also deter bees from excavating nests.

3.5 Conclusions

Attempting to explain the patterns in insect visits between sites and burn classes was a challenge. Measures of species richness and diversity were likely too coarse a scale for detecting a burn effect, as this method considered trends in community composition and not factors such as species specific responses to environment. Patterns in insect abundance and activity became clearer as the influences of larval habitat and climate were considered. If insects that pollinated flowers required a thick litter layer in which to nest, for example, it would be logical that the presence of this species in a burned site would be affected. This effect was detected in some sites: bees that nested below the ground were more frequently observed in newer sites, while bumble bees that nested in the duff were more associated with mature sites. Other insect groups, such as the syrphids, appeared to be more influenced by standing water than by fire.

It was interesting that there were no differences in phenology and abundance for insects by burn class as was detected for plants in Chapter 2. Commonly visiting insects appeared to have season-long flight periods with similar peaks in activity within years. The assumption that frequency of visits by burn class would differ due to mortality as was hypothesized in my objectives did not appear to be the case in this study.

Given that the insect community was influenced in some ways by climate and disturbance, it is possible that the interactions between insects and flowering plants might differ between sites and years. Investigation of plant-pollinator interactions at the network scale could reveal how well the community withstands variations in environmental conditions as well as the impacts of disturbance.

APPENDICES

Appendix 3.1. Sampling hours for pan traps in 2010 and 2011. There was an additional sample date in early May in 2010.

2010	Sample date									Total hours per site
	1	2	3	4	5*	6	7	8	9	
# bowls	30	30	30	30	30	30	30	30	30	
New 1	6.50	7.83	8.83	7.42		6.33	6.92	7.58	7.08	58.50
New 2	6.25	8.33	7.42	7.83	6.33	6.50	7.00	7.68	7.27	64.62
Mature 3	5.75	8.75	7.25	7.50	6.08	6.58	7.25	7.75	7.28	64.20
Mature 4	6.25	9.17	7.17	7.50		6.17	7.50	7.58	6.92	58.25
Old 5		7.83		7.33		9.25		7.33		31.75
Old 6		7.25		7.25		9.17		7.33		31.00
Avg hours per day	6.19	8.19	7.67	7.47	6.21	7.33	7.17	7.54	7.14	308.32
										Total hours for season
										Avg per day for season
										7.21

2011	Sample date								Total hours per site
	1	2	3	4*	5	6	7	8	
# bowls	15	30	15		15	30	15	30	
New 1	7.83	8.38			7.07	8.43	8.42	7.33	47.47
New 2	7.67	7.90	7.67		7.17	8.32	8.10	8.42	55.23
Mature 3	7.50	7.50	7.00		7.33	7.47	8.17	7.80	52.77
Mature 4	7.20	7.20			7.17	7.33	7.80	8.62	45.32
Avg hours per day	7.55	7.75	7.33		7.18	7.89	8.12	8.04	200.78
									Total hours for season
									Avg per day for season
									7.69

*Poweshiek Skipperling flight period

Appendix 3.2. Insect guilds used for PCA of site associations by site and year. Members of each guild, explanations of groupings, and references are shown. Numbers correspond to PCA variables.

PCA#	Guild/Family	Members	Explanation	References
1	Coleopteran plant feeders	<i>Diabrotica</i> (Chrysomelidae), Curculionidae	Leaf beetles, seed borers, crop pests	1
2	Coleopteran predators	Cantharidae, Coccinellidae, Lampyridae	Flower visiting beetles with predatory adults and/or larvae	1
3	Elateridae	Click beetles	Adults feed on plant tissue, larvae habits variable	1
4	Mordellidae	Tumbling flower beetles	Plant feeders, common on flowers though probably not substantial pollinators	1
5	Aquatic larvae: Stratiomyidae	<i>Odontomyia</i> , <i>Stratiomys</i>	Larvae require standing water or high moisture, includes species with specific flight periods	2
6	Aquatic larvae: Syrphidae and Culicidae (2010)	<i>Eristalis</i> , <i>Tropidia</i> , <i>Helophilus</i> , Culicidae <i>Eupeodes</i> , <i>Heringia</i> , <i>Melanostoma</i> , <i>Sphaerophoria</i> , <i>Syrphus</i> ,	Larvae require standing water or high moisture, present most of season	1, 3, 4
7	Predatory larvae: Syrphidae	<i>Toxomerus</i> , <i>Ocyptamus</i> , <i>Platycheirus</i> , <i>Paragus</i>	Larvae feed on soft bodied insects, usually aphids	3, 4, 5, 6
8	Parasitic flies	<i>Archytas</i> , <i>Siphona</i> , <i>Cylindromyia</i> , <i>Peleteria</i> , <i>Poecilanthrax</i> , <i>Villa</i>	Various flower visitors, larvae parasitic to Arthropoda	7, 8
9	Saprophagous: Syrphidae and Strats	<i>Nemotelus</i> , <i>Syrpita</i> , <i>Spilomyia</i> , <i>Ceriana</i>	Larvae feed on decaying material, adults floral visitors	2, 5
10	<i>Estheria</i> spp.	Heavily visiting genus in Tachinidae	Parasites of arthropods, a frequently encountered Tachinid genus	7
11	Anthomyiidae	Anthomyiid flies	Larvae feed on plant tissues	1, 9
12	Calliphoridae	<i>Lucilia</i> , <i>Cynomya</i> , <i>Pollenia</i> , Calliphorids obs to family	Adults visit flowers, larvae feed on living/dead vertebrate tissue.	10
13	Muscidae	Muscid flies	Saprophagous larvae, adults were floral visitors	11
14	Ulidiidae	Picture winged flies	Probably all saprophagous larvae	12
15	Under/ground surface: <i>Bombus</i> spp.	<i>rufocinctus</i> , <i>vagans</i> , <i>giseocolis</i> , <i>Bombus</i> spp.	<i>Bombus</i> that may nest at the ground surface or slightly below ground, includes obs only made to genus	13, 14,
16	Underground: <i>Bombus</i> spp.	<i>borealis</i> , <i>ternarius</i> <i>Ancistrocerus</i> , <i>Apis</i> , Megachilidae, <i>Polistes</i> , <i>Hoplitis</i> ,	<i>Bombus</i> that nest underground	13, 14
17	Cavities	<i>Parancistrocerus</i> , Sphecidae <i>Andrena</i> , <i>Augochlorella</i> , Crabronidae, <i>Dialictus</i> , <i>Dufourea</i> ,	Bees that nest in cavities	13, 15, 16, 17
18	Underground	<i>Lasiosglossum</i> , Halictid/Anrenid, <i>M. perihirta</i> , <i>Melissodes</i>	Bees and wasps that nest deep underground	1, 13, 16, 18, 19
19	Stems	<i>Hylaeus</i> , <i>Ceratina</i>	Bees that nest in pithy stems	13, 16
20	Parasitic wasp	Unidentified parasitic Hymenoptera, Ichneumonidae	Observed probing florets with ovipositor	20
21	Hemipteran plant feeders	Cicadellidae, Cercopidae, <i>Adelphocoris</i> , <i>Lygus</i> , Miridae, Rhyparochromidae, Thyreocoridae	Plant feeders, either leaf tissues or seeds	1
22	Hemipteran predators	<i>Phymata</i> , <i>Nabis</i>	Predatory, intercept flower visitors on open blooms or leaves	1, 21
23	Hesperiidae	Hesperiidae, <i>Oarisma</i> , <i>Erynnis</i> , <i>Euphyes</i> , <i>Polites</i> , <i>Thymelicus</i> <i>Lycaena dorcas</i> , <i>Phyciodes morpheus</i> , <i>Phyciodes cocyta</i> ,	Skippers, small butterflies with larvae at base of grasses	22
24	Small Butterfly 19-38mm	<i>Phyciodes batesii</i> <i>Colias philodice</i> , <i>Boloria selene</i> , <i>Pieris rapae</i> , <i>Colias</i> spp.,	Size category: may determine dispersal	22
25	Med Butterfly 32-54mm	<i>Pontia occidentalis</i> , <i>Boloria bellona</i> , <i>Boloria</i> spp. <i>Danaus plexippus</i> , <i>Speyeria aphrodite</i> , <i>Papilio polyxenes</i> ,	Size category: may determine dispersal	22
26	Large Butterfly 51-105mm	<i>Limnitis archippus</i> , <i>Speyeria cybele</i>	Size category: may determine dispersal	22
27	Med Moth 28-38mm	Moth sp. 1, Moth sp. 3, <i>Cisseps fulvicollis</i> , Moth sp. 2	Size category: may determine dispersal	23
28	Chrysopidae	Chrysopid larvae	Predatory larvae, mainly feed on soft-bodied insects	1
29	Tephritidae	Fruit flies	Feed on plant tissue	24
30	Cleptoparasite	<i>Sphecodes</i>	Hosts are Halictid bees	16, 19
31	Tenthredinidae	Sawflies	Plant tissue, seemed to be consuming pollen and/or nectar	1
32	Microlepidoptera	Moths < 1 cm	Size category: may determine dispersal	NA
33	Small Moth 15-24mm	<i>Thyris sepulchralis</i> , <i>Pterophoridae</i> (cf. <i>Geina</i> sp.), <i>Schinia lucens</i>	Size category: may determine dispersal	NA

Guild placement based on information from ¹Triplehorn and Johnson 2005, ²James 1981, ³Vockeroth and Thompson 1987, ⁴Vockeroth 1992, ⁵Thompson 2011, ⁶Gilbert 1993, ⁷Wood 1987, ⁸Hall 1981, ⁹Huckett 1987, ¹⁰Marshall et al. 2011, ¹¹Huckett and Vockeroth 1987, ¹²Steyskal 1987, ¹³Richards et al. 2011, ¹⁴Colla et al. 2010, ¹⁵Buck et al. 2008, ¹⁶Sheffield et al. 2013, ¹⁷Bartlett et al. 2004, ¹⁸Sheffield et al. 2011, ¹⁹Packer et al. 2007, ²⁰Bartlett et al. 2004a, ²¹Eiseman et al. 2009, ²²Layberry et al. 1998, ²³Coin et al. 2004, ²⁴Foote and Steyskal 1987

Appendix 3.3. Insect observations used for PCA. Observed visits were doubled in 2010 to standardize data. Data was log(n+1) transformed.

Guild/Family	2010					2011			
	PCA #	New 1	New 2	Mature 3	Mature 4	New 1	New 2	Mature 3	Mature 4
Coleopteran plant feeders	1	0	4	2	4	0	2	0	1
Coleopteran predators	2	2	6	4	0	8	1	4	7
Elateridae	3	8	4	6	0	5	3	1	0
Mordellidae	4	26	10	70	32	17	15	17	17
Aquatic larvae: Stratiomyidae	5	62	66	26	18	82	100	24	46
Aquatic larvae: Syrphidae and Culicidae	6	508	306	484	136	29	113	22	9
Predatory larvae	7	170	160	226	64	337	236	285	186
Parasitic flies	8	16	6	14	4	7	4	7	3
Saprophagous	9	8	6	14	0	11	3	9	5
Estheria spp.	10	2	0	56	24	40	23	60	4
Anthomyiidae	11	2	6	0	2	11	0	1	1
Calliphoridae	12	0	10	6	0	1	6	4	4
Muscidae	13	18	4	6	36	17	5	10	15
Ulidiidae	14	12	86	12	0	7	49	3	2
Bombus-under/ground surface	15	68	2	36	6	39	11	23	6
Bombus-underground	16	14	2	30	18	6	9	15	6
Cavities	17	2	0	0	4	0	4	1	5
Underground	18	42	78	34	24	77	69	38	44
Stems	19	4	0	16	0	6	2	7	9
Parasitic wasp	20	0	0	8	0	1	0	2	0
Hemipteran plant feeders	21	0	16	2	2	0	1	5	4
Hemipteran predators	22	0	0	2	0	0	2	5	2
Hesperiidae	23	0	0	2	2	6	2	2	1
Small Butterfly 19-38mm	24	0	0	2	2	13	0	8	3
Med Butterfly 32-54mm	25	0	0	8	2	8	23	10	14
Large Butterfly 51-105mm	26	0	4	0	0	2	1	4	7
Med Moth 28-38mm	27	0	4	0	0	5	2	3	0
Chrysopidae	28	0	2	0	0	0	0	0	0
Tephritidae	29	2	20	0	0	0	0	0	0
Cleptoparasite	30	0	6	0	0	0	0	0	0
Tenthredinidae	31	0	0	0	0	1	13	1	1
Microlepidoptera	32	0	0	0	0	0	1	6	2
Small Moth 15-24mm	33	0	0	0	0	1	0	7	0

4. THE STRUCTURE OF POLLINATION NETWORKS

4.0 Chapter summary

Interactions that take place between plants and insects can be summarized by pollination networks. Binary metrics, such as nestedness and connectance, can be used to understand the structure and stability of a network. However, it is important to also examine the driving forces of network assembly, or “building blocks”, that influence how and why interactions occur. I assessed species composition and abundance, phenology, and trait matching to help understand network structure in sites of differing burn ages. I also assessed pollen loads to determine the quality of plant-insect interactions. I found indications that network building blocks were important determinants of structure, yet no single building block fully explained how each network was structured on its own. Instead, the combination of building blocks contributed to interactions, with the networks showing significant nestedness as a result of high generalization. Additionally, while assessing pollination networks in their entirety can provide information about structure and stability, I found that assessing the impact of individual visits through interaction strength and pollen loads offered greater insight into the importance of particular interactions. This is important from a conservation perspective, as some interactions might require more protection or consideration during management activities.

4.1 Introduction

Ecosystems are assembled and maintained by interactions among organisms and interactions between organisms and their environment (Schowalter 2000). Understanding these interactions, and how they maintain the structure and stability of a given community, has become

an important part of pollination biology. The use of pollination networks, or summaries of the interactions that take place between a plant and a pollinator, examine the relationships between insects and flowering plants at the community level. Pollination network ecology borrowed from previous studies of food webs (Jordano 1987, Memmott 1999), which sought to understand how species contribute to web integrity (see Solé and Montoya 2001, Dunne et al. 2002). Now, the resilience of a pollination community can be measured in part by matrix-based assessments of plant-insect interactions.

Binary metrics are commonly used when assessing the presence or absence of interactions in a matrix. Connectance and nestedness are two such metrics. Connectance reveals the proportion of realized interactions, or links, that occur between the insect and plant species observed in the community (Dupont et al. 2009). A nested structure results from a strongly interacting core of generalist species (Bascompte et al 2003). Specialist species interact with these generalists, resulting in specialists that are “nested” within the generalist core (Jordano et al. 2006).

Communities that are significantly nested are believed to be more resistant to disturbance and extinction events by providing redundancies in links through highly interacting generalists (Bascompte et al. 2003, Memmott et al. 2004). Determining how a structure is established can help us understand how nested communities are maintained following disturbance. Interactions are dependent on several structural constraints; the “building blocks” of a community that dictate the occurrence of interactions. As reviewed by Vázquez et al. (2009), interactions may be driven by a combination of factors including species composition, abundance, phenology, and trait-matching.

Species composition refers to the identity of species in the community that are available for interactions. Insect species composition can vary between locations or seasons, as can their preferences for certain flowers (Petanidou et al. 2008). Abundance refers to the number of individuals in the community. The probability of interaction is expected to be higher for abundant plants and insects than for species that are rare (Vázquez et al. 2009). Phenology is important, because interacting species must have overlapping life-cycles in order for an interaction to occur (Olesen et al. 2008). Non-overlapping species are a major contributor to “forbidden links”, i.e., links that cannot occur due to biological constraints (Olesen et al. 2011). Lastly, trait-matching can contribute to the probability of interactions by limiting access to floral rewards (ex. a long corolla tubed flower and an insect with short mouthparts), or by pollinator preferences for certain traits, such as floral colour (McCall and Primack 1992, Vázquez et al. 2009).

While understanding the influence of these building-blocks on community structure is important, recent studies have also begun to consider the quality of individual interactions. There is increasing evidence that not all plant-pollinator interactions are mutualistic, and that previous studies that relied on only the presence or absence of interactions might overlook the impacts of insect visitors on plant fitness (see Alarcón 2010, Popic et al. 2013). A greater depth of understanding can be achieved by considering interaction strength and pollen loads on insects.

Interaction strength considers the influence an insect species might have on a plant, and vice versa. For example, a generalist plant species that receives many insect visits may not rely heavily on a particular infrequent visitor (low interaction strength), but if that insect visitor was the only visitor to another species, the interaction strength for the second plant species would be high (Vázquez et al. 2007). This can also be defined as the degree of dependence one species has

on another (Bascompte et al. 2006, Vázquez et al. 2009). Calculations of interaction strength rely on observations of interaction frequency (Blüthen 2010), and previous studies have linked floral fitness to both the number of visits made by a pollinator, and the effectiveness of that pollinator in terms of pollen deposition (Vázquez et al. 2005, reviewed by Ne'eman et al. 2009).

Sampling the pollen loads carried on the body of an insect can provide additional insight into the impact of a particular visit. Not all insects that land on a flower will contribute to fertilization (Alarcon 2010, Ne'eman et al. 2009). Quantifying pollen loads can help determine which insects have the potential to be the most the effective pollinators, giving a better estimate of their importance to the flowering community (Forup and Memmott 2005, Alarcon et al 2010) and revealing the importance of pollinator identity (King et al. 2013, Popic et al. 2013).

In my previous chapters, I've demonstrated how fire and climate can have an effect on certain aspects of the community. Flowering phenology was significantly earlier in new burns in the first year (Chapter 2) which could be reflected by the uncoupling of plant-insect interactions by burn age. A greater abundance of flowering plants in the season following fire (Chapter 2) could also attract a greater variety of pollinators from surrounding refugia. The occurrence of certain insect taxa related to nesting biology and fire mortality might determine the identity of interacting partners due to burn history (Chapter 3). Annual variation in precipitation increased the abundance of syrphids whose larvae developed in aquatic habitats (Chapter 3). If these changes have a negative effect on interactions in the community, this could decrease nestedness. If the community is adapted to these changes from disturbance or climate, then the community should be nested and stable despite burn age or year.

Chapter 4 places emphasis on my second thesis objective:

- 1) I was to assess pollination networks using binary metrics. If newly burned sites experienced increased interactions due to greater floral resources over the length of the season, I expected that that connectance would be lowest in those sites. I also expected that temperature would be high (low nestedness) in recently burned sites, as interactions could be uncoupled by changes in flowering phenology and the mortality of certain pollinator guilds. The frequency of visits from frequently visiting pollinating flies differed with annual precipitation, so I expected to see a difference in nestedness values between years.
- 2) I assessed structural elements, or building-blocks, of plant-pollinator networks by site and burn class to see if burn age may change their contributions to network structure. This was followed by assessing pollinator quality through pollen loads, as this would provide greater insight into the impact of particular interactions. I investigated the following assumptions:
 - a. I expected that the most abundant flowers would receive the greatest number of visits. If recently burned sites have higher floral abundance, I should detect a greater number of visits in those sites.
 - b. I expected that changes in flowering phenology by burn class would influence interacting partners through phenological uncoupling. This could be indicated by differences in interacting partners between burn classes and years.
 - c. I expected that insects would exhibit preferences in floral colour. If consistent, preferences should be similar between counts in pan traps and observed visits and could determine the likelihood of interaction between species.

- d. I expected that pollen loads would differ by taxa, and that frequently visiting species would be some of the most high quality pollinators.

My third objective is to assess temporal variation in plant and pollinator diversity.

Pollination network structure is expected to exhibit stability across our sample seasons, while temporal differences in links between pollinator taxa related to the presence of particular flowering species are expected to vary with burn age.

4.2 Materials and Methods

4.2.1 Study sites and insect observations

Site location, pan trapping, and insect observation methods follow those of Chapter 2 and 3.

4.2.2 Analysis of interaction matrices

Matrices were used to assess the influence of species composition, abundance, phenology, and trait matching on interactions and community structure. Interaction matrices were established for each site and burn class, with plant species in columns and insect species in rows. Matrices were arranged with the most frequently interacting species present in the upper left corner, with infrequent interactions visualized further to the right. In order to assess interactions at a fine scale, insects were identified to the lowest taxonomic unit possible. In cases where differentiation between species in the field was difficult, species groups were combined to morphospecies, genus, or family.

Connectance (I/AP) was calculated following Dupont et al. (2009), where I represented the number of realized links in the matrix, A represented all insect species observed, and P all

plant species involved in interactions. Connectance was first used in the assessment of plant-pollinator interactions by Jordano (1987), who transferred the metric from food web ecology. Connectance typically decreased with an increasing number of species in the community and with the prevalence of specialist interactions (Jordano 1987).

Nestedness measures the “heat of disorder” in a system (or community) as temperature (0-100°), and in earlier studies was used as a tool to better understand extinction events in fragmented island communities (Atmar and Patterson 1993). At 0°, the system was cold, and all extinctions that could occur would follow a repeatable, predictable pattern (Atmar and Patterson 1993). A warmer system meant that more random factors were influencing extinction patterns, making the loss of species harder to predict (Atmar and Patterson 1993). Therefore, a nested community (closer to 0°) was considered more stable, and a hotter community more chaotic. This idea was adapted to explain the interactions of generalists and specialists that composed the nested core of plant-insect networks. A nested community tolerates extinctions, and so maintains stability, due the redundancy of interactions by strongly interacting generalists (Memmott et al. 2004).

Nestedness is calculated through three main steps (Atmar and Patterson 1993, outlined by Rodríguez-Gironés and Santamaría 2006). An isocline of perfect order is calculated which would separate interactions (1s) from non-interactions (0s) in a perfectly nested matrix. Interacting species nearest to this line would be most affected by extinctions, while those further from the line would be expected to be more resilient. Next, the interactions are reordered in an attempt to achieve the most nested structure for the data. Finally, the distance to the isocline for both present and absent interactions in the nested matrix are calculated. The distances are summed and standardized to fit the 0-100° temperature scale.

To test whether the nestedness was a result of community structure and not random interactions, matrices were assessed using Monte Carlo simulations (Rodríguez-Gironés and Santamaría 2006). The simulations determine the probability of a random matrix having a lower temperature than the matrix from the sampled community (Rodríguez-Gironés and Santamaría 2006). Null models were used for this comparison, and selection of the most appropriate models had been assessed by Rodríguez-Gironés and Santamaría (2006). The null model that performed the best (based on a formula suggested by Bascompte et al 2003) was incorporated in the statistical package BINMATNEST (Dormann et al. 2013).

Network metrics were calculated in RStudio v. 3.0.0. Nestedness was analyzed using the *networklevel* function in the *bipartite* package v. 2.02 (Dormann et al. 2013). Nestedness was also calculated in R using *binmatnest*, based on the C++ program previously coded by Rodríguez-Gironés (Rodríguez-Gironés and Santamaría 2006). This function compares the matrix with null models to determine significant nestedness from random networks. Null model 3 was used to test for significance and the recommended setting of 100 nulls was used (Rodríguez-Gironés and Santamaría 2006, Petanidou et al. 2008).

4.2.3 “Building blocks” of a pollination network

The correlation between floral abundance and the number of visits received was assessed to determine if abundance was related to number of visits. Comparisons were made by site over each season and by peak dates of flowering by burn class and site using SAS v.9.1.3. Sample dates 2 and 7 represented peak flowering in the new burn sites, and dates 3 and 6 represented peak flowering in the mature burns. Flower species that were counted by quadrats and the

flower species that were visited by insects were correlated. Abundance and visit frequency was $\log(n+1)$ transformed and was tested using Pearson or Spearman correlation where appropriate.

The relationship between floral peaks and peaks in insect activity was investigated qualitatively by graphing flowering phenology and frequency of visits. Of the insects observed in the community, Dipterans in the family Syrphidae were the most common visitors, followed by Hymenoptera, particularly Apidae and Halictidae.

The two common groups of visiting syrphids, one with aquatic larvae (*Eristalis* and *Helophilus*) and one with predatory larvae (*Toxomerus* and *Sphaerophoria*) were used for syrphid comparisons due to a high frequency of visits and the relationship between larval habits. Activity was plotted with floral abundance (quadrats) for six flowering species: *Hypoxis hirsuta*, *Zizia aptera* [(A. Gray) Fernald], *Dalea purpurea*, *Solidago rigida*, *Dasiphora fruticosa*, and *Symphyotrichum laeve* [(L.) A. Löve & D. Löve]. These species had significantly earlier flowering in newly burned sites, represented early, mid, and late flowering peaks, and were apparently favoured by insects in the new burns. These comparisons were repeated for Halictidae and Apidae, the two most frequently visiting families of bees. Data shown was pooled by burn class following investigation at the site level.

Insect preference for colour was assessed in two ways. First, preference was determined by comparing the abundance of pan trapped syrphids and bees by bowl colour (yellow, blue, or white). Second, preferences in flower colour were examined using the number of visits received by flowering species within the same three colour categories. Syrphids were grouped using the most commonly visiting taxa with predatory or aquatic larvae, and bees were grouped by the two most commonly visiting genera (*Lassioglossum* and *Bombus*.) when comparing floral visits.

Comparisons were made by site for both years. Preference was assessed using Chi Square goodness of fit tests in SPSS v. 17.0.

4.2.4. Interaction strength and pollen load

Interaction strength was calculated from the perspective of six flowering species that had been selected for hand pollination in 2011 (data not included in thesis), as well as an additional three species that were frequently visited. Interaction strength (b_{ij}) was calculated based on the equation by Blüthgen (2010):

$$b_{ij} = a_{ij} / A_i$$

where a_{ij} is the number of interactions between the focal plant or insect, and A_i is the total number of visits for the plant or insect overall. The number of interactions was pooled over both years.

Insects that were observed visiting the six hand pollinated flowering species were collected as vouchers for pollen load sampling. I attempted to sample a variety of taxa in order to determine their potential influence as a pollinator. However, only the data from the most common visitors (aquatic and predatory syrphids, halictids and *Bombus* spp.) will be shown.

Vouchers were euthanized and swabbed with fuschin jelly cubes ($\sim 3\text{mm}^3$) suspended on insect pins (see Semmler 2013). The regions swabbed included the ventral abdomen and thorax, mouthparts, legs, and tarsi. Pollen that was collected in the scopa or corbicula was not sampled, as this pollen was removed from contact with stigmas during foraging (Forup and Memmott 2005). Used cubes were contained in individual vials.

Jelly cubes were placed on microscope slides with cover slips. The jelly was gently melted and the slides were sealed with clear nail polish.

Pollen was counted by compound microscope. When pollen was dense, counts were taken from five random fields of view at 10x magnification and pooled, then averaged by the number of vouchers for each insect species. When pollen was sparse, counts were taken from the entire slide and averaged in the same manner. Counts were used to place pollinators into three pollen load categories: High (500+ grains), Moderate (499-100 grains), and Low (99-0 grains). These categories were intended to create a qualitative scale of pollinator quality.

A pollen library was established by collecting flowers within sites throughout the summer. Anthers were swabbed with fuschin jelly cubes and mounted on slides. Grains were assessed by taking measurements of size using Image Pro Express (Media Cybernetics Inc. 2002). For each species, 10 grains of similar spatial orientation were measured by length and width. The measurements were averaged and used to assist in the identification of grains that were similar in appearance. The pollen library ensured that the pollen counted belonged to the flowering species that was visited, and also allowed for the identification of other species that were visited by the insect.

4.3 Results

4.3.1. Analysis of interaction matrices

Total interactions for each matrix are shown in Table 4.1, with 2011 standardized for visits per one hour. Results from both R programs are shown with similar outcomes, so I will discuss the bipartite results only.

Connectance in new and mature sites ranged from approximately 9-11% in 2010 and from 6-8% in 2011 (Table 4.1). The relatively lower connectance in newly burned sites in 2010 matched my predictions, but connectance was similar between all sites in 2011. Burn class

connectance was similar within and between years, ranging 3% between the mature burn classes annually (Table 4.1).

New and mature sites and burn classes were all highly nested, with similar temperatures below 10° (Table 4.1). Nestedness was slightly lower in 2011. Within the old burns, Old 2 had the highest connectance score of 15.3%, while Old 1 was more comparable to other sites at 9.2%. Old sites were also highly nested at 5.71°.

4.3.2 Analysis of community “building blocks”

a) Species composition and abundance

Newly burned sites had significantly higher floral abundance when compared to mature burn sites in 2010, but abundance was similar in 2011 (see Chapter 2). The number of visits observed showed a similar trend at the level of burn class (Table 4.1), with more visits in the new burn in both years. However, no significant difference between burn classes in peak activity was found for insects in 2010 (see Chapter 3).

Sites contained one or two flowering species that were highly abundant during the sampling season (based on floral counts in quadrats, see Chapter 2), with the proportional abundance of most species quickly declining (Table 4.2, 4.3). The range of relative abundances within the top five could extend from approximately 50% for the most abundant, to ~5% for a species ranked 5th within upland sites.

Table 4.1. Connectance, nestedness, and total interactions by site and burn class. Total interactions in 2011 were standardized to visits per hour for comparison in the table.

	Connectance bipartite	Nestedness bipartite	Nestedness binmatnest*	Total interactions
Site/Burn class	2010			
New 1	0.09	8.04	7.62	497
New 2	0.09	5.17	5.30	411
New burn	0.07	4.29	4.20	908
Mature 3	0.11	9.26	8.61	533
Mature 4	0.11	8.40	8.24	193
Mature burn	0.09	7.15	6.82	726
	2011			
New 1	0.08	8.09	6.76	373
New 2	0.07	4.14	4.18	353
New burn	0.06	4.69	4.43	726
Mature 3	0.07	3.90	3.97	294
Mature 4	0.08	6.57	6.33	209
Mature burn	0.06	3.57	3.52	503

*Binmatnest produces p values. All results of nestedness analysis were significant at $p = 0.000$.

Some abundant flowering species were consistently frequently visited. *Dasiphora fruticosa* was typically both well visited and abundant in sites in both years (Table 4.2, 4.3), particularly in the old burn class. In 2011, *Galium boreale* was abundant and was frequently visited in Mature 4 on the third sample date (Table 4.3).

Flowering species within the top five most visited showed the same pattern as those that were abundant, in that the top five ranged by proportion (Table 4.2, 4.3), with the top visited plant scoring less than 30%, to ~3% for a plant ranked 5th.

Insect visitors utilized abundant species, but many flowering species that were ranked as a top five most visited were ranked well below the top five most abundant, such as *Zizia aptera* (25/28 flowering species, New 1 2010) or *Solidago altissima* L. (20/28 flowering species, New 2 2010) (Table 4.2). Flowering plants that were the most visited (within the top two) were typically yellow, with the exception of *Symphyotrichum laeve* in New 1 2010 (Table 4.2).

Table 4.2. Comparative ranks of top five most abundant flowering species and top five most visited flowering species based on pollinator observations and floral counts by quadrats, 2010.

Abundance rank by site	Most abundant flowering species	Relative abundance (%)	Most visited flowering species	Relative number of visits (%)	Abundance rank of most visited by site
New 1					
1	<i>Parnassia glauca</i>	32.42	<i>Symphyotrichum laeve</i>	28.17	4
2	<i>Hypoxis hirsuta</i>	16.02	<i>Dasiphora fruticosa</i>	11.07	5
3	<i>Galium boreale</i>	12.52	<i>Zizia aptera</i>	9.86	25
4	<i>Symphyotrichum laeve</i>	5.26	<i>Solidago rigida</i>	8.65	12
5	<i>Dasiphora fruticosa</i>	5.13	<i>Crepis runcinata</i>	7.44	20
New 2					
1	<i>Hypoxis hirsuta</i>	42.48	<i>Zizia aptera</i>	16.06	10
2	<i>Symphyotrichum boreale</i>	7.30	<i>Solidago altissima</i>	13.38	20
3	<i>Sisyrinchium mucronatum</i>	7.30	<i>Zizia aurea</i>	11.92	7
4	<i>Symphyotrichum ericoides</i>	5.97	<i>Symphyotrichum boreale</i>	8.76	2
5	<i>Solidago rigida</i>	4.87	<i>Solidago rigida</i>	8.52	5
Mature 3					
1	<i>Parnassia glauca</i>	36.56	<i>Solidago riddellii</i>	16.32	17
2	<i>Polygala senega</i>	7.89	<i>Krigia biflora</i>	13.88	11
3	<i>Dasiphora fruticosa</i>	6.45	<i>Solidago altissima</i>	10.13	20
4	<i>Sisyrinchium mucronatum</i>	6.09	<i>Symphyotrichum boreale</i>	7.32	8
5	<i>Hypoxis hirsuta</i>	5.73	<i>Zizia aurea</i>	7.13	9
Mature 4					
1	<i>Galium boreale</i>	48.21	<i>Helianthus nuttallii</i>	20.21	7
2	<i>Sisyrinchium mucronatum</i>	6.70	<i>Zizia aptera</i>	18.13	3
3	<i>Zizia aptera</i>	6.70	<i>Solidago rigida</i>	7.77	10
4	<i>Symphyotrichum ericoides</i>	5.80	<i>Symphyotrichum ericoides</i>	7.25	4
5	<i>Hypoxis hirsuta</i> *	5.36	<i>Solidago canadensis</i>	7.25	n/a
5	<i>Dasiphora fruticosa</i> *	5.36			
Old 5					
1	<i>Dasiphora fruticosa</i>	77.06	<i>Dasiphora fruticosa</i>	80.26	1
2	<i>Lysimachia quadriflora</i>	14.68	<i>Symphyotrichum ericoides</i>	11.40	n/a
3	<i>Hypoxis hirsuta</i>	3.67	<i>Spirea alba</i>	2.19	n/a
4	<i>Asclepias ovalifolia</i>	1.83	<i>Symphyotrichum boreale</i>	1.75	6
5	<i>Sisyrinchium mucronatum</i>	1.38	<i>Galium boreale</i> *	1.32	7
			<i>Senecio spp.*</i>	1.32	n/a
Old 6					
1	<i>Dasiphora fruticosa</i>	58.45	<i>Dasiphora fruticosa</i>	67.80	1
2	<i>Lysimachia quadriflora</i>	14.08	<i>Asclepias ovalifolia</i>	5.65	6
3	<i>Lobelia kalmii</i>	9.86	<i>Euthamia graminifolia</i>	4.52	7
4	<i>Parnassia glauca</i>	8.45	<i>Zizia aptera</i>	4.52	n/a
5	<i>Sisyrinchium mucronatum</i>	2.82	<i>Dalea purpurea</i>	2.26	n/a
			<i>Elaeagnus commutata</i> *	2.26	n/a
			<i>Hypoxis hirsuta</i> *	2.26	8
			<i>Solidago ptarmicoides</i> *	2.26	n/a

*Indicates tie. Total flowering species by site: New 1, 28; New 2, 28; Mature 3, 30; Mature 4, 18; Old 5, 7; Old 6, 10.

Indicates species that were both top 5 visited and abundant.

Table 4.3 Comparative ranks of top five most abundant flowering species and top five most visited flowering species based on pollinator observations and floral counts by quadrats, 2011.

Abundance rank by site	Most abundant flowering species	Relative abundance (%)	Most visited flowering species	Relative number of visits (%)	Abundance rank of most visited by site
New 1					
1	<i>Hypoxis hirsuta</i>	25.47	<i>Zizia aptera</i>	22.42	8
2	<i>Dasiphora fruticosa</i>	16.51	<i>Dasiphora fruticosa</i>	16.64	2
3	<i>Galium boreale</i>	10.85	<i>Krigia biflora</i>	10.07	10
4	<i>Sisyrinchium mucronatum</i>	10.38	<i>Solidago nemoralis</i>	10.07	9
5	<i>Lithospermum canescens</i>	5.66	<i>Solidago ptarmicoides</i>	5.23	20
New 2					
1	<i>Hypoxis hirsuta</i>	39.17	<i>Zizia aurea</i>	25.25	3
2	<i>Sisyrinchium mucronatum</i>	22.50	<i>Zizia aptera</i>	16.74	9
3	<i>Zizia aurea</i>	8.75	<i>Dasiphora fruticosa</i>	15.46	6
4	<i>Viola spp.</i>	6.25	<i>Solidago gigantea</i>	8.23	8
5	<i>Symphyotrichum ericoides</i> *	5.83	<i>Solidago altissima</i>	3.69	n/a
5	<i>Dasiphora fruticosa</i> *	5.83			
Mature 3					
1	<i>Parnassia glauca</i>	20.62	<i>Dasiphora fruticosa</i>	26.06	2
2	<i>Dasiphora fruticosa</i>	20.00	<i>Zizia aptera</i>	12.61	26
3	<i>Dalea purpurea</i>	7.69	<i>Zizia aurea</i>	10.05	27
4	<i>Sisyrinchium mucronatum</i>	7.08	<i>Symphyotrichum ericoides</i>	5.96	12
5	<i>Lysimachia quadriflora</i> *	5.23	<i>Parnassia glauca</i>	5.11	1
5	<i>Prunella vulgaris</i> *	5.23			
Mature 4					
1	<i>Galium boreale</i>	56.04	<i>Dasiphora fruticosa</i>	21.05	7
2	<i>Pedicularis lanceolata</i>	7.69	<i>Zizia aptera</i>	16.27	n/a
3	<i>Hypoxis hirsuta</i>	6.59	<i>Galium boreale</i>	12.20	1
4	<i>Symphyotrichum ericoides</i>	6.04	<i>Senecio spp.</i>	8.37	n/a
5	<i>Viola spp.</i>	5.49	<i>Helianthus nuttallii</i>	7.42	14

*Indicates tie. Total flowering species by site: New 1, 28; New 2, 16; Mature 3, 33; Mature 4, 15.

Indicates species that were both top 5 visited and abundant.

Correlation analysis did not show a strong relationship between floral abundance and visits received by site in 2010 (Table 4.4). Results for Spearman correlation were non-significant in with the exception of New 2 ($r = 0.36$, $n = 39$, $p \leq 0.02$). However, abundance and visits were significantly correlated for sites in 2011 except in Mature 4 (Spearman, $r = 0.20$, $n = 28$, $p > 0.25$). No correlations were found within sites by peak dates of flowering in both years except in

New 2 Date 7, 2010 (Pearson, $r = 0.60$, $n = 11$, $p < 0.05$). Spearman correlation was also significant for Date 7 in the New burn class, 2010 ($r = 0.64$, $n = 13$, $p < 0.02$), but the remaining burn classes were non-significant in both years.

Table. 4.4. Correlation analysis of floral abundance and visits received. Spearman correlation with correlation coefficient (Shapiro-Wilk) and p-value (Kolmogorov-Smirnov).

	2010 Cor. Coeff. (p)	2011
New 1	0.316 (0.057)	0.412 (0.008)*
New 2	0.363 (0.023)*	0.369 (0.025)*
Mature 3	0.145 (0.384)	0.312 (0.039)*
Mature 4	0.216 (0.289)	0.207 (0.291)

* significant at $p < 0.05$

The most frequent visiting insects were discussed in Chapter 3, showing a dominance of fly visits, followed by bees. Those insects that were responsible for the most visits generally also had the most interactions with different flowering species. Frequently visiting generalists were mainly syrphids, particularly *Toxomerus marginatus* (Say, 1823). A genus of tachinid fly, *Estheria* spp. was also a frequent visitor with many species interactions in upland and old sites, as was *Nemotelus* spp. (Stratiomyidae).

b) Phenology

Both insects and plants showed a roughly bimodal distribution through the season, with an early and late season peak in activity or flowering. Certain flowering species were not present on all sample dates as a result, yet many of the most generalized insect species were present throughout the summer with varying degrees of abundance.

Peak activity in predatory syrphids showed some asynchronies with peaks in open blooms in 2010. The peak in activity on Date 3 and Date 4 in 2010 was later than the floral peaks of *Hypoxis hirsuta* and most of the bloom period of *Zizia aptera* in new burns (Fig. 4.1a) but peak flight of predatory syrphids coincided with those two flowering species in the mature burn (Fig. 4.1b). The peak activity of predatory syrphids in 2011 was similar between classes and lined up with the onset of flowering for *Dasiphora fruticosa* (Fig. 4.1 c and d). The peak in aquatic syrphids was in line with late flowering asters such as *Symphyotrichum laeve* in 2010 (Fig 4.1 a and b), but aquatic syrphids were much less abundant in 2011 (Fig 4.1 c and d).

Toxomerus marginatus visited a variety of flowering species in both years that included flowers at their peak blooms or flowers that were abundant. *Dalea purpurea* was visited on Date 4 during peak flight in New 2010 (Fig. 4.1a), but other flowering species, such as *Solidago ptarmicoides* [(Torr. & A. Gray) B. Boivin] and *Solidago altissima*, were also frequently visited on that date. *Zizia aptera* and *Solidago rigida* were frequently visited by the predatory group in the mature burns in 2010 (Fig. 4.1b), but *Krigia biflora* [(Walter) S.F. Blake] was the most highly visited flowering species in the early to mid season. In 2011, *Toxomerus marginatus* frequently visited the flowering species shown for the new burn class (Fig. 4.1c), but also heavily visited *Zizia aurea*, *Krigia biflora*, and *Solidago ptarmicoides*. The peak in visits on Date 4 was due to visits to *D. fruticosa*, despite this species reaching its greatest peak on Date 6. Peaks in visits strongly overlapped with peaks in flowering for the species shown in the mature burn (Fig. 4.1d). *Dasiphora fruticosa* was a preferred mid season species for the predatory group, but again *Solidago ptarmicoides* was also a frequently visited species.

The majority of interactions within the aquatic group were the result of visits during a late summer peak in fly abundance. *Helophilus* spp. and *Eristalis* spp. frequently visited

Dasiphora fruticosa, *Symphytotrichum ericoides* (L.) and *Symphytotrichum boreale* [(Torr. & A. Gray) Prov.] in the late season on Date 7 in New 2010 (Fig. 4.1a), while also visiting most of the flowering species available during their peak in activity in the mature class in 2010 (Fig. 4.1b). Observations of the aquatic group were less frequent in both burn classes in 2011, though one peak on Date 2 in the new burn was the result of visits to *Zizia aurea* [(L.) W.D.J. Koch] (Fig 4.1c).

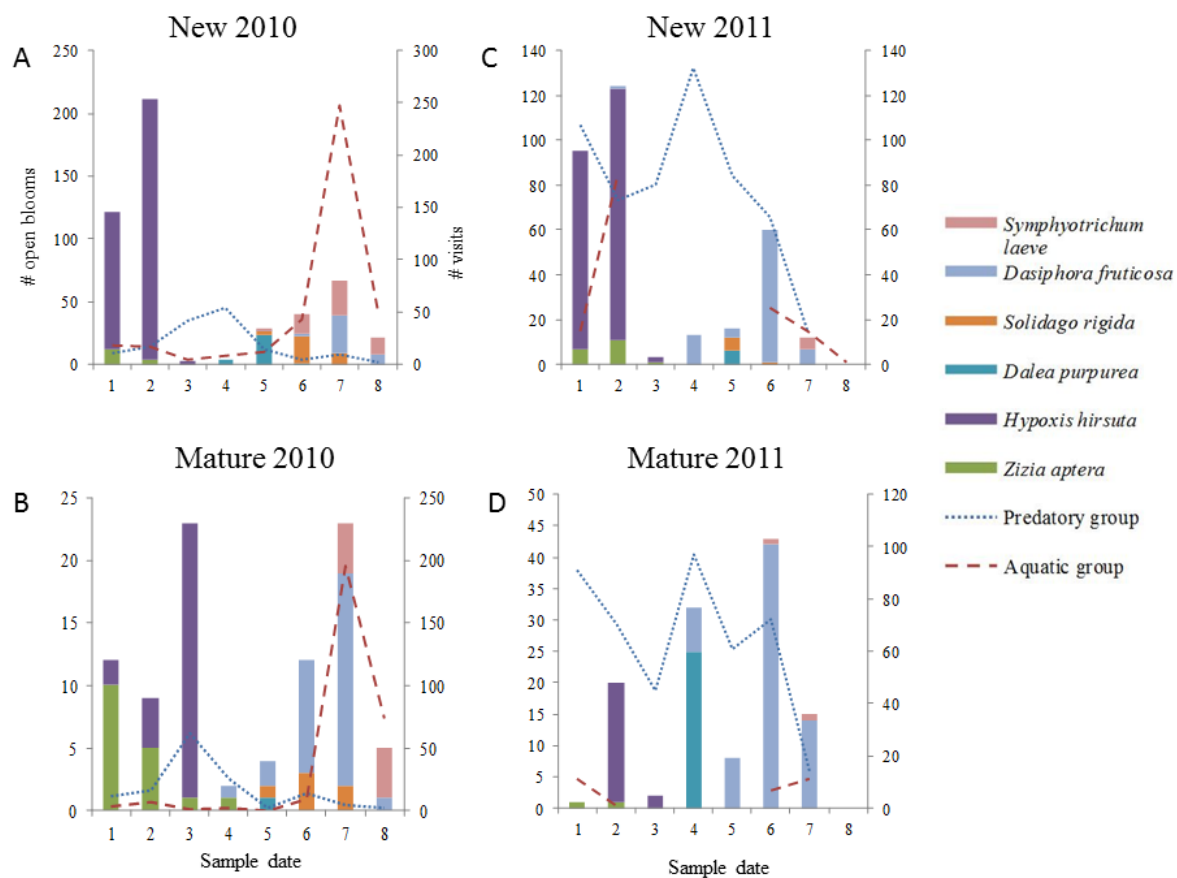


Figure 4.1. The relationship between floral abundance and frequency of visits by syrphids for 2010 (A-B) and 2011 (C-D). Bars (scale on left) represent floral abundance by quadrat and lines (scale on right) represent frequency of visits for the new burn class (A and C) and mature burn class (B and D). The data are not standardized to visits per hour, so observation time in 2011 are double that of 2010.

Peaks in bee activity appeared to overlap with peaks in flowering within burn classes in both years. In general, Halictid bees were most frequently observed in the early season, particularly in the new burn class (Fig. 4.2 a, c). The Apid bee group was mainly composed of visits by *Bombus* spp., and these bees typically increased in observed visits toward the end of the season (Fig. 4.2 a-d).

In the new burns in 2010, halictid activity was greatest in the early season, with most visits received by *K. biflora* and *Zizia* spp. (Fig. 4.2a). A small plateau around Date 5 resulted from generalized visits to several species beyond those six selected for comparison. Relationships between visits and peaks were not obvious for Halictids in the early season in the mature class. A few visits from *Lasioglossum* spp. suggested a preference for *Parnassia glauca* in the late season in Mature 3 (Fig 4.2b).

Bumble bee activity in the early season in 2010 did not appear to be related to floral peaks, with relationships between visits and peak flowering emerging later in the the season, particularly in the mature burn (Fig 4.2, a, b). *Bombus* spp. frequently visited *Solidago rigida* on Date 6 in the new burn, but no peak in activity was observed for *Bombus* spp. by site in New 2. (Fig. 4.2a). *Bombus* spp. showed increased visits to several late season species in the mature burn: *Dalea purpurea*, *Symphyotrichum laeve*, *Solidago rigida*, *Dasiphora fruticosa*, and *Helianthus nuttallii* (Torr. & A. Gray) (Fig. 4.2b).

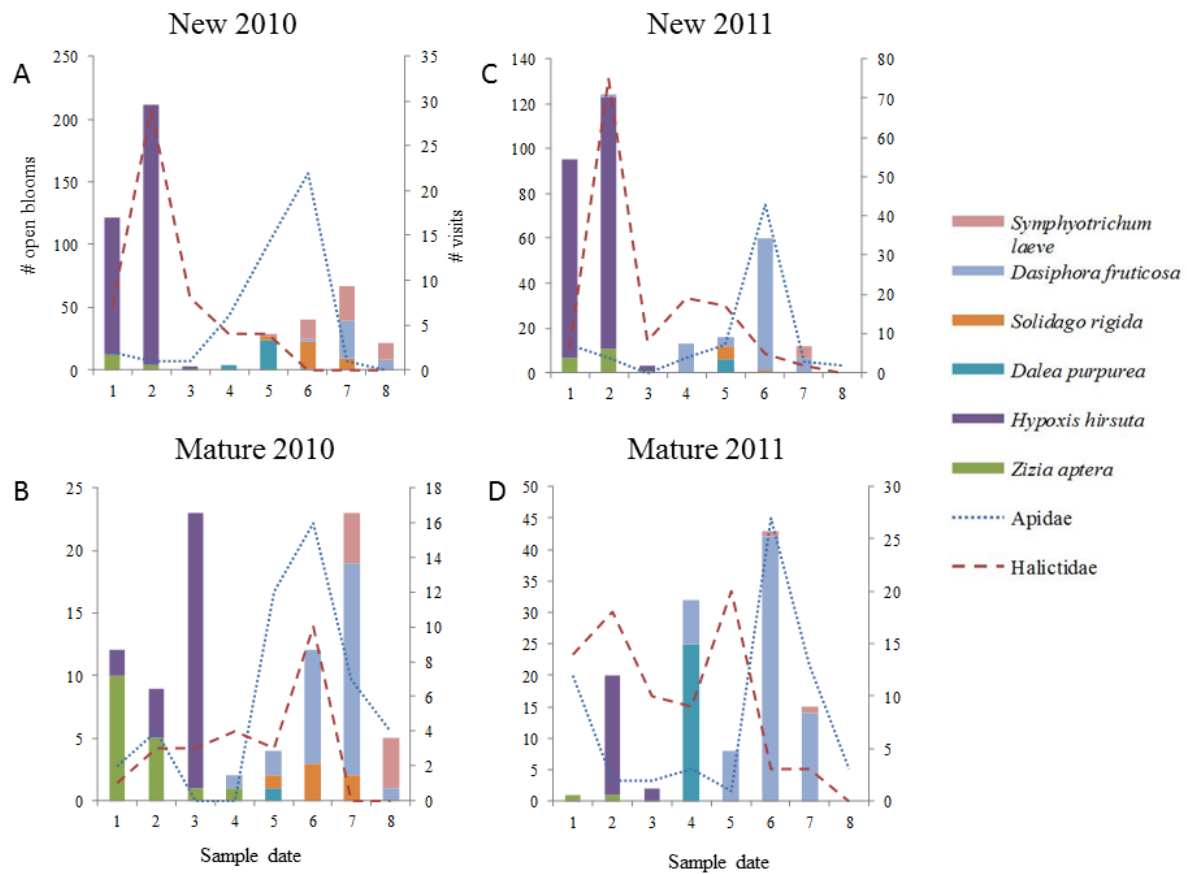


Figure 4.2. The relationship between floral abundance and frequency of visits by bees for 2010 (A-B) and 2011 (C-D). Bars (scale on left) represent floral abundance by quadrat and lines (scale on right) represent frequency of visits for the new burn class (A and C) and mature burn class (B and D). The data are not standardized to visits per hour so that observation time in 2011 is double that in 2010.

The overlap in peaks in visits and floral abundance was most obvious in the new burns in 2011, particularly for halictids. Halictid bees visited *Zizia* spp. on Date 1 and Date 2, also preferring to visit *Krigia biflora* (Fig 4.2c). *Dasiphora fruticosa* was highly visited on Date 4 and Date 5 (Fig. 4.2c). *Dalea purpurea* and *Solidago* spp. were visited during their peaks, but more

in New 1. *Bombus* spp. visited *Pedicularis canadensis* in the early season and frequently visited *Solidago* spp. in the late season (Fig. 4.2c).

There were two peaks in activity in the mature class in 2011 for halictid bees, with *Bombus* spp. once again increasing as the season progressed. The first halictid peak on Date 2 was due to visits to a variety of species in Mature 4 and a high number of visits to *Zizia* spp. in Mature 3 (Fig 4.2d). The peak on Date 5 was directly due to a large number of visits to *Dasiphora fruticosa*. *Bombus* spp. started the season with most visits to *Lithospermum canescens* and *Pedicularis canadensis* (Fig. 4.2d). The greatest peak in visits was on Date 6, where bumble bees favoured *Dasiphora fruticosa* and *Solidago gigantea*.

d) Trait-matching

There were very few insects that could be designated as a specialist based on observations. The most specialized fly visitor appeared to be *Odontomyia pubescens*, with all or the majority of visits observed on *Zizia* spp. in 2010 and 2011. The flight period and flowering period of these species were restricted to the early season. For bees, halictids in the genus *Dufourea* were known specialists, and the interactions observed matched those of previous records of floral hosts (*Dasiphora* and *Helianthus*) (Dumesh and Sheffield 2012).

The majority of insects were generalists, with insects visiting plants from multiple families. Flowering species also appeared to be generalists, receiving visits from numerous species within Hymenoptera, Diptera, Coleoptera, and Lepidoptera.

Chi square analysis of *Lasioglossum* spp. from pan traps showed no preference for colour (Table 4.5). Only in the mature burn in 2010 were *Lasioglossum* spp. trapped beyond expected amounts in yellow pans. Bumble bees were always more frequently trapped in blue pans in both years (Table 4.5).

Colour preference for flowers was slightly different than pans. *Lasioglossum* spp. were frequently observed on yellow flowers, with Chi square analysis showing more visits than expected in both years (Table 4.6). Results differed from pans for floral preference in *Bombus* spp. in 2010, with the majority of visits divided between yellow and blue flowers (Table 4.6).

Both predatory and aquatic syrphids showed a strong preference for yellow when assessed by either counts in pans (Table. 4.5) or visits to flowers (Table 4.6).

Table 4.5. Pan colour preference in the most frequently observed insect taxa.

2010					2011		
HYMENOPTERA							
New burn	Pan	# in pans	Chi Square	<i>p</i>	# in pans	Chi Square	<i>p</i>
<i>Lasioglossum</i> spp.	Yellow	97	0.194	0.908	35	0.182	0.913
	Blue	93			32		
	White	99			32		
<i>Bombus</i> spp.	Yellow	2	19.000	0.000	17	33.405	0.000
	Blue	19			71		
	White	5			43		
Mature burn							
<i>Lasioglossum</i> spp.	Yellow	80	40.933	0.000	24	2.632	0.268
	Blue	29			19		
	White	26			14		
<i>Bombus</i> spp.	Yellow	7	65.148	0.000	55	20.738	0.000
	Blue	50			114		
	White	4			83		
SYRPHIDAE							
New burn							
Aquatic group	Yellow	40	39.263	0.000	5	39.308	0.000
	Blue	2			0		
	White	15			0		
Predatory group	Yellow	47	24.074	0.000	52	39.308	0.000
	Blue	12			15		
	White	22			11		
Mature burn							
Aquatic group	Yellow	24	21.160	0.000	0	22.944	0.000
	Blue	1			0		
	White	0			0		
Predatory group	Yellow	50	34.462	0.000	50	22.944	0.000
	Blue	18			14		
	White	10			25		

Table 4.6. Floral colour preference in the most frequently observed insect taxa.

2010					2011		
HYMENOPTERA							
New burn	Flower	# Obs	Chi Square	<i>p</i>	# Obs	Chi Square	<i>p</i>
<i>Lasioglossum</i> spp.	Yellow	20	14.727	0.000	100	154.850	0.000
	Blue	0			8		
	White	2			5		
<i>Bombus</i> spp.	Yellow	18	0.857	0.355	52	64.277	0.000
	Blue	24			9		
	White	0			4		
Mature burn							
<i>Lasioglossum</i> spp.	Yellow	15	3.857	0.050	49	86.808	0.000
	Blue	0			1		
	White	6			2		
<i>Bombus</i> spp.	Yellow	24	9.294	0.010	45	32.000	0.000
	Blue	20			5		
	White	7			0		
SYRPHIDAE							
New burn							
Aquatic group	Yellow	179	55.290	0.000	125	194.851	0.000
	Blue	154			4		
	White	64			12		
Predatory group	Yellow	111	127.734	0.000	437	512.896	0.000
	Blue	22			32		
	White	10			91		
Mature burn							
Aquatic group	Yellow	199	169.134	0.000	22	6.533	0.011
	Blue	26			4		
	White	66			4		
Predatory group	Yellow	108	130.088	0.000	336	348.940	0.000
	Blue	7			48		
	White	22			65		

4.3.3. Interaction strength and pollen loads

Interaction strength (IS) varied by species combination (Table 4.7). Most IS scores for Halictid bees were low (<0.1). Bumble bees had scores above 0.1 for *Solidago rigida* and *Liatris ligulistylis* (A. Neslon), with the highest IS for *Dalea purpurea* (0.41). The aquatic group of syrphids had higher IS with *Sisyrinchium mucronatum* and *Solidago rigida*, with the highest IS for *Symphyotrichum laeve* (0.48). The predatory syrphid group had the highest IS scores overall, particularly with *Sisyrinchium mucronatum* (0.76), *Hypoxis hirsuta* (0.67), *Dasiphora fruticosa* (0.45), and *Dalea purpurea* (0.41).

Pollen loads differed by taxa (Table 4.7). The highest pollen loads were collected from bees, but the larger aquatic Syrphids also fell within the High category. Smaller halictid bees (subgenus *Dialictus* spp.) fell within the Moderate category. Small predatory syrphids had the lowest pollen loads.

Table 4.7. Interaction strength for nine species of flowering plants and common pollinators. Categories of pollen loads are included with each insect species. Data represents 2010 and 2011 combined.

Insect group	Flies - predatory larvae		Flies - Aquatic larvae		Small Halictidae	Large Halictidae	Apidae	Total visits to plant species	Total proportion of interactions to plant species
	<i>Toxomerus</i> spp.	<i>Sphaerophoria</i> spp.	<i>Eristalis</i> spp.	<i>Helophilus</i> spp.	<i>Lasioglossum</i> (<i>Dialictus</i>)	<i>Lasioglossum</i> spp.	<i>Bombus</i> spp.		
Pollen load	low	low	high	high	moderate	high	high		
Plant species									
<i>Hypoxis hirsuta</i>	0.67	0.05	0.01	0.01	0.05	0.06	0	125	0.85
<i>Sisyrinchium mucronatum</i>	0.76	0	0	0.14	0.07	0	0	29	0.97
<i>Zizia aptera</i>	0.23	0.02	0.04	0.01	0.05	0	0	593	0.35
<i>Parnassia glauca</i>	0.05	0.04	0.04	0.01	0.02	0.06	0	100	0.22
<i>Dalea purpurea</i>	0.41	0.02	0	0.06	0.07	0	0.41	86	0.97
<i>Solidago rigida</i>	0.16	0.02	0.3	0.05	0	0.03	0.2	133	0.76
<i>Liatris ligulistylis</i>	0.13	0.02	0.04	0.02	0	0	0.15	47	0.36
<i>Dasiphora fruticosa</i>	0.45	0.12	0.04	0.07	0.07	0.01	0.02	581	0.78
<i>Symphoricarum laeve</i>	0.02	0.01	0.48	0.31	0	0	0.06	207	0.88

*High (500+ grains), Moderate (499-100 grains), Low (99-0 grains)

4.4 Discussion

Assessments of overall network structure showed that connectance was low and that networks were strongly nested and similar between sites and years, with little evidence of differences between burn classes. From this, we might assume that sites had relatively high numbers of species, and that sites included a highly interactive generalist core, with specialists that tended to interact with generalists. But networks are more complex than their binary metrics convey. Closer inspection of the building blocks of network structure revealed important information pertaining to relationships between abundance and number of visits, phenological overlap, pollinator host preference, and the quality of interactions.

4.4.1 Networks and species composition and abundance

Chapters 2 and 3 offered some insight into the species of flowering plants and insect pollinators that might be present by site. For flowering plants, each site was consistent across years in terms of the identity of species present; what differed was the flowering phenology and abundance of certain flowering species depending on the year or burn age. For insects, frequent visiting species were fairly consistent between sites, meaning that a fly that was a top visitor in one site was typically a top visitor in another. However, abundance was influenced by annual variation in weather for some dipterans and lepidopterans, while the presence of certain hymenopterans was more likely to be influenced by fire-altered habitat.

If the species present in a site had an impact on the structure of the pollination network, we should see evidence of this in network metrics. Connectance values were certainly low within this study and similar between sites, even though there was increased potential for interactions due to higher species richness in some sites. To explain this, we have to remember

that observations were taking place in high quality tall grass prairie habitat with a diverse assemblage of flowering species and insects. When the potential to observe a great variety of life is high, the opportunity to observe all potential interactions is limited (Jordano 1987). In addition, all sites contained a variety of generalist flowering species whose rewards were not restricted to specific pollinators, as well as similar assemblages of insect generalists. Therefore, low connectance could stem from the large number of species available for unrestricted interactions, again making it difficult to record each interaction during a one or two hour survey. Connectance has been described as a measure of generalization, showing that as species are added to the system, the average number of observed interactions per species declines (Jordano 1987, Olesen and Jordano 2002). This generalization as shown by low connectance is certainly supported by the high nestedness in this study.

The species with the most frequently interacting individuals were typically generalists, either flies and/or bees in the case of the insects, or flowering plants in the Asteraceae and *Dasiphora fruticosa* (Rosaceae). Generalist species establish the core of a pollination network with which specialists interact, creating a higher likelihood of persistence for specialist species (Jordano 1987, Bascompte et al. 2003, Vázquez and Aizen 2004, Olesen et al. 2008, Olesen et al. 2012). Generalists also create links between subsets of species in a community which increases the resilience to disturbance or extinctions (Memmott 2004, Martin-González et al. 2009).

There were examples of links between generalists and specialists in the interaction matrices. Bees in the genus *Dufourea* have specific floral hosts, and two species were encountered in this study. *Dufourea marginata* was observed on its host genus *Helianthus* (*nuttallii*), and *Dufourea fimbriata* was observed on its host, *Dasiphora (fruticosa)* (Dumesh and Sheffield 2012). Both plant species were generalists that were often in the top five most visited

flowers. These two flowering species could be assumed to be at a low risk of extinction due to the number of pollinators contributing to seed set, offering a stable resource for specialized bees. Without these generalized species, the persistence of *Dufourea* in this habitat might be limited.

Specialized plants were more difficult to identify, but there were some indications that flowering plants required specific visitors. The difficulty in confidently assigning the label of “specialist” came from the patchy or infrequent records of certain plants along transects. A plant that was infrequently sampled might appear specialized because few visits were observed.

Pedicularis canadensis (L.), however, appeared to receive consistent visits from a particular taxa. *Pedicularis canadensis* bloomed in early spring, and was more commonly encountered in Mature 3. The yellow pea-like flowers were those that required a visit from a bee to push open the petals and trigger the exposure of an anther (Córdoba and Cocucci 2011). Bumble bees were observed to be the primary visitor to this flowering plant, particularly queens who had recently emerged from hibernation. If disturbance in the previous season were to disrupt the abundance of *Bombus* spp. queens in early spring, it could have negative effects on this species in terms of pollen limitation and reduced seed set.

The abundance of individuals within a species had been thought to influence the likelihood of interactions by increasing the probability that a link will occur (Vázquez et al. 2009). When frequency of visits was used to represent abundance in tall grass prairie communities, syrphids were the most active dipteran visitors, and halictids and apids the most active of the hymenopterans. For syrphids, the dominance of visits from syrphids with aquatic larvae was limited to the first sample season. When ratios between syrphid genera from pan traps (Chapter 3) were compared with frequency of visits, syrphids in the aquatic group were not the most abundant in sites, even though they were the most active visitors. Though abundance

and frequency did not appear to be related in this case, ratios in pan traps for bees in 2010 and 2011, and syrphids for 2011, did show that the more abundant genera were usually visiting flowers in the greatest frequency. This effect of abundance was certainly observed in the old burn class, with the most abundant flies and flowering species making the majority of links in those sites.

Many flowering species that were abundant in the community also received many visits, indicating that relative abundance does influence network structure. However, abundance did not appear to dictate all interactions, as many of the flowering species that had the most links did not occur in high abundance during floral surveys by quadrats. Correlation analysis did not show a relationship between floral abundance and visits by site in 2010 (exception of New 2), but did show a stronger relationship in 2011 (exception of Mature 4). There may have been greater evidence for this relationship due to the increased sample effort in 2011 which resulted in more observations.

Abundance does not always appear to fully predict the likelihood of interactions, but it can play a significant role in creating a nested structure. Abundant species can contribute to 60-70% of nestedness patterns in some communities following comparisons of simulated and real networks (Krishna et al. 2008). This was a result of increased probability of interaction due to abundance (Krishna et al. 2008) and their asymmetric interaction strengths (Vásquez et al. 2007). Asymmetric interactions occur when the effect of one species on another is disproportionate; one species relies heavily on its interacting partner while that partner could still function if that particular interaction was lacking. The most abundant species have the strongest reciprocal effects when interactions occur, but show asymmetry when interacting with less abundant species or specialists (Vásquez et al. 2007). An interesting result of the study by Vásquez (et al.

2007) was that the influence of abundance on interaction asymmetry was more pronounced for consumers than hosts in pollination networks, which may partially explain why pan trap abundance appeared to be more related to insect visit frequency than quadrat abundance was related to visits received in my study.

4.4.2 Networks and phenology

Chapter 2 showed that flowering plants had peaks in blooms about two weeks earlier in newly burned sites in 2010, but that this effect of fire did not continue into 2011. Insects, however, did not show a difference in phenology between burn classes in either year, but the abundance of certain species did appear to be related to disturbance and temporal variation in climate.

Syrphid Activity – Syrphids did not appear to have peaks in activity related to differences in flowering phenology. Graphs showing six common species that bloomed over the eight sample days showed that Syrphids visited flowering species whether at peak flowering or not. Many of the flowering species that were heavily visited during syrphid peaks were not necessarily abundant (ex. *Z. aurea*) or were locally abundant for a short period of time (ex. *K. biflora*). Correlation analysis between flowering abundance and visits received for peak dates by year and sites further showed a lack of a relationship, but it should be noted that small sample sizes for this analysis limited statistical power.

In general, syrphid populations are quite stable, and syrphid species with larvae that feed on a variety of aphids were some of the most abundant in suburban gardens (Owen and Gilbert 1989). However, this stability is still dependent on resources (Owen and Gilbert 1989) and on the ability to access to floral rewards based on insect and flower morphology (Gilbert 1981). The

similar dates of peak visit rate between years, the commonness of aphid feeding syrphids, and abundant floral (and aphid) resources in the preserve explain the high number of predatory syrphid observations, at least for the two years examined. The general stability in predatory syrphid populations might explain why burn age did not have a significant effect on peak activity.

The observed consistency of peaks in visit rates in predatory syrphids might also be due to the life cycle of *Toxomerus marginatus*. Late instar larvae overwinter and pupate in the soil in spring, with adults emerging in summer (Coin et al. 2004, Milne and Milne 2007). It could be that date 4 lined up with the emergence of the majority of adult flies, and not with bloom times for abundant species.

A similar argument could be made for the aquatic group of syrphids; species of *Eristalis* and *Helophilus* can have more than one generation per season (Wojcik 2014). Date 6 could simply be a representation of a mass emergence of adult flies following a successful season of larval development due an abundance of habitat. The only significant results for the relationship between floral abundance and visit rate by date fell on the late season peak associated with the aquatic group. Additionally, it would be interesting to know if late season emergence of a second brood falls in this time period as a rule for the aquatic group. It would seem a good reproductive strategy, as two abundant and frequently visited flowering species, *Dasiphora fruticosa* and *Symphyotrichum laeve*, had reached peak flowering at that point in the season.

Bee Activity – Bees appeared to have a closer relationship with the six flowering species selected, but patterns were also related to burn age. As was discussed in Chapter 3, the frequency of visits by bees appeared to be partly explained by nest sites. Bees that nested deep below

ground, such as Halictids, were often associated with newly burned sites. For *Bombus* spp., which nested close to the ground surface, the association appeared to be with the mature sites.

Investigating these patterns using flowering phenology offered a different perspective. Bees did appear to visit the six species selected during their peaks, as well as other species like *Krigia biflora* and *Pedicularis canadensis*. Floral constancy was introduced in Chapter 3, and this behavior might explain the apparent relationship in peak flowering and visit rate.

Floral constancy occurs when a pollinator shows a preference for a particular flower species even when other species are available. Many hypotheses for why this might occur have been put forward, such as limits or specializations in cognitive ability or reducing inter-colony competition (reviewed by Grüter and Ratnieks 2011). However, the best explanation for floral constancy is probably the quality of the reward (Grüter and Ratnieks 2011). The “costly-information hypothesis” (Chittka et al. 1999) predicts that flowers with a certain level of reward will be continuously visited, with alternatives to this flower investigated as the reward declines (Chittka et al. 1999, Grüter and Ratnieks 2011). From the plant perspective, nectar rewards can be costly, but necessary, in order to ensure that a pollinator visits the flower, spends enough time consuming the reward for pollen to adhere, and convinces the pollinator to continue to visit similar flowers (Zimmerman 1988).

Floral constancy may have occurred in the tall grass prairie, but was difficult to detect by species within interaction matrices. However, one species of common bumble bee, *Bombus rufocinctus* (Cresson 1863), was observed on 4/8 sample dates in New 1 in 2010. This species was observed making single visits to a variety of flowering species, such as *Sanicula marilandica* L. and *Liatris ligulistylis*. For other flowering species, visits for *B. rufocinctus* were

consistent, with the only observed visits made to *Dalea purpurea* in July, and 9/12 visits to *Solidago rigida* on 10 Aug. Most bee species were observed visiting flowers on each sample date, making it hard to track the changing preferences within a bee species. In addition, floral constancy can change by individual bee, so the visits of a specific bee would need to be followed to prove that floral constancy was the cause of this trend.

These data suggest that flowering phenology might determine the frequency of bee visits in cases where a rewarding species blooms at a particular time. Since bees will select these species based on reward, slight shifts in phenology based on disturbance should not have an overly negative impact on bee populations. Bees will seek out and visit species that offer rewards, and switch to another host, if available, when resources become scarce. In the case of the specialist *Dufourea* spp., however, changes in phenology might become more of an issue if bloom times changed, but the abundance of blooms might be of greater concern to a specialist without a limited flight period.

Interactions that cannot occur in the community should also be considered when interpreting network structural elements. Forbidden links are the result of non-overlapping phenophases, morphological mismatching, or a lack of suitable floral rewards (see Olesen et al. 2011). Analyses of networks which include entire sample seasons will create forbidden links due to the limited flowering times or flight times of some species. Temporal variation in the abundance of certain taxa may also play a role. With both plants and insects showing some degree of bimodal peaks in abundance or visit frequency in my study, it might be useful to assess the tall grass prairie community by early and late season separately to reduce the influence of forbidden links on network metrics. Additionally, more sampling within smaller blocks of time might offer more insight into the temporal variation of interacting partners.

4.4.3 Networks and trait matching

The colour preferences I observed for bumble bees and syrphid flies in pan traps were consistent with previous studies. Yellow, and to a lesser extent white, is commonly preferred by syrphids (Haslett 1989, Dicks et al. 2002), while blues or purples are preferred by bumble bees (Lunau and Maier 1995, Dicks et al. 2002, Raine and Chittka 2007). Emphasis on colour preference for *Lasioglossum* spp. is less prominent in the literature. Work by Stephen and Rao (2005) showed a more even representation in pan traps, Campbell and Hanula (2007) found the greatest abundance in blue, and Wilson et al. (2008) found the most *Lasioglossum* spp. in yellow pans. I observed a fairly even representation of *Lasioglossum* spp. between pan colours, with the exception of the mature burn in 2010 where more bees were trapped in yellow pans.

The colour preferences found in pans did not necessarily match those observed during surveys depending on taxa. When preference was based on observed visits, almost all visits by *Lasioglossum* were observed on yellow flowers. Bumble bees were always trapped in the greatest numbers in the blue pans, but observations of flowers showed no difference between yellow and blue in 2010. By comparison, syrphid colour preference between pan traps and floral visits was consistent.

Pan traps have been used in many studies involving flower visiting insects, but they might not provide an accurate subsample of the community on all occasions. They allow for the collection of large numbers of insects with low cost materials and limited investments of time and energy on the part of the researcher (Cane et al. 2000). Pan traps are also a long used sampling method, with the technique showing prevalence in the literature since the early 90s. However, confidence in pan traps can vary. Some studies have shown the pans fail to trap a large

number of individuals and species when compared to aerial nets (Cane et al. 2000, Roulston et al. 2007), while others showed little difference in abundance between nets and pans, but found lower species richness in pans (Wilson et al. 2008). Pans also seems to show bias for certain species over others (Roulston et al. 2007, Wilson et al. 2008), and in some instances specialist bees were trapped in pans that were a different colour than their host (Cane et al. 2000, Wilson et al. 2008). The abundance of flowers in the community might also have an impact on pan trap success, with fewer bees trapped in areas where floral abundance was high (Baum and Wallen 2011). These variable results could explain why pan and observed colour preferences were not identical, though preferences found in pans in the tall grass prairie matched some of those found in other studies.

Insects use cues beyond colour when determining where to forage which should be considered during interpretation. Attractants exist that were not included in this study, such as petal markings in the ultra-violet spectrum (Silberglied 1979), abundance of floral rewards (Thomson 1988), and floral scent (Raguso 2008), all of which would be lacking in pans. It would also be useful to consider the ratio of floral colour in the habitat at the time of sampling. If the community was dominated by yellow flowers, would the pollinators prefer this colour, or would they be attracted to an alternative? Ultimately, the use of pans in combination with other methods (ex. nets) has been suggested (Wilson et al. 2008) and in my study observations were able to offer additional information on floral use.

The morphology of yellow flowers and purple flowers could also affect insect preference. For Syrphids that primarily feed on pollen and have short, sponging mouthparts, flowers that lacked a deep corolla would be preferred (Gilbert 1981). Yellow flowers in this study offered easily accessible rewards for both syrphids and bees. Frequently visited flowers such as *Solidago*

spp. and *Zizia* spp. had floral masses or umbels with exposed anthers, short and small nectar tubes, and large areas for landing and foraging. *Dasiphora fruticosa* had multiple individual flowers with ample space for foraging and easily accessible anthers. Halictids, which are short-tongued bees, might also have little trouble accessing nectar from smaller flowers while at the same time easily gathering pollen on the surface of the inflorescence. Long-tongued bees like *Bombus* spp. could also access these rewards, but they could also forage on flowers with longer corollas (Fontaine et al 2006). Purple flowers such as *Liatris ligulistylis* and *Monarda fistulosa* (L.) have long nectar tubes that would be inaccessible to flies and small bees, and purple vetches like *Vicia americana* (Muhl. Ex Willd.) require a heavy bee to spring the release of the anther while finding nectar (Córdoba and Cocucci 2011). This could mean that syrphids and small bees visited flowers with easily accessible rewards based on morphology, which could be a more reliable determinant of interaction than colour alone (Stang et al 2006). For bumble bees, the inherent attraction to blue combined with the ability to access the rewards in longer corollas could predict interaction. This relationship between floral morphology and bee vs. fly visits was shown by Fontaine et al. (2006). Syrphids were less effective visitors of flowers with long nectar tubes, resulting in lower fruit set with fly visits when compared to open flowers. Bumble bee visits resulted in higher fruit set in flowers with long nectar tubes. Interestingly, bee visits resulted in few seeds per fruit in open flowers, suggesting that a combined bee and fly effort would be most ideal for floral reproduction (Fontaine et al. 2006).

4.4.4 Interaction strength and pollen loads

Investigating interaction strength can give an indication of the reliance of one species on another, and at times show how skewed that reliance may be. A combination of IS and pollen loads on insects provided more information than IS alone by assessing the quality of the visit

from a pollinator (Alarcón 2010). Pollen loads help determine whether or not an insect has as great an impact on floral reproduction as assumed, which could be important when considering their impact on the ecosystem or network (Popic et al 2013). An important caveat is that pollen loads should not be considered equal to information that could be provided by quantifying pollen deposition on the stigma (King et al. 2013), but pollen loads can at least act as a surrogate for pollen deposition, assisting in improved assumptions about the quality of interactions.

Each plant selected for interpretation of pollen loads and IS received visits from several insect species while in bloom, suggesting that pollinator identity or specialization did not have a strong influence on reproduction in the plant species examined. However, the identity and abundance of individual pollinators will still affect reproductive success of the plant, particularly if pollinators vary in the amount of conspecific pollen they carry (Alarcón 2010).

Toxomerus marginatus was responsible for many visits to flowers, yet assessments of pollen loads showed that these visits were not necessarily beneficial to the plant. *Toxomerus marginatus* had high IS with most species examined, particularly *Hypoxis hirsuta* and *Sisyrinchium mucronatum*. In the case of the genus *Hypoxis*, related species in Africa reproduce by apomixis and sexual reproduction (Zimudzi 2013), and plants in the genus *Sisyrinchium* have been found to be self-incompatible (Montgomery 2009). Preliminary pollination experiments carried out during my study showed that the two species produced significantly fewer seeds in the absence of pollinator visits (data not included). This means that insect visits must be important to these species, so an abundance of visits by *Toxomerus marginatus* might be beneficial. However, the pollen counts on *Toxomerus marginatus* were very low, suggesting that little pollen was actually transported between flowers.

Flies with aquatic larvae appeared to be important pollinators for a small number of flowering plants, meaning that annual variation in visitor abundance could have a negative impact on those species. *Symphyotrichum laeve* received almost all visits from *Eristalis* spp. and *Helophilus* spp. in 2010. Both syrphids had high pollen loads in combination with high IS with *Symphyotrichum laeve*. However, low precipitation in 2011 resulted in reduced larval habitat causing visits to *Symphyotrichum laeve* to plummet from triple to single digits in the following summer. No alternative insect species were observed as compensation for the extreme frequency of visits in 2010. In this case seasonal variability could have a strongly negative impact on seed production in 2011.

Comparisons of visits by bumble bees and *Toxomerus marginatus* revealed that bumble bees were important pollinators, even if observed visits were lower than those of syrphids. *Liatris ligulistylis* and *Dalea purpurea* appeared to benefit from interactions with syrphids and bees based on the high IS between those species. High pollen loads revealed that *Bombus* spp. was a more important pollinator for *Liatris ligulistylis* and *Dalea purpurea* despite the comparable IS from *Toxomerus marginatus*. This greater understanding of the contribution of bumble bees to floral reproduction for particular species is important, as my study showed that in newly burned sites, bumble bees might require additional time for recolonization. Flowers with nectar in a longer corolla tube, such as *Liatris ligulistylis*, would be more accessible to insects with longer tongues such as bumble bees (Armbruster and Guinn 1989), yet *Liatris ligulistylis* present in newly burned sites could suffer from a lack of bumble bee pollination.

Halictid bees generally had low IS for the species examined, yet pollen loads showed that these infrequently observed visits were of higher quality than those of more frequent visitors. Bees in the genus *Lasioglossum* contributed relatively few observed visits when compared to

Toxomerus marginatus, but pollen loads were 10-100x that of *Toxomerus marginatus*. Therefore, while IS was low between *Lasioglossum* and flowers such as *Hypoxis hirsuta* and *Sisyrinchium mucronatum*, each individual interaction had a greater potential contribution to floral reproduction.

Some of the flowering species had very low combined IS for the pollinators considered despite being selected for their abundance and/or high frequency of visits in the community. The selected insects made up to 75% of the visits to six of the nine flowering species examined. However, not all interactions from the network were included in this table, so low IS should not necessarily be interpreted as poor quality interactions. For example, small halictid bees in the subgenus *Dialictus* visited 37 different species in 2010 and 2011 combined. Larger halictid bees in the genus *Lasioglossum* visited a variety of flowering species in several families in this study, and they have been reported from at least 22 different families of plant (McGinley 1986). This generalist behaviour would make it difficult to observe all interactions that took place, while also lowering apparent IS to individual flowering species. Some of the plants selected were also frequently visited by insects that were not part of the bee and syrphid guilds. *Zizia aptera* had low IS with the majority of insects selected, but what wasn't shown was that *Odontomyia pubescens* (Day, 1882), an early season soldier fly, was responsible for over 50% of the visits received. *Odontomyia pubescens* was only observed visiting *Zizia aptera* in New 1, 2010. *Parnassia glauca* was an interesting case where the low IS more accurately reflected that few insects were observed visiting this species with regularity. Insects that did prefer this flower were less "traditional" pollinators, such as Tachinid (parasitoid) or Ulidiid (picture-winged) flies.

Other studies have attempted to classify interaction quality in the absence of pollen loads. A high frequency of visits by pollinators has been related to a greater overall contribution to

plant reproduction (Vázquez et al. 2005, Ne'eman et al. 2009), but this seemed to be a combined effort from multiple pollinator species and not necessarily between a specific interacting pair (Vázquez et al. 2005). However, many poor visits, rather than infrequent good visits, might be beneficial in some communities. This could be an important alternative following disturbance such as fire, particularly when high quality interacting partners might experience post-fire declines in abundance. A small amount of cross-pollination might at least reduce the negative effects of pollen limitation, inbreeding depression, self-fertilization or no fertilization on seed set (Aizen and Harder 2007).

Using the combination of pollen loads and IS offered greater insight into the quality of interactions than IS alone. Had IS been used as the only measure of the importance of particular links in the network, the actual importance of an interaction, or the dependence of one species on another, could have been exaggerated.

4.5 Conclusions

Despite differences in burn age and temporal variation in climate, all pollination networks were highly nested. This nested structure is thought to offer ecosystem stability by providing interaction redundancies that buffer against species extinctions or the uncoupling of interactions. This suggests that tall grass prairie pollination networks are resilient and adapted to both disturbance and annual variations in environmental conditions.

Building blocks that considered abundance, phenology, and colour preference offered insight into network structure, but did not always explain all interactions. Frequently visited

species were not necessarily the most abundant in sites, and insects that were pan trapped in greater abundance were not always the most frequent visitors. In the case of phenology, peak flowering and visits received were not correlated, and syrphid flies visited flowers whether in peak bloom or not. Bees were more likely to visit flower at their peaks, but this could have been related to the floral preferences developed by bees based on rewards. While the phenological overlap between insects and flowers was important, it appeared that insects were quite plastic in their preference for hosts and could adjust foraging patterns to suit the changes in phenology brought about by fire and annual variation. However, more statistical analysis should be completed to make further conclusions on the role of phenology. When trait matching was considered, colour preference was an important predictor of interaction, particularly for bumble bees and syrphids in pan traps. These preferences were not always comparable for flowers, but traps were lacking the same attractants that would have been found in nature.

The interactions taking place in the network could not be assumed to be equal in terms of quality. Interaction strength in combination with pollen loads revealed that frequently visiting species may contribute very little to pollination if few pollen grains were carried per visit. Infrequently visiting species with high pollen loads could have a greater impact on floral reproduction in some cases. This emphasized the importance of assessing interactions at the species level, and that quantifying the impact of interactions offers greater insight into the relationships between plants and insects.

While assessing pollination networks may provide greater confidence in the stability of the ecosystem, understanding individual interactions could be very important for conservation of tall grass prairie. While much of the interactions were due to generalists, strong preferences for particular flowering species were detected for some pollinating insects. More specialized

interactions could be lost through reductions in habitat, fragmentation, and overly intensive management. Efforts to increase the area of protected habitat and lengthened fire intervals could assist in maintaining the generalist species that make up the stable core of interactions in this rare habitat.

5. CONCLUSIONS AND FUTURE DIRECTIONS

An attempt was made to disentangle a great deal of ecological complexity in this study. The tall grass prairie ecosystem contains innumerable interactions between flowering plants and their insect pollinators. Efforts to describe the structure of the community as a whole through network analysis, or to quantify the impact of individual visits, required a variety of approaches. The combination of diversity measures, multivariate analyses, and network metrics were a good start, yet the data collected could certainly be tested and manipulated in other ways. However, the trends and significant results determined here have begun to reveal how the tall grass prairie community persists through fire and annual variation in environmental conditions.

This study showed the value of combining binary metrics with assessments of community composition. Binary metrics determined that the sites sampled were significantly nested, which implied stability of community structure and resilience to disturbance. While this thesis was being written, more research was done on the prominence of nestedness in communities in a variety of habitats. It was determined that nestedness was inherent of pollination networks with greater than 50 interacting species (Bascompte et al. 2003, Olesen et al. 2012). Yet it is still interesting to look closely at the community beyond the metric to determine how it becomes nested.

By examining the potential effects of fire on plants and insects separately, I was able to better understand how these interacting partners might contribute to network structure. The flowering plant community showed adaptation to fire. The perennial community increased floral abundance and accelerated flowering in the first year following fire, yet the species richness in sites was site-specific and did not appear to be affected by burning. This suggests that floral

richness was more reliable in sites than anticipated, likely a positive finding in terms of pollinator resources. Insect species composition was also dependent on site, but bees that nested in litter were more likely to decline following fire. Important pollinating flies did not show an effect of fire; their abundance appeared to be strongly related to the presence of larval habitat. The changes in peak flowering due to fire did not appear to influence peak insect activity through the summer. On the whole, the flowering and insect communities seem to be resilient to disturbance, with their presence being more related to previous land use or the availability of unburned refugia.

Network building blocks helped to explain some of the interactions in the community, yet some of the assumptions about the building blocks were not met. Abundance of species only partly explained the likelihood of interactions, with some less abundant species being more frequently visited. Overlaps in peak flowering and peak insect activity occurred in some cases, but many of the interactions might have been better explained by pollinator preference. Pollinators were found in greater frequency on particular colours, with bumble bees preferring blues and halictid bees and syrphids preferring yellow in most cases. Ultimately, there are floral cues, overlapping phenologies, and patterns in species abundance and richness that all contribute to network structure.

Pollination networks in tall grass prairie were maintained by a high proportion of generalist interactions. Generalists act as a connection between different subsets of interacting partners (Martin-González et al. 2009), essentially acting as hubs of the community where species are sewn together through their interactions. Generalists increase the likelihood of links persisting between species, particularly if extirpation or extinction events occur (Memmott et al 2004). Generalists also maintain specialists in the community. An example was the relationship

between *Dufourea* spp. and their hosts *D. fruticosa* and *Helianthus* spp. Both flowering species were generalists that supported these specialist insects.

Interaction strength combined with assessments of pollen loads showed that not all interactions carried equal weight in the community. Interaction strength can act as an indication of the quality of interactions, with high IS reflecting high quality. However, IS can be misleading without the addition of pollen loads. Pollen loads showed that interaction quality was exaggerated in some cases, with some frequent, generalist visitors having poor pollen loads and so a reduced likelihood of pollen deposition. However, this method is a surrogate to deposition, and further study is needed for the actual impact of pollinator visits on floral reproduction.

Pollinator quality is certainly an important component of network studies, as it reveals more about the network than binary metrics alone. Forup and Memmott (2005) showed how pollen loads can be used to determine the success of prairie restorations. They concluded that similar pollen loads between restored and remnant habitat indicated success. Alarcón (2010) used pollen loads to warn of making assumptions that all insects contribute equally to pollination in a network, which was supported and expanded upon by Popic et al. (2013). King et al. (2013) assessed pollinator quality by quantifying pollen deposited on stigmas from single visits, showing that not all flower visitors should be considered functional pollinators in a network. Most recently, Ballantyne et al. (2015) combined counts of pollen deposits on stigmas (pollinator effectiveness) with visit frequency to create a measure of pollinator importance. They determined that visitation networks overlooked specialized interactions between insects and plants, and that combining visit data with assessments of pollinator quality are the best way to develop the most realistic networks (Ballantyne et al. 2015).

By adding assessments of pollinator quality to network analysis, we can improve our understanding of the relationship between visits and pollinator services to plants. This is important to consider in rare, fragmented habitat such as tall grass prairie. Fires are an important prairie management tool as they mimic natural disturbance. While this disturbance is close to what would have been experienced historically, the size of tall grass prairie has changed dramatically. Flowering plants in disturbed, fragmented habitat could be susceptible to reductions in reproductive success from limited pollen deposition, including generalist species (Aizen et al. 2002, Ashworth et al. 2004, Aizen and Harder 2007). Under pollen limitation, poor pollen deposition by pollinators results in reduced seed production (Ashman et al. 2004). This can result from an inadequate frequency of visits from pollinators, poor pollen deposition by the insect visitor, or by the deposition of conspecific pollen (Ashman et al. 2004). Gaining a better understanding of pollen transport in prairies could help mitigate the possibly negative effects of management on species contained in prairie fragments.

Studies of plant-insect interactions have resulted in greater public awareness of pollinator conservation. Recommendations for planting the most beneficial flowers for pollinators creates new habitat and resources for pollinating insects, while at the same time increasing crop production when created between agricultural fields (Wratten et al. 2012, Robson 2014). More studies are interested in creating and assessing the functionality of urban bee habitat for conservation (McFrederick and LeBuhn 2004, Hernandez et al. 2009). Also, groups supporting the protection of arthropods and their habitat are sharing research with the public to improve their knowledge of insect conservation (ex. The Xerces Society, Pollinator Partnership, David Suzuki Foundation).

Conservation of prairie and pollinating insects is extremely important, yet a large scale assessment of plant-insect interactions in the Tall Grass Prairie Preserve had not been undertaken. This study has created baseline data for the responses of the flowering and insect communities to fire and annual variations in climate. I have documented thousands of interactions occurring in this habitat, and have attempted to highlight the major trends in community composition, network structure, and species specific interactions that occur. It is recommended that surveys of plant-pollinator interactions be expanded upon to include different habitat characteristics, as well as changes in community composition through time.

By gaining a better understanding of network dynamics, we can better preserve the plant and insect community in tall grass prairie.

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