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**PLANT-POLLINATOR MUTUALISMS:
COMMUNITY STRUCTURE, FUNCTION AND MANAGEMENT**

A Dissertation in

Ecology

by

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ABSTRACT

This thesis combines both experimental work and theoretical models to address the taxonomic and phenological structure of mutualistic interactions between plants and pollinators, implications for management and conservation of ecosystem services in agroecosystems, and potential impacts of perturbations on the structure of these interactions. I explore the utility of network theoretical models in quantifying, visually representing, and analyzing mutualistic community structure, as well as the potential for such models to direct management objectives.

The first chapter is an introduction that gives an overview and definitions of frequently used terms in the work. It is followed by an investigation of the importance of taxonomy in structuring mutualistic interactions (chapter two). Using a large legacy dataset of plants and insect visitors, I provide evidence that specialization at the regional level is lineage-specific and find that patterns of significant taxonomic conservatism are common; in other words, closely related species are more likely to interact with similar partners. I also identify groups of plant species and their insect visitors that have correlated taxonomies, suggesting subsets of strongly interacting groups within the whole.

Communities of wild bees have been shown to be capable of fully supplying the pollination requirements of agricultural fields. For this reason, many land managers seek to supplement the pollination services provided by honeybees by supporting the diversity and abundance of wild bees. In chapter three, I identify plant species capable of provisioning communities of wild bees with floral resources, and demonstrate how floral

provisioning habitat can be targeted to support individual species, or groups of species, over time.

In chapter four, I construct theoretical models to explore the impact of the integration of a novel plant species into a community of interacting plants and pollinators. I find that the impact of a novel species on community structure is mediated both by the number and type of interactions it forms with native species. Testing experimental data from a community of plants and pollinators, I find that certain network properties are reliably predicted by these interaction models, while more complex properties are dependent on link identity.

Though much of this work focuses on communities of pollinators specifically adapted to the collection of floral resources, I also demonstrate that groups of lesser-known taxa can contribute to pollination services, and that their association with plants can be important for one or both of the interacting organisms. In chapter five, I therefore emphasize the importance of capturing and examining groups of species other than those which are commonly thought to be the most important floral visitors as a way of quantifying the diversity of arthropods.

I have also found that disturbances, such as species invasions, do not always necessarily have negative connotations for mutualistic partners. Indeed, I show in chapter six that the weed species that persist in marginal habitat may be strongly preferred by groups of threatened pollinators, and therefore may contribute significantly to the persistence of pollinator communities. Thus, the invasion of non-native species may have both detrimental and beneficial effects.

Finally, in chapter seven, I find that structural properties of the interactions between plants and insects can be manipulated on a community level. In addition, I find that subtle manipulations can lead to large changes in the composition of plant-pollinator communities. I also show that insects forage opportunistically and respond rapidly to perceived changes in the quality and attractiveness of flowers. Thus, the identity of the species in the community does strongly influence the structure of interactions.

Using community-level approaches, as I do in this thesis, demonstrates that networks can be a useful tool for studying emergent ecosystem services, such as pollination. I synthesize my findings in a conclusion chapter (chapter eight). Overall, I find that the structure of mutualisms between plants and insect visitors can provide insights into both fundamental ecological questions and applied contemporary challenges.

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Chapter 1

Introduction

The study of ecological communities dates back to the very origin of ecology as a field; the term was coined in the early 20th century (Warming 1909), but, conceptually, community ecology existed long before that. Even Ernst Haeckel (commonly attributed with the origin of the term “oecology” in the 1860’s) was interested in communities of plants and animals; certainly, Darwin before him was fascinated by the interactions between species, especially mutualisms.

An ecological community is a “set of species that live together in some place” (Loreau 2009). Though not extremely useful as a strict definition, this expresses the flexibility of what constitutes a “community”; namely, that it depends on the system and the questions being asked. Despite the lability of this term, there are some fundamental concepts associated with ecological communities. They must comprise more than one species, and those species must interact. In addition, the spatial scale of the community is critical, and is often used to define the threshold between what constitutes an ecological community, within which biotic interactions have a dominant influence on the distribution and dynamics of populations and species assemblages, and a region, where biogeographical factors may play a stronger role (Webb et al. 2002). That said, there are ecological communities on every spatial scale, from the microbes living and interacting within a drop of water, to forests that cover large areas of land and the plants and animals

that live within them. For example, the symbionts living within the gut of a human being can be considered a “community” (Hooper and Gordon 2001).

Communities are also quite distinct from ecosystems in that they focus on the biotic interactions between groups of species in a given time and space rather than the combination of biotic and abiotic factors that lead to energy and nutrient flows. These biotic interactions, which comprise ecological communities, can be divided into loose categories: antagonistic (beneficial for one organism, negative for the other), commensal (one organism benefits, there is no effect on the other), amensal (negative for one and neutral for the other), competitive (negative for both organisms) and mutualistic (beneficial for both organisms). A community can be defined as a subgroup of the total by the type of interactions it comprises. Hence, a mutualistic community is one comprising mutualistic interactions between organisms in a given location.

Mutualistic interactions are ubiquitous in nature. Indeed, life as we know it would not exist without mutualisms. For example, eukaryotes are incapable of fixing nitrogen from the air. Certain species of plants, however, are able to fix nitrogen via a mutualistic symbiosis with nitrogen-fixing bacteria that live in specialized root nodules (Kiers et al. 2003). However, in spite of their ubiquity, mutualisms are relatively understudied compared to competitive or antagonistic interactions, and their implications for natural selection and coevolution are often poorly understood (Chapter 2).

The mutualistic interactions between entomophilous plants (78-94% of terrestrial angiosperms (Ollerton et al. 2011)) and their insect pollinators are diffuse and generalized (Waser et al. 1996). Darwin himself was fascinated by this mutualism, and he wrote about it at great length (e.g. (Darwin 1862)). Indeed, he was able to predict the

existence of a moth by observing the morphology of a flower (to the best of my knowledge, this is the only species whose existence has been successfully predicted before it was discovered) (Arditti et al. 2012). Though extremely specific interactions, such as the one between the Long-spurred Malagasy Orchid and Morgan's Sphinx, do exist, the vast majority of plant-pollinator interactions are far less specific. In aggregate, however, this mutualism has arguably led to the impressive diversity of both floral forms and insects specially adapted for the collection of floral resources (e.g. bees) (Cardinal and Danforth 2013). I address the taxonomic structuring and conservatism of plant-pollinator mutualisms in a large legacy dataset (Chapter 2).

When studying pollination mutualisms, it is common to focus on the superfamily Apoidea (bees). This superfamily has specific adaptations for collecting pollen, which is the primary protein source for the developing young. Bees are evolutionarily derived from predatory wasps, replacing insect prey with pollen (Cardinal and Danforth 2013). Their specific adaptations include branched body hairs (which pollen clings to) and often specialized pollen baskets or scopa (the location of which depends on the bee and is usually reserved for females). Bees are effective vectors for the dispersal of the gametic phase of the otherwise sessile plants (Free 1970). At the same time, pollen and nectar are resources which have fueled the radiation of the bees (Cardinal and Danforth 2013). Thus, several of the chapters of this thesis focus on bees specifically (i.e. Chapter 3, 6, and 7). However, there are many other insect species that visit and pollinate plants; the broader insect community is addressed in Chapters 2, 4, and 5.

In many ways, mobile mutualisms, where one or both interacting organisms are able to move away from each other, such as plants and their pollinators or seed

dispersers, are more difficult to study than intimate mutualisms, where one organism lives within the body of the other (e.g. coral animals and *Symbiodinium*) (Thompson 2005). The exchange of resources and population level dynamics of the mobile partner are extremely difficult to track, especially when they are rare in a large area. Thus, sampling effort and resolution remain challenges for the standardization and communication of data on these systems. As part of an effort to address the logistical challenges of characterizing insect diversity in agricultural systems, I develop a new trap for aerial arthropods in Chapter 5.

The study of mutualistic communities is particularly important with regard to several contemporary ecological challenges. For example, mutualisms are critical for the establishment and spread of invasive species, which become integrated into native communities (Chittka and Schurkens 2001) (Chapters 4 and 6), mutualistic interactions are key to the conservation of many species (Christian 2001) (Chapters 3, 4, and 6), and, in addition, the development of many of the agricultural crop species that we depend on relies on pollination from insect mutualists (Klein et al. 2007) (Chapters 3, 6, and 7).

To address the impact of invasive species on mutualistic communities, many studies have compared invaded and uninvaded communities (e.g. (Aizen et al. 2008)) and even experimentally removed species (Lopezaraiza-Mikel et al. 2007), but the experimental addition of species is rare (largely because deliberately introducing an invasive species without allowing it to spread or escape is logistically challenging). In Chapter 6, I address this gap by demonstrating the impacts of the experimental introduction of an invasive thistle on the insect community, with a special focus on the response of the bee community.

Of particular contemporary concern is the collapse of honeybees as agricultural pollinators (Williams et al. 2010). The relationship between humans and one species of domesticated bee (*Apis mellifera*) dates back to at least 2400 BC in Egypt (Buchmann 2006), but recently this species has been declining, due to a number of threats (including increased pesticide usage, as well as susceptibility to introduced pathogens and parasites) (vanEngelsdorp and Meixner 2010). Many land managers have shifted their focus from honeybees to communities of wild bees (Greenleaf and Kremen 2006). These native species of bees are capable of fully supplying the pollination requirements of agricultural fields, but they require sufficient habitat along field margins (Kremen et al. 2002). The precise landscape and in-farm management strategies that will best promote these wild bees are under investigation, with many promising potential avenues (Chapter 3).

One of these relatively novel approaches to conserving and managing communities of wild bees is the use of network theory. The usage of networks to visually represent and analyze ecological communities has become increasingly prevalent (Bascompte 2007, Ings 2009). In particular, the representation of mutualistic communities as bipartite networks has become common (Bascompte and Jordano 2007). Bipartite networks are a natural model of mutualistic interactions: they comprise two sets of nodes (representing two different sets of species) which are, in this case, joined by bidirectional links (representing mutually beneficial interactions).

There are both advantages and disadvantages to the application of this method. Many have brought up concerns addressing the sensitivity of network theoretical methods to sampling effort. Indeed, many network-level measures are sensitive to both the size of the community (i.e. number of species) and the frequency of interactions (Dormann et al.

2009, Rivera-Hutinel et al. 2012). Both of these factors in turn are strongly affected by the sampling effort: species rarefaction curves demonstrate that the number of species observed increases with sampling effort (Gotelli and Colwell 2001), and more frequent interactions are more likely to be observed (Bluthgen 2010). On the other hand, there are network-level attributes that are robust to sampling effort, as well as community size and interaction frequency (Nielsen and Bascompte 2007, Dormann et al. 2009, Rivera-Hutinel et al. 2012).

Along the same vein, network representations of mutualistic communities tend to be temporal and/or spatial snapshots, while, in reality, mutualisms such as pollination are labile and dynamic in nature (Petanidou et al. 2008, Carlo and Yang 2011). Indeed, the limited phenologies of both interacting partners lead to constraints on possible interactions (i.e. partners that are not active at the same time of year will not have the opportunity to interact, hence it is a forbidden link) (Vázquez et al. 2009). To explore the importance of seasonality in an applied setting, I address the matching of floral resource provisioning to pollinator phenology in agricultural settings in Chapter 3.

Attempts are being made to address the challenges facing network theoretical approaches to community ecology. The many possible network-level measures are being evaluated in depth for their sensitivity to sampling (Dormann et al. 2009, Hegland et al. 2010, Rivera-Hutinel et al. 2012) and networks are being characterized with increasing information about interactions (e.g. pollen-based networks (Ackerman and Roubik 2012), character traits (Eklöf et al. 2013). All of these challenges are opportunities for new avenues of research to improve the applicability of these measures, with promising implications for our understanding of community structure and dynamics.

The conserved properties of plant-pollinator networks also make them useful for making predictions about the impacts of species introductions on the structure of mutualisms. This is important both because it will help us to understand the impacts of invasive mutualists, but also because species are being moved deliberately for conservation purposes, to protect them in the face of a changing climate. I develop several theoretical models predicting the impact of species additions in Chapter 4.

Regardless of these challenges, much has already been learned from the application of networks to pollination mutualisms. Observational and comparative studies worldwide have shown that some structural properties of mutualistic communities are remarkably conserved (Olesen and Jordano 2002, Bascompte et al. 2003, Jordano et al. 2003, Vazquez and Aizen 2004, Bastolla et al. 2009, Thébault and Fontaine 2010). For example, pollination networks tend to be highly nested, but not compartmentalized (Bascompte et al. 2003). They are asymmetric (Bascompte et al. 2006), generalized (Waser et al. 1996), and robust to the random loss of species (Kaiser-Bunbury et al. 2010). The implications of these attributes are being thoroughly investigated with theoretical studies (e.g. (LaBar et al. 2013, Campbell et al. 2011, 2012), but empirical and experimental works are less common. Thus, in this thesis, I address both the question of whether networks of species interactions can be experimentally manipulated, and whether these manipulations do indeed result in a desired effect on community function in Chapter 7. Because of the importance of pollination mutualisms to many plant and insect species, the ability to deliberately manipulate these complex networks of interactions also has implications for conservation and management.

I summarize these chapters and suggest new avenues for research in Chapter 8.

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Chapter 2

The taxonomic structuring and conservatism of plants and their insect visitors

submitted

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Abstract

Despite the ubiquity of plant-pollinator mutualisms, questions remain about how communities of plants and pollinating insects are structured. To detect and quantify taxonomic structuring in such mutualisms, we analyzed a 33-year legacy dataset comprising over 13,000 unique interactions between 1,174 insect species and 455 plant species in a tallgrass prairie ecosystem. We applied two statistical analyses: first to test the hypothesis that there is taxonomic conservatism in plant-insect interactions, and second to test the hypothesis that there are correlations among their taxonomic hierarchies. For the first time, we provide evidence that specialization at the regional level is lineage-specific, and that the architecture of this community is taxonomically

structured. We find that patterns of significant conservatism are common in this community of plants and insect visitors; in other words, closely related species are more likely to interact with similar partners. Finally, our methods identify groups of plant species and their insect visitors that have correlated taxonomies, suggesting subsets of strongly interacting groups within the whole.

Introduction

Charles Darwin predicted the existence of a moth with a 33-cm proboscis based on his observations of the corolla length of the Long-spurred Malagasy Orchid (*Angraecum sesquipedale*) (Arditti et al. 2012); he suggested that natural selection might act on the characters that facilitate interactions between tightly linked mutualistic partners, leading to reciprocal selection and coevolution (Darwin 1877). Although interactions of this extreme specificity are rare in nature (Johnson and Steiner 2000), there are clear adaptations among flower-visiting insects for pollen and nectar collection, as well as adaptations among entomophilous plants for attracting insect visitors (Leppik 1957). Indeed, morphology-based taxonomy accounts for traits in insect species, especially among the flower-visiting insects, that directly relate to their interactions with flowers (e.g. tongue length, scopal position, hair branching). Additionally, taxonomy of plant species is strongly influenced by traits that directly relate to their interactions with insects (e.g. corolla length and other floral characters).

However, the main driver behind the structure of interactions between plants and their floral visitors is unknown. For example, the pollinator syndrome hypothesis predicts

clear segregation of pollinators based on floral morphology (Faegri and van der Pijl 1979, Ollerton et al. 2009), yet most insects visit a wide range of floral morphologies (Bosch et al. 1997, Ollerton 1998, Hingston and McQuillan 2000, Ollerton et al. 2009, Danieli-Silva et al. 2011). In addition, plant-pollinator communities tend to (1) include mostly generalist plant and pollinator species, which have large numbers of mutualistic partner species (Waser et al. 1996, Bascompte and Jordano 2007, Petanidou et al. 2008), (2) be asymmetric, with specialist plants and pollinators that tend to interact with partners that are generalists (Vázquez and Aizen 2004), and (3) have mostly weak interactions with occasional rare strong interactions (Fortuna and Bascompte 2006).

Our key question is the extent to which plant-insect associations segregate according to morphology (and hence taxonomy), as opposed to being taxonomically generalist, diffuse, weak, and unstructured. Here, we use two different statistical analyses to describe taxonomic patterns in networks of plants and floral visitors. First, we test the hypothesis that there is lineage specificity in plant-insect floral interactions. Lineage specificity (i.e. specific adaptations along a given line of descent) means that species tend to interact in a taxonomically structured fashion, and has been demonstrated in other mutualistic interactions, such as orchid-mycorrhizal systems (Jacquemyn et al. 2011), and in host-parasite interactions (Aguileta et al. 2009). Second, we test the hypothesis that the taxonomic hierarchies of interacting groups of species are correlated in their structure. Lineage specificity and correlated taxonomic hierarchies in plant-insect interactions can indicate some degree of trait-based matching, because many of the key characters used to distinguish taxa are precisely the traits that are important for plant-insect interactions (e.g. pollen is carried on abdominal scopa of females in the genus

Megachile, but in tibial baskets in the genus *Apis*). This would suggest that, for the taxa considered, plant-pollinator interactions are based on the phylogenetic conservation of ecological niches (Wiens et al. 2010).

Importantly, the correspondence of forms between different species that access similar resources is a community-level phenomenon: an emergent property that arises from the whole rather than from individual interactions (Strauss and Irwin 2004). Hence, the structure of these interactions can be diffuse and generalist, and therefore less reliant on tightly correlated taxonomic hierarchies and strict morphological matching. Thus, to detect taxonomic structuring, it is necessary to analyze communities with several higher-order taxa, and look for the effects at all taxonomic levels.

Because detection of taxonomic structuring requires taxonomically-broad data, we used a legacy dataset that incorporates the interactions between 1,174 insect species and 455 plant species over a period of 33 years (Robertson 1929, Marlin and LaBerge 2001). The large area, long time scale, and comprehensiveness of our interaction data offer an unprecedented view into the relationships between plant-insect interactions and taxonomic structure at the regional scale (Webb et al. 2002), and permit statistical testing at significance levels unavailable from smaller datasets. Our approach also allows us to evaluate the same community from two perspectives, those of the insects and those of the plants, whereas most studies have been plant-centered (Strauss and Irwin 2004). In this community of potentially mutualistic partners, we find that a correlation between taxonomic structure and interaction structure is common, but not universal. In addition, our methods allow us to directly detect which groups of interacting species have correlated taxonomies. We conclude that there is a strong relationship between the

taxonomy and interaction structure of insect and plant species in this tallgrass prairie ecosystem.

Materials and Methods

Dataset

We used a dataset originally compiled by Charles Robertson, who observed insects visiting flowers in a tallgrass prairie ecosystem from 1884 to 1916 over an area of more than 225,000 hectares in central Illinois (USA) “...for the purpose of ascertaining the different kinds of insect visitors” and self-published his data in *Flowers and Insects* (Robertson 1929, Parks 1936). This dataset has been analyzed independently elsewhere for other questions (e.g. Memmott and Waser 2002), and for a large portion of this dataset, one of us previously updated the taxonomy of the plant species and several taxa of insect visitors (Tooker and Hanks 2000, Tooker et al. 2002, Tooker et al. 2006, Graham et al. 2012).

Our effort here addresses a subset of Robertson’s full dataset comprising parasitoid wasps (Tooker and Hanks 2000), Lepidoptera (butterflies, skippers, and moths, Tooker et al. 2002), Diptera (flies) including those of importance for biological control (Tooker et al. 2006), Coleoptera (beetles, Graham et al. 2012), Hemiptera (bugs), and long- and short-tongued bees (Table 2-1). The only group omitted from the original dataset is the predaceous wasps, the taxonomy of which has not been updated. Taxonomy of short-tongued bees relies on Robertson’s original nomenclature, whereas the long-tongued bees (Apidae and Megachilidae) were updated using two references

(Krombein et al. 1979, Michener 2000) and then verified by Dr. Charles Michener (Kansas University). Taxonomy of Hemiptera was updated using primary literature and the Planetary BioDiversity Inventory for Plant Bugs (<http://research.amnh.org/pbi/>). Our dataset contains 13,106 unique interaction types between 1,174 insects of five different orders and 455 plants of 37 different orders. The broad geographic and temporal scales of Robertson's observations make them a useful tool for evaluating community-level dynamics between plants and floral visitors (Parks 1936). Because Robertson did not provide data on strictly defined pollination events, we use visitation events as a proxy for a mutualistic interaction, though we recognize that visits do not necessarily imply pollination.

From the list of interactions provided in the Robertson dataset, we created a matrix of species interactions, where rows represent insect species and columns represent plant species. The interactions are binary, representing presence or absence of interaction between a plant species and an insect species. From the binary matrix, we next generate two matrices of ecological dissimilarities using a Jaccard algorithm (Chao et al. 2004). The algorithm calculates a value quantifying the similarity of interactions between pairs of insect species or pairs of plant species. This process results in two square matrices: one of insect dissimilarities and one of plant dissimilarities. Using insects as an example, a 0 in the dissimilarity matrix represents insect species with complete overlap in plant species visitation and a positive value implies some overlap; an index of 1 indicates that the two insect species visit no common plant species.

We also generated two square matrices of taxonomic distances (one for plants and one for insects). These have decreasing values for increasing relatedness between

species. A value of 0 indicates that two species are congeners, 1 that they belong to the same family but not genus, 2 that they belong to the same order but not family, 3 if they are in different orders (Rezende et al. 2007). We used taxonomic distance matrices as proxies of phylogenetic relatedness. Analyses using complete community-level phylogenies would yield higher resolution insights, but such data are not currently available.

Statistics

We use two complementary statistical tests: one to address the correlation between the taxonomic history and interaction structure of a given group (lineage specificity), and the other to address the correlation of relationships between interacting partners. The simplest way to look for an association between evolutionary history and ecological function is to use a permutation test to compare taxonomic distance and ecological dissimilarity matrices (Mantel 1967). We therefore first did separate tests using the insect matrices and plant matrices. Statistical significance was calculated from permutation tests as implemented in the “vegan” package for R (Oksanen et al. 2011, R Core Development Team 2008). The permutation test shows whether more taxonomically related insect or plant species are significantly more likely to interact with similar partners than expected by chance alone.

We examined correlations between taxonomic and ecological dissimilarity matrices for the complete dataset of interactions (including all orders of plants and insects), and for select subsets of the data. Specifically, we tested each of the five insect orders and their associated plant species separately, as well as the families of short- and long-tongued bees, parasitoid wasps (for which we had subfamilial data) (Table 2-1), and

the three largest orders of plants (Asterales, Lamiales, and Fabales). For each subsample of the complete dataset, we tested for correlations within both plant and insect species. We applied a Holm-Bonferroni correction (a sequential Bonferroni test, Ludbrook 1998) to our significance values to correct for multiple hypothesis tests.

The Mantel permutation test is the standard way to investigate if taxonomies correlate with ecological function (and as we show below it is a good method to do just that for insect and plant species). However, it does not address the question of whether the taxonomy of plant species is related to the taxonomy of visiting insect species. In other words, groups of taxa within the entire community may have correlated taxonomic hierarchies. In addition, it is still possible for groups of species within the whole to interact more strongly than others. To address these questions, we used Legendre's (1997, Legendre et al. 2002, and see ter Braak et al. 2012 for an additional alpha correction) fourth-corner method.

The fourth-corner method “offers a way of analyzing the relationships between the supplementary variables associated with the rows and columns of a binary (presence or absence) data table” (Legendre 1997). In the original application of the method, the supplementary variables were behavioral traits or characteristics of species, and the binary data table was a matrix of presences or absences of those species in given habitat types. We applied this method to a similar set of matrices, though their contents are importantly different from the original application. Essentially, we had a binary (presence or absence) matrix of interactions, and two matrices of hierarchical taxonomic distances that we arranged in a square with a missing corner (Fig. 2-1). We created three data matrices of differing dimensions: \mathbf{L} which represents the interactions between the n

plant species and m insect species, \mathbf{R} which is the $n \times n$ matrix representing taxonomic distances between plant species, and \mathbf{Q} which is the $m \times m$ matrix representing taxonomic distances between insect species. The test itself searched for relationships between the taxonomic classifications of insect and plant species by generating a contingency table. The significance of values within the table was calculated by permuting the rows of the interaction matrix \mathbf{L} to create a distribution of chi-squared statistics. We applied the fourth-corner method to identify correlations at both the order and family levels. For each test, we ran 999 permutations to determine significance of the correlation. The source code for all tests used is in Appendix A.

While the Mantel test allows us to compare one taxonomic hierarchy with one set of interactions, we are really interested in estimating the ecological associations among the two different taxonomic hierarchies as is provided by the fourth-corner method. To the best of our knowledge, our application of the fourth-corner method to test for correlated taxonomic structures is novel, and our results show that it is a useful method for this task.

Results

Mantel test

In the full dataset, there was significant correlation ($P < 0.001$, standardized Mantel correlation statistic = 0.075) between plant taxonomy and interaction structure and between insect taxonomy and interaction structure (Table 2-2). Thus, across the entire region studied by Robertson (1929), taxonomic relatedness between species is a significant predictor of overlap in their floral or insect partners. We also found

significant relationships between taxonomy of the plant species and their interaction structure, and between the insect species and their interaction structure in most order-level subsets of the data, with few exceptions (Table 2-2). For the exceptional cases (parasitoid wasp species, including braconids, plant species visited by Coleoptera, Hemiptera, and Diptera [Table 2-2]), only one of the interacting partners of the subset had a significant correlation between taxonomic and interaction structure, thus demonstrating asymmetric interactions. For all tests, the significant correlation statistics varied between 0.03 (insect order Coleoptera) and 0.26 (plant order Fabales). These significant correlations indicate that closely related species tend to interact with similar partners, but the correlation statistics are constrained to small values by the nature of the method, and the size of the input matrices (Urban et al. 2002). Thus, strength of our correlation statistics should not be compared between subtests. However, our results were not sensitive to changes in the weighting of interaction frequencies (1, 3, 5 vs. 1, 2, 3) or changes in the weighting of taxonomic distances (1, 2, 3 vs. 1, 5, 10).

Fourth corner method

The fourth-corner method detected a number of positive and significant correlations between the orders (Fig. 2-2) and families (Fig. 2-3) of insect and plant species. At the order level, we found 22 significant ($P < 0.001$) correlations and, on the family level, there were 162 significant correlations at the $P < 0.01$ level and 313 significant correlations at the $P < 0.05$ level (ter Braak et al. 2012). After controlling for the number of families per order, there were disproportionately more significant correlations in Hymenoptera (86 correlations) than any other insect order, and more significant correlations within Apiales (35) than any other plant order. The insect family

with the largest number of significant correlations was Apidae (honeybee family) (20) and the plant family with the largest number of significant correlations was Apiaceae (carrot family) (38).

Many groups traditionally considered to be pollinators share similar interactions and are distinct from groups less likely to be considered pollinators. For example, Syrphidae (hoverfly family) and Andrenidae (mining bee family) share a significant correlation ($P < 0.01$) with Caprifoliaceae (honeysuckle family), while Braconidae (parasitoid wasps) is significantly correlated with Berberidaceae (barberry family) ($P < 0.01$) (Fig. 2-4).

The fourth-corner method as applied here is a distinct test, but the derived results are consistent with the results of the Mantel test. The results from both types of tests show that taxonomy and interaction structure are interrelated and, in some cases, strongly correlated.

Discussion

We performed two complementary permutation analyses to test for lineage specificity and correlated taxonomic structure among flowering plant species and their visiting insect species in a tallgrass prairie ecosystem, across multiple habitat types and many years. We found evidence of significant taxonomic conservatism, and also that the architecture of this community is taxonomically structured. Because we analyzed taxonomic relationships that are partly morphologically based, evidence of lineage specificity has the potential to imply some degree of trait-based matching. This is because

many of the key characters used to distinguish taxa are also important for ensuring that plants and their insect visitors are mutually compatible (e.g. tongue length in bees and corolla depth in flowers).

Our results indicate that the interaction structure of plant species and the insect species that visit them are correlated with the respective taxonomies, at multiple different taxonomic levels. This finding highlights the importance of organizational level in analyses of correlated taxonomic hierarchies. For example, there was a significant correlation between the interaction structures and taxonomies of all the insect and plant species in our full dataset, but when subgroups were evaluated separately, significant correlations were intermittent. There are two possible, mutually compatible explanations for these findings: 1) there are constraints limiting interactions between plant and insect species related to morphological traits that separate taxa, and 2) interactions exert some selective pressure on the interacting partners. In most taxa, plant and insect species that were closely related tended to interact with similar partners.

Our study has shown that lineage specificity may be a common outcome of plant-insect interactions, and also illustrated that it is contra-indicated for certain taxon pairs. We also found asymmetrical signals in some groups. For example, the groups of plant species visited by insects in the orders Hemiptera, Diptera, and Coleoptera did not have correlated taxonomies and interactions, while their insect partners did. In some cases, the absence of taxonomic conservatism may provide insight into the importance of observed interactions. Asymmetric interactions between groups with lineage specificity have been found in other mutualistic communities as well (e.g. Jacquemyn et al. 2011). The asymmetric signal in our dataset was unexpected, but not completely surprising, given the

different selective forces acting on partners in the mutualism (Graham et al. 2012). For example, insect visitation is required for reproduction of many plant species, but visits from bugs, flies, and beetles may not result in pollination. On the other hand, insect species visiting certain groups of flowering plant species may be visiting to gather specific nutrients or because flower morphology allows easy access to pollen and nectar, among other reasons. Nevertheless, it seems reasonable to suspect that, across the whole region and all taxa, selective forces acting directly on reproductive interactions may have stronger effects than those which act on food gathering.

One group that exemplifies this concept is the braconid wasps and their floral hosts. There was not a significant correlation between the taxonomic history and interaction structure of braconid wasps, which may suggest that they are not tightly tied to the plant species that they visit. This particular result makes sense because braconids do not provision their young with pollen. Instead, adults tend to visit flowering plant species to drink nectar and perhaps eat pollen to power their daily activities (Tooker and Hanks 2000, Pucci and Jones 2010). However, the set of plants visited by braconids does have a significant correlation between its taxonomic distances and interaction structure, hinting that braconids may be pollinating some plant species, even though braconids are not commonly implicated as pollinators (van Emden 1963, Faegri and Van der Pijl 1979, Jervis et al. 1993).

Using a novel application of the fourth-corner method (Legendre 1997), we also identified significant positive correlations between taxonomies of plant species and visiting insect species at order and family levels. Some of our results were intuitive: for example, that the insect family Apidae had the largest number of significant correlations

with plant families. Because the interaction with plants is critical to members of this insect family (the pollen they gather supports larval development), it is logical that there should be strong evidence of taxonomic conservatism and that its taxonomic history should closely match its interacting partners. It is also intuitive that families with similar ecological functions (e.g. those considered pollinators) share similar correlations, distinct from families with different ecological roles (such as the parasitoid wasps). In addition, some of our results provide support for the hypothesized importance of the interactions between specific groups: for example, an important interaction has been suggested between the braconids and Apiaceae (van Emden 1963, Tooker and Hanks 2000), and our results support this hypothesis. There were also some non-intuitive results, including significant correlations between groups where one would not necessarily expect a strong effect of interaction on the taxonomy of the visitors, such as the significant correlation between the taxonomy of the order Hemiptera and its interaction structure. Such non-intuitive results prompt new questions, and could drive future hypotheses and studies. Similarly, a signal was absent in some cases where it might be expected: for example, between the bee family Apidae and the plant family Apiaceae (both derived from the Greek word for bee: “Apis”). In particular, the fourth-corner method allowed us to narrow the scope of the correlation, thereby highlighting groups for which the interaction has a greater importance.

High-resolution molecular phylogenies could provide branch lengths that might demonstrate cospeciation and increase the power of both methods to detect correlations. Unfortunately, such phylogenies are not available at the broad taxonomic scale considered here. Importantly, even without such detailed information, we were able to

find many significant and interesting correlations among taxa. The strength of the signal can only be improved with more information regarding distributions, character traits, and morphological complementarity.

Permutation methods, such as the Mantel test we applied here, have been used to infer coevolution in the host-parasite literature (e.g. Legendre et al. 2002) and the Mantel test has been used to seek pairwise coevolution between Euglossine bees and the orchids they pollinate (Ramirez et al. 2011). In both cases, the studies focused on one very specific group of interacting organisms where a signal of coevolution was expected. In the case of the Euglossine bees, the findings were consistent with the hypothesis of asymmetric coevolution (Ramirez 2009), where the taxonomy of the orchids was significantly correlated with their interaction structure, but the taxonomy of the bees was not (Ramirez et al. 2011). However, to the best of our knowledge, there is no prior application of the fourth corner method to test for broad correlations between the taxonomic hierarchies of interacting mutualists. In our effort, we detected many significant relationships in a far larger and more complex community, without any *a priori* assumptions of which groups might display signals of taxonomic structuring. Our results also highlight some interesting new candidate groups in which reciprocal selection may have exerted a stronger influence than has been previously recognized (e.g. between braconids and their floral hosts). On the whole, the presence of the many (and sometimes asymmetric) relationships in a dataset of this size suggests that, though diffuse on small taxonomic scales, the associations between floral hosts and their insect visitors have a clear structuring at higher levels of taxonomic organization.

Our study provides novel evidence that there is significant lineage specificity and taxonomic conservatism, and that the associations between plants and insects are structured by their taxonomy at the regional level. Instead of searching for the rare, strong species-species interactions that gave rise to Darwin's early hypotheses on coevolution, we find that communities of insects and flowering plants have weak interactions among many generalist species. We show that, even with such weak and diffuse interactions, there is a significant, strong, and ubiquitous correlation between the taxonomies of interacting partners and their interaction structures and we identify significant positive correlations between taxonomic groupings of plant species and visiting insect species.

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Author contributions

L.R. motivated the research, performed the analyses, and wrote the initial draft of the paper, J.T. provided and updated the data, O.B. suggested the fourth corner method, and A.M. and K.S. assisted with the analyses and motivated the research. All authors discussed the results and wrote the manuscript.

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Tables

Table 2-1 Families represented in subgroups of the Robertson dataset.

Short Tongued Bees	Long Tongued Bees	Parasitoid Wasps
Andrenidae	Apidae	Bethylidae
Colletidae	Megachilidae	Braconidae
Dufouridae		Chalcididae
Halictidae		Chrysididae
Macropididae		Eucoilidae
Nomiidae		Evaniidae
Panurgidae		Figitidae
Prosopididae		Ichneumonidae
		Mutillidae
		Sapygidae
		Scelionidae
		Scoliidae
		Thynnidae
		Tiphiidae

Table 2-2 Results of Mantel permutation tests on subsets of the full dataset. Two tests were run on each subset, one from the perspective of insect species and one from the perspective of plant species. Non-significant P values are highlighted in bold. Alpha values are determined by a Holm-Bonferroni correction (a sequential Bonferroni, Ludbrook 1998). The standardized Mantel statistic (r) is reported here, but is sensitive to the size of the square matrix, here the number of species. The statistic is constrained to relatively small sizes by the nature of the test (Dutilleul *et al.* 2000, Urban *et al.* 2002).

Subset tested	Perspective	Mantel statistic, r	Significance, P	Size
Full dataset	Insect	0.07514	0.001	1174
Coleoptera	Insect	0.0328	0.012	154
Diptera	Insect	0.08954	0.001	449
Hemiptera	Insect	0.154	0.012	24
Hymenoptera	Insect	0.1653	0.001	450
Lepidoptera	Insect	0.141	0.001	97
Insect Subgroups				
Parasitic Wasps	Insect	0	0.854	151
Braconidae	Insect	0.01915	0.314	29
Apoidea	Insect	0.194	0.001	299
Short Tongue Bees	Insect	0.2196	0.001	156
Long Tongue Bees	Insect	0.1289	0.001	143
Plant Subgroups				
Asterales	Insect	0.119	0.001	574
Fabales	Insect	0.09192	0.001	291
Lamiales	Insect	0.09134	0.001	412
	Perspective	Mantel statistic, r	Significance, P	Size
Full dataset	Plant	0.1291	0.001	455
Coleoptera	Plant	0.03208	0.067	153
Diptera	Plant	0.02192	0.035	298
Hemiptera	Plant	-0.03875	0.921	134
Hymenoptera	Plant	0.108	0.001	445
Lepidoptera	Plant	0.1286	0.001	244
Insect Subgroups				
Parasitic Wasps	Plant	0.1086	0.001	111
Braconidae	Plant	0.1828	0.01	25
Apoidea	Plant	0.1121	0.001	437
Short Tongue Bees	Plant	0.09884	0.001	339
Long Tongue Bees	Plant	0.09221	0.001	374
Plant Subgroups				
Asterales	Plant	0.2235	0.001	95
Fabales	Plant	0.2605	0.001	40
Lamiales	Plant	0.2449	0.001	46

Figures

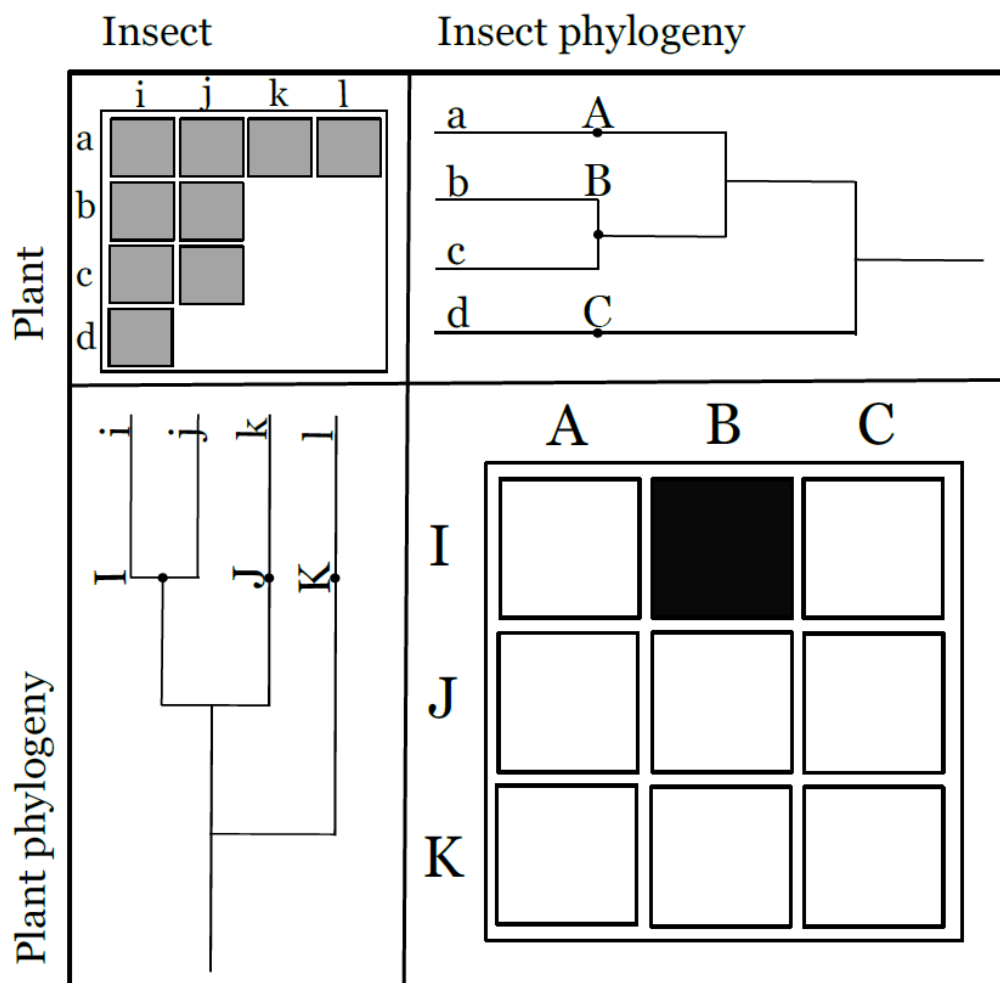


Figure 2-1 A heuristic diagram demonstrating the fourth-corner application for our problem. An interaction matrix among plant and insect species (top left, with species labeled as lowercase letters) has both a taxonomic tree for insect species (top right) and plant species (bottom left), both with genera labeled as capital letters. Using the fourth-corner method, we can directly test for correlations in the taxonomies of interacting partners. The matrix in the bottom right shows a significant relationship between genus I of the plants and B of the insects.

	Coleoptera	Diptera	Hemiptera	Hymenoptera	Lepidoptera
Alismatales					
Apiales	■				
Aristolochiales	■				
Asparagales					
Asterales					■
Brassicales	■		■		
Caryophyllales					
Celastrales					
Commelinales				■	
Cornales					
Cucurbitales					
Dipsacales	■				
Ericales					
Fabales			■		
Gentianales					
Geraniales	■				
Lamiales					
Laurales					
Liliales					
Magnoliales					
Malpighiales					■
Malvales				■	
Myrtales	■				■
Nymphaeales					■
Oxalidales				■	
Poales					
Polygalales	■		■		
Polygonales					■
Primulales	■				
Ranunculales					
Rosales					
Santalales					
Sapindales			■		
Saxifragales					
Scrophulariales	■				
Solanales		■			
Vitales					

Figure 2-2 Results of the application of the fourth-corner method to test for significant positive correlations between orders of insects (columns) and plants (rows). Dark-grey rectangles represent a significance of $P < 0.01$, light gray squares represent a significance of $P < 0.05$, and white squares represent values where $P > 0.05$ (ter Braak et al. 2012).

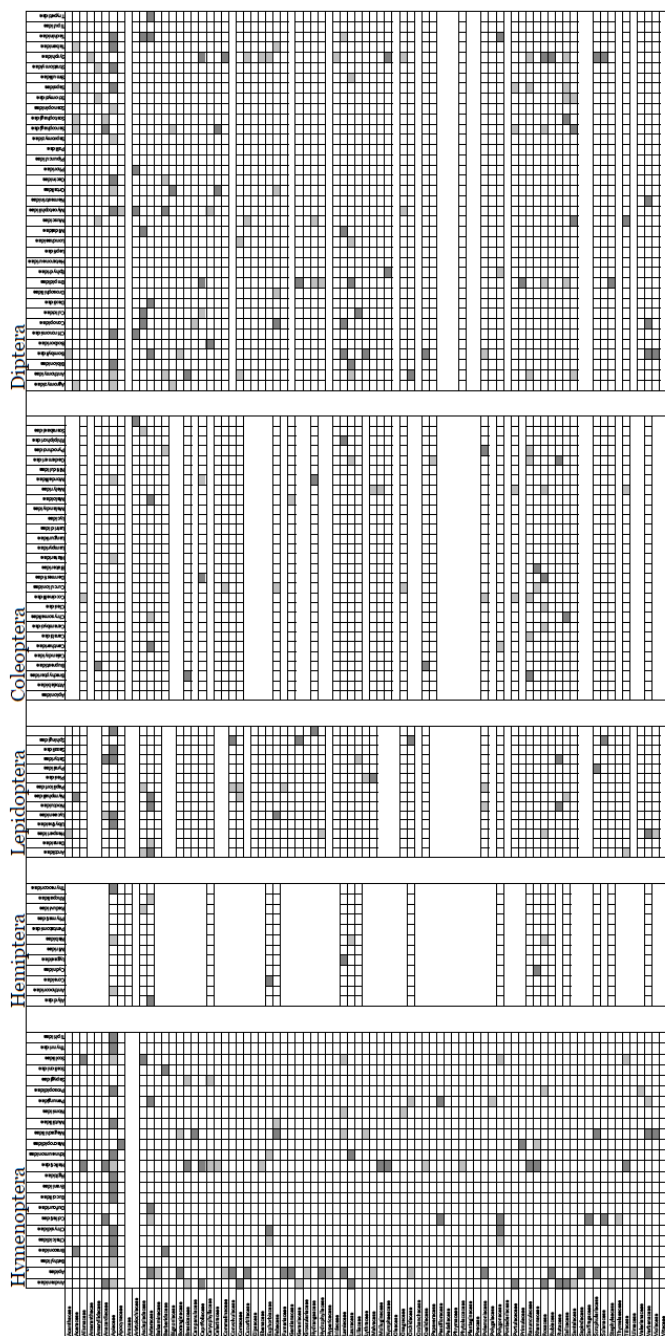


Figure 2-3 Results of the application of the fourth-corner method to test for significant positive correlations between families of insects (columns) and plants (rows). Dark grey squares represent a significance of $P < 0.01$, light grey squares represent a significance of $P < 0.05$, and white squares represent values where $P > 0.05$ (ter Braak et al. 2012).

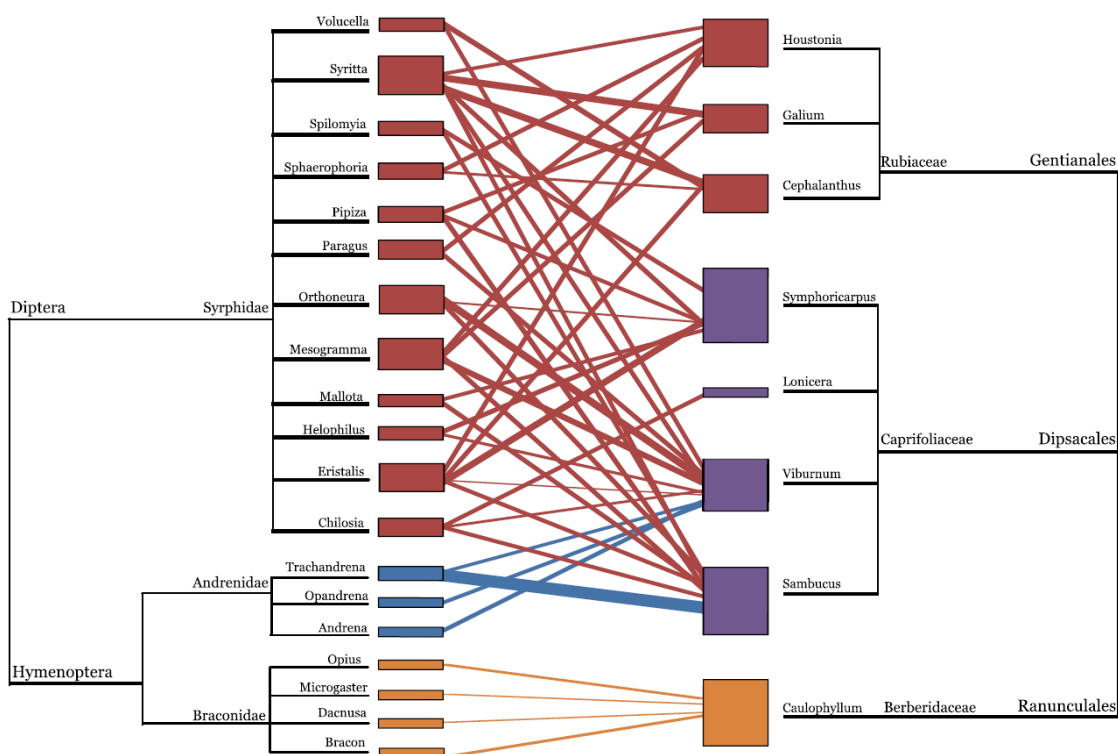


Figure 2-4 A subset of the full dataset selected to illustrate the significant correlations ($P < 0.01$) between the genera of three insect families (boxes on left) and three plant families (boxes on right), with observed interactions as lines connecting them. Syrphidae was correlated with both Rubiaceae and Caprifoliaceae, while Andrenidae was correlated with Caprifoliaceae, and Braconidae was correlated with Berberidaceae. The taxonomies of both are illustrated, but syrphid genera of degree 1 were omitted for clarity.

Chapter 3

Supporting crop pollinators with floral resources: network-based phenological matching

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Abstract

The production of diverse and affordable agricultural crop species depends on pollination services provided by bees. Indeed, the proportion of pollinator-dependent crops is increasing globally. Agriculture relies heavily on the domesticated honey bee; the services provided by this single species are under threat and becoming increasingly costly. Importantly, the free pollination services provided by diverse wild bee communities have been shown to be sufficient for high agricultural yields in some systems. However, stable, functional wild bee communities require floral resources, such as pollen and nectar, throughout their active season, not just when crop species are in flower. To target floral provisioning efforts to conserve and support native and managed bee species, we apply network theoretical methods incorporating plant and pollinator

phenologies. Using a two-year dataset comprising interactions between bees (superfamily Apoidea, Anthophila) and 25 native perennial plant species in floral provisioning habitat, we identify plant and bee species that provide a key and central role to the stability of the structure of this community. We also examine three specific case studies: how provisioning habitat can provide temporally continuous support for honey bees (*Apis mellifera*) and bumblebees (*Bombus impatiens*), and how resource supplementation strategies might be designed for a single genus of important orchard pollinators (*Osmia*). This framework could be used to provide native bee communities with additional, well-targeted floral resources to ensure that they not only survive, but thrive.

Introduction

There are 87 globally important commercial crop species that depend on insect pollination (Klein *et al.* 2007). Among the insect pollinators, bees are the most important pollinating agents (Free 1993). Of the bees, honey bees (*Apis mellifera* L.) are the single most important crop pollinators, contributing not only to the diversity, but also to the affordability of many agricultural food products (Losey & Vaughan 2006; Klein *et al.* 2007; Garibaldi *et al.* 2013). In the United States alone, the services of honey bees were valued at 14.6 billion US\$ in 2000 (Morse & Calderone 2000) (19.3-40.3 billion US\$ when adjusted for inflation in 2012), and demand for pollination services is increasing as ever larger areas are devoted to pollinator-dependent crops (Aizen *et al.* 2008).

However, honey bee populations are threatened by a suite of hazards, including pesticides, diseases, the mite *Varroa destructor* (vanEngelsdorp *et al.* 2009), and the potential indirect effects of loss of habitat (Potts *et al.* 2003; Winfree *et al.* 2007) and host plants resulting from herbicide drift (Mortensen *et al.* 2012). These threats have highlighted the dependency of modern agriculture on increasingly threatened pollination services (Gallai *et al.* 2009).

While honey bees are traditionally considered to be the most valuable pollinators (Free 1993), they are not the most efficient pollinators for all crops. Native bees are often efficient and sometimes superior pollinators, and contribute significantly to crop yield (Klein, Steffan-Dewenter, & Tscharntke 2003; Losey & Vaughan 2006; Greenleaf & Kremen 2006). For example, for “Red Delicious” apples, flowers visited by *Osmia cornuta* were five times more likely to set fruit than honey bee-visited flowers, and resulting fruits were larger when flowers were visited by *O. cornuta* (Vicens & Bosch 2000). The value of pollination services provided by wild bees has been estimated at approximately 3.07 billion US\$ in the United States alone (Losey & Vaughan 2006) and they were globally valued at 248 billion US\$ in 2009 (Gallai *et al.* 2009). Native bees are capable of fully supplying the pollination services required by certain crops (Winfree *et al.* 2007), but require sufficient habitat with floral and nesting resources to maintain a population size large enough to be effective crop pollinators (Kremen *et al.* 2004; Cane 2008). In response to habitat loss, for example, there have been declines in native bee populations in the north eastern United States (Bartomeus *et al.* 2013). Supplementing habitat for native bees may provide the additional benefit of supporting managed bee populations (Carvalho *et al.* 2012). For example, Carvalho *et al.* (2012) found

increased honey bee contribution to the pollination of mangoes when floral provisioning resources were provided for native bees.

Both in response to threats to honey bees, and in recognition of the potential benefits of augmenting wild bees, methods are being developed to conserve native and domesticated bee populations. One strategy involves managing agricultural field edges to increase the diversity of floral provisioning resources (Winfree *et al.* 2008; Egan & Mortensen 2012) and the abundance of specific floral hosts (Isaacs *et al.* 2009). Current recommendations for selecting floral provisioning species are often based on pollinator syndromes, without incorporating information about actual insect visitation frequencies (e.g. NAPPC Planting Guides 2011). However, selecting the best plants for provisioning wild pollinators with nectar and pollen resources can be difficult because visitation rates often depend on multiple complex floral characters (Thompson 2001). The quality and quantity of resources provided by flower species can vary significantly, and quantifying these resources can be challenging (Kearns & Inouye 1993). For this study, we asked the question: given the threats to pollinators, how can we promote stability and diversity of bee communities that provide pollination services to crops?

To make a more informed decision about the plant species that might be used to conserve bee communities, and to identify bee species that might also visit a wide variety of crop species, it is necessary to observe interactions between bees and plants. These observations can then be used to capture community interaction structure in the form of bipartite mutualistic networks (Memmott 1999). Such networks have recently gained attention in the scientific literature as a vital tool for understanding how ecological communities form and function (Memmott 1999; Olesen *et al.* 2008; Campbell *et al.*

2011). Here we show that these methods can also be used to address the real-world management problem of how to improve floral provisioning with the objective of conserving native and managed bee populations to provide crop pollination services.

Specifically, we use network measures to assess the stability of community interaction structure over time and the role of individual species. These measures allow us to investigate the roles of individual plant species in the connectivity of the pollinator community, and we thus are able to rank them. Our analyses include one novel “node duration” measure to demonstrate how phenology relates to the importance of species, but we also show how interaction phenology can be used to match pollinators with a suite of plants that provide continuous floral provisioning resources throughout the season and how the phenology of the interacting species relates to the stability of the community as a whole over time.

We investigate three case studies to demonstrate how our framework might be used to target management objectives. First, we demonstrate how floral resources might benefit the domesticated honey bee (*A. mellifera*). We show that the provisioning habitat could be used to complement and supplement crop species, and to provide continuous resources throughout the active season. Second, we demonstrate how a generalist bee species, the common eastern bumblebee (*Bombus impatiens*), could be supported by multiple floral provisioning species throughout the summer. *A. mellifera* has long been used in agriculture, while *B. impatiens* has only been recently domesticated (i.e. in the 1970s, Velthuis & Doorn 2006); both might benefit from additional and varied resources (Carvalho *et al.* 2012).

Next, we focus on the genus *Osmia*; some *Osmia* species have gained attention because of their potential to be managed as orchard pollinators (Vicens & Bosch 2000; Bosch & Kemp 2002; Gruber *et al.* 2011). For example, *Osmia lignaria*, *O. cornifrons*, and *O. cornuta* are sometimes managed as pollinators of almonds, cherries, plums, pears, and apples (Bosch & Kemp 2002). In most cases, the bloom period of these crop plants corresponds directly with the natural activity period of native *Osmia* spp. (Bosch & Kemp 2002). Though they prefer flowers of orchard trees when available, they require other resources when orchards are not flowering (Bosch & Kemp 2002). Floral resource provisioning in an orchard setting could help to sustain *O. lignaria* populations when crop flowering is poor (e.g. when a hard freeze or pest infestation kills buds or blossoms) and also help to build local populations over time. Because other *Osmia* spp. may also be effective crop pollinators, we have selected them as a target group to illustrate the application of our framework (Gruber *et al.* 2011).

Material and Methods

Experimental Design

We established floral provisioning habitat 25 m from the edge of a 6 hectare corn field in the Russell E. Larson research farm, Centre County, PA (coordinates; 40.712019,-77.934192). The experiment consisting of 25 native perennial species (Table 3-1) was established in 2007 in a randomized complete block design, with 4 blocks of the 25 species. We chose native plant species because they represent appropriate taxa for floral provisioning with native pollinators and require less maintenance than plants not

adapted to the local climate (Isaacs *et al.* 2009). Each block consisted of individual plants, separated by 3 m, within a 12 m x 12 m grid. Blocks were aligned in a single row and positioned 6m apart. The effect of blocks was not significant, and we subsequently pool visitors from each species and across both years that bees were collected (2008 and 2009). The purpose of the randomized complete block design was therefore to ensure that each plant was treated as an individual experimental unit, and also to ensure a relatively even spatial distribution of the species.

In the summers of 2008 and 2009, we vacuum sampled the flowers every other week from May to October with a modified leaf blower (Craftsman USA, model #358794760) (Tuell *et al.* 2008; DeBarros 2010). On a sampling day, each individual plant was vacuumed for 15 seconds in a randomized sequence from 0900-1200 EST and again from 1300-1600 EST. Therefore, there were a total of 18.3 sampling hours on this community between the two years.

We also measured a number of other plant characteristics, such as the area of each individual flower and the total number of flowers across both seasons. The average planar floral area of an individual blossom was measured for each species by taking digital photographs of 10 representative blossoms or blossom clusters on each individual plant, keeping a metric ruler in each photographic frame for reference. We then used Adobe Photoshop CS4 Extended (Version 11.0, Adobe 2008) to calculate the area of the blossom in each photograph, and averaged across the ten photographs for each individual. The total floral area for individual plants was then estimated for each week by multiplying the number of observed flowers by the average blossom area for the species (DeBarros 2010). Floral visitors of the superfamily Apoidea were pinned and identified

to the species level, except for 62 *Lasioglossum* specimens that could only be resolved to morphospecies because they were too damaged to be identified or were males. Males of the genus *Lasioglossum* are not well resolved, and are often impossible to separate taxonomically (S. Droege, pers. comm.). Thus, they likely represent more than one species. Nonetheless, due to their impact on the visitation rates of plants, it was inappropriate to leave the specimens out of the analysis entirely. For all species, we deposited voucher specimens in the collections at the Pennsylvania Department of Agriculture in Harrisburg, PA.

Network Construction and Analysis

From floral visitation events, we constructed both weighted and unweighted bipartite networks with plant and bee nodes, using a visitation event as an interaction (Fig. 3-1), with the “bipartite” package in R (Memmott 1999; Dormann, Gruber, & Fründ 2008). The interaction network comprised visitation events from both years and all blocks. A weighted version was scaled by the abundance of bees collected on flowers, while an unweighted version of the network documented the presence or absence of species interactions only.

To evaluate the effect of phenology on network structure, we separated the season into early (May and June), middle (July and the first half of August), and late (from the second half of August through the first half of October) summer. These periods were chosen based on the flowering phenology of the plant species in the floral provisioning habitat. For each of these periods of the summer, we evaluated the size, nestedness, and connectance of the network. The size of the network at any given time is the sum of the species richness of interacting plant and bee species. Nestedness is a measure of order in

a network, and has been shown to relate to species and community persistence (Bascompte *et al.* 2003) and stability and robustness (Thébault & Fontaine 2010; Pocock, Evans, & Memmott 2012). In addition, nestedness is not sensitive to network size (Nielson & Bascompte 2007). Connectance has been theoretically shown to relate to the complexity and robustness of a community to species loss (Dunne, Williams, & Martinez 2002) and stability (Thébault & Fontaine 2010). However, it is sensitive to small network sizes (Dormann *et al.* 2009).

On a node level (i.e. individual species), there are many ways to rank the species in the context of the community. Here, we evaluated the specialization, relative abundance, centrality, and duration of plant-pollinator interactions. Because we were especially interested in identifying key plant species for floral provisioning, we also examined the number of visits each flower species received relative to their floral display, and ranked the plant species by their functional complementarity (Devoto *et al.* 2012). We tested for correlations between separate rankings using a Spearman's correlation coefficient.

We first identified generalist plants and pollinators as those species with the highest degree (i.e. largest number of species interactions; Memmott 1999); in network parlance, this can be referred to as degree centrality (Opsahl, Agneessens, & Skvoretz 2010). Generalist plants support pollinator diversity, which in turn has been shown to provide increased crop yields (Klein, Steffan-Dewenter, & Tschardtke 2003; Garibaldi *et al.* 2013), especially given year to year variation in native bee populations. In turn, generalist pollinators are assumed to be more likely to visit not only plants in the floral

provisioning habitat, but also many crop species because they are less selective about where they obtain floral resources (Memmott 1999; Tylianakis *et al.* 2010).

We then identified plant species that supported large numbers of floral visitors by weighting interactions with interaction frequency to generate a quantitative network (Memmott 1999). The weighted degree of a node is the total abundance of all its interactions. Nodes with a high weighted degree are visited more. Highly visited plants likely support pollinator population growth and improve pollination services, especially if they provide both nectar and pollen, although they may also function as mating or nesting sites. In addition, some plants received a high frequency of visits despite having a relatively small floral display. We identified such species as those that were outside of a 95% confidence interval of the correlation between floral display and visitation frequency. In other words, they had more visitors than would be expected given the relationship between floral area and visitation frequency.

We used two additional separate measures of centrality in addition to degree centrality, both of which are common in the literature, for identifying the importance of individual nodes to network structure and stability (Jordán, Benedek, & Podani 2007). Betweenness centrality ranks species as connectors between other species in the community, while closeness centrality ranks species relative to their proximity to other species in the community (Martín González, Dalsgaard, & Olesen 2010). Because the interactions between plants and pollinators generate bipartite networks, we made single mode projections of the plant species and pollinator species before calculating centrality. In the single mode projections, links are formed between species that share interacting partners (e.g. plant species that share one or more pollinator species).

We used duration of activity through the season of the plant and bee species as another measure of importance. For example, pollinators that actively forage for longer periods will likely visit multiple crop species with differing floral phenologies. Similarly, plant species that provide floral resources for longer periods can support pollinators when crops are not flowering. We define this new measure, which we term node duration, as the number of times out of the total number of samples that a species participates in the network. This measure is distinct from other measures of node dynamics in the literature (e.g. phenophase as defined by Olesen *et al.* 2008) because it only accounts for the presence of the species within the network. For example, the phenophase of a plant species is the period between the opening of its first flower and the senescence of its last flower (Olesen *et al.* 2008), while node duration is strictly defined by the number of times the plant species interacts with floral visitors in the community.

Finally, we calculated the functional complementarity of the plant species. Functional complementarity is a measure of how individual plant species support separate functional groups of pollinators in the community (Devoto *et al.* 2012). Plants that are visited by distinct groups of pollinators will therefore increase the functional complementarity of the community more than species that share the same species of pollinators. If there are constraints on the number of plants available for floral provisioning habitat, one might select a combination that maximizes functional complementarity to support the largest diversity of pollinators. As suggested by Devoto *et al.* (2012), we use branch lengths in a functional dendrogram based on a distance matrix generated from an interaction matrix. Here we removed the species one by one in such a way as to maximize the functional complementarity of the community at each

number of species. Thus, the order that the species are removed reflects a gradient from the species that are least critical to complementarity to those that are most critical.

Example with target groups

To demonstrate how the floral provisioning species could complement and supplement flowering crop species, we created a separate phenology graph incorporating the interaction phenology of *A. mellifera* and the plant species it visited over the season. We compared the interaction phenology of these species with the approximate flowering phenology of five pollinator-dependent crop species commonly found in the study area. We performed a similar analysis with *B. impatiens* for comparison. (Readers interested in seeing the phenology-oriented visitation for any other of the 64 bee species found in this provisioning habitat can visit the following website for additional figures: www.floralprovisioningforpollinators.wordpress.com.)

With the objective of conserving *Osmia* spp., we created a separate interaction network of the four *Osmia* spp. found in our provisioning habitat (*O. atriventris*, *O. bucephala*, *O. cornifrons*, and *O. pumila*) and the flowers they visited. We also evaluated the interaction phenology of the *Osmia* spp. to determine when they visited the provisioning habitat.

Results

Over the two summers that the floral provisioning habitat was sampled, 64 bee species were captured while visiting the 25 perennial plant species. There were a total of 1,651 specimens captured, representing a total of 261 unique insect-flower species

interactions. We performed an interaction rarefaction to estimate the completeness of our sampling (a Chao 1 estimator, see Chacoff *et al.* 2012), and found that we captured 60.5% of the maximum number of expected interactions, a result consistent with other similar plant-pollinator communities, despite differing sampling methods (e.g. Chacoff *et al.* 2012, Devoto *et al.* 2012).

The community was dynamic across the summer, changing in size, nestedness, and connectance (Table 3-2). The community was largest in both the number of plant and insect species in the middle of the summer, and smallest early in the summer. The nestedness followed the same trend, being highest in the middle of the summer and lowest early in the summer, but the connectance followed an opposite trend, being lowest in the middle of the summer and highest early in the summer. When the interactions were pooled across the whole season, the nestedness was also maximized, but the connectance remained lower than the separate periods of the summer. However, the network size was below 50 species for the early division of the summer, and the measure of connectance for that time may therefore be less reliable (Dormann *et al.* 2009).

We assessed the importance of plant and bee species in the context of the full network (Fig. 3-2) with five network measures: unweighted degree, weighted degree, betweenness, and closeness centrality, as well as node duration. Unweighted degree and weighted degree are correlated to each other and to both other measures of centrality, as well as functional complementarity in the plants ($p \ll 0.01$ for all except floral area and node duration, for which $p \sim 0.02$, Table B1 in Appendix B). However, none of the node measures are significantly correlated with floral area or node duration in the plants (Table B1). In contrast, all measures are significantly correlated to each other in the bee species

($p \ll 0.01$, Table B2). Despite these significant correlations, each measure results in a substantially different ranking.

To demonstrate the relationships between these measures, we show the 25 plant species ranked by unweighted degree (Fig. 3-3, Table 3-3). Among the 25 species, *Veronicastrum virginicum* (culver's root) was visited by the greatest number of bee species, while *Eupatorium perfoliatum* (common boneset) had the highest abundance of bee visitors, and *Tradescantia ohiensis* (Ohio spiderwort) had the longest duration of activity, from May to September. Interestingly, the plant species were not well separated by either betweenness or closeness centrality. Indeed, nine species shared the top value for closeness centrality (Fig. 3-3). Five plant species had more visitors than would be expected given the relationship between floral area and weighted degree: *E. perfoliatum*, *Pycnanthemum tenuifolium* (narrowleaf mountainmint), *Conoclinium coelestinum* (blue mistflower), *Eurybia macrophylla* (bigleaf aster), and *V. virginicum* (Fig. 3-4). In contrast, seven plant species had fewer visits than would be expected, given their floral area: *Symphotrichum novi-belgii* (New York aster), *T. ohiensis*, *Desmodium canadense* (showy ticktrefoil), *Lysimachia quadrifolia* (whorled yellow loosestrife), *Lespedeza capitata* (roundhead lespedeza), *Senna hebecarpa* (American senna), and *Phlox divaricata* (wild blue phlox).

We also ranked the 64 bee species that visited the floral provisioning habitat by unweighted degree (Fig. 3-5). The unresolved *Lasioglossum* spp. have the largest of four of five of the measures (unweighted degree, node duration, and both measures of centrality). As a group, they interact with the largest number of species, have the longest duration of activity, and the highest of both betweenness and closeness centrality.

However, because they are unresolved, they likely represent more than one species. The interpretation is therefore that the genus *Lasioglossum* as a whole is an important group in the community, despite the fact that individual species tend to be specialists, or rare. This poorly known group may therefore require more study in the future, including the development of better guides for the identification of male specimens. If we remove the effect of the unresolved *Lasioglossum* spp., *Bombus impatiens* was both the most generalist species and most abundant. Its abundance was more than twice that of any other bee species. However, *Ceratina calcarata* had the highest ranking in both measures of centrality and *A. mellifera* had the longest duration of activity, from June to October. Importantly, though we found significant correlations between weighted degree, unweighted degree, betweenness, and closeness centrality, and node duration, ($p \ll 0.01$), each measure ranks the species differently.

Only three plant species, *Asclepias tuberosa* (butterfly milkweed), *E. perfoliatum*, and *E. macrophylla*, always appeared among the top ten species when ranked separately by unweighted degree (number of unique interactions), weighted degree (including abundance), betweenness, and closeness centrality, and node duration (duration of interactive interval) (Table 3-3). Of these, *E. perfoliatum* and *E. macrophylla* still rank in the top ten, even when we controlled for floral display. In contrast, there were seven bee species (*Bombus impatiens*, *B. bimaculatus*, *Halictus ligatus*, *A. mellifera*, *Augochlora pura*, *Ceratina dupla*, and *C. calcarata*) that appeared important by all five measures (Fig. 3-5).

The method of ranking may be adapted according to specific conservation goals. To show how one might select species to provide continuous floral resources for a target

pollinator with a long duration of activity, we use the species visited by the honey bee (*A. mellifera*) as an example. The honey bee shifts its frequency of visitation from one species to another throughout the season; to ensure continuous resources, a manager would choose plants from each of the three periods (Fig. 3-6, A). Floral resources could thus be available for this important domesticated pollinator when crop species are not in flower. In addition, we provide the approximate flowering times for five pollinator-dependent and high yield crops in Pennsylvania (Fig. 3-6, B). In contrast to the honey bee, the bumblebee (*B. impatiens*) was found to be extremely generalist, visiting multiple floral provisioning species within each time period (Fig. 3-7). This suggests that the bumblebee relies less on any single floral provisioning species and may be well-supported by a wide variety of species.

Although *Osmia* spp. were not frequent visitors in our floral resource provisioning habitat, we provide preliminary analyses as to the species that they visited. Our network demonstrates that the four *Osmia* spp. in the floral provisioning site visited only three plant species, and three of them visited only *Penstemon digitalis* (foxglove penstemon) (Fig. 3-8), though this plant was a minor node of the full network (Fig. 3-2). In our provisioning habitat, there were few plants in flower at the time that the *Osmia* spp. were active, and because the visitation frequency from these species was low, we do not suggest that *P. digitalis* is the ideal resource for all *Osmia* spp.; empirical confirmation would be necessary. However, the example demonstrates how our framework might be used to identify such a resource for a particular objective.

Discussion

The benefits of a diverse bee community for agricultural yields have been convincingly demonstrated (e.g. Garibaldi *et al.* 2013). However, in order to have a stable, functional wild bee community, it is necessary for there to be sufficient habitat (Winfrey *et al.* 2007) to provide floral resources, such as pollen and nectar (Isaacs *et al.* 2009). We show how network theoretical methods incorporate plant and pollinator phenologies to target floral provisioning efforts to conserve and support native and managed bee species. Although many have suggested that networks can be used to direct conservation and management objectives (e.g. Tylianakis *et al.* 2010), our study demonstrates how network theory might have a practical application to a real and urgent problem. Understanding the temporal dynamics of community level interactions in a plant-pollinator network is critical for maximizing the provisioning of floral resources for crop pollinators and targeting conservation efforts at species that provide pollination services. In particular, knowledge of phenological constraints on plant-pollinator interactions, and plant species used by bee visitors, will be essential to managers that have bee conservation as an objective.

Another advantage of our method is that it allows us to design the floral provisioning habitat with local bee assemblages in mind. This multispecies framework emphasizes the importance of key interactions. Stable, natural communities have particular structural aspects that we want to design or maintain to conserve diversity and functionality. In other words, we can select highly generalist species that are abundant and active over long periods of time, while sustaining rare interactions between

uncommon or specialist species. In addition, by keeping the phenology of the whole community in mind, we can see where it might be more vulnerable, or less stable. For example, the nestedness of this community was lowest early in the summer, and highest in the middle of the season. That might pinpoint an opportunity to strengthen the bee community by providing more flower species with an early phenology in provisioning habitat. In contrast, the connectance of the community was highest early in the spring, though this was potentially influenced by network size.

To explore the phenology of individual species, we developed a novel network measure, node duration, or the activity of interacting species over time. Node duration can provide useful information for managers concerned about relative flowering times of floral provisioning plants and crops. Some studies have shown a “magnet species” effect where pollinator visitation rates to nearby species are enhanced by the presence of a species with large floral rewards (Molina-Montenegro, Badano, & Cavieres 2008). In this case, it would be advantageous to synchronize the flowering of crop and nearby attractive wild plants. However, there is also some evidence for the opposite effect, where plants compete for pollinators (Feinsinger 1987). In this situation, it would be ideal to have plant species that support pollinators when crop species are not in flower. Interaction phenology allows species in the community to be selected on the basis of the duration or seasonality of their activity (Fig. 3-2), as relevant to the focal management situation; such insights will help with the design and tractability of field trials or provisioning applications. Toward this end, we explore the case study of the interaction phenology of *A. mellifera* relative to the phenology of a selection of pollinator-dependent crop species (Fig. 3-6); to provide continuous resources for this bee species, a manager

might select one or more provisioning species flowering in each of the three periods of the summer. In contrast, the bumblebee (*B. impatiens*) visited several species in each period of the summer, and may therefore be less reliant on any one plant species (Fig. 3-7).

We identified generalist plant species, such as *V. virginicum*, that attracted a large number of bee species, but also *E. perfoliatum* that attracted a large abundance of bees, and *T. ohioensis* that provided attractive resources over a long period of time. This demonstrates that different plant species might be used for different provisioning objectives. In addition, three species (*A. tuberosa*, *E. perfoliatum*, and *E. macrophylla*) among the plants of our floral provisioning habitat were consistently ranked highly in all categories measured, and *E. perfoliatum* and *E. macrophylla* still rank in the top ten species even when we control for the size of the floral display. These species might be the strongest candidates for resource provisioning; their efficacy as resources should then be the target of field trials. In contrast, seven species had fewer visitors than would be expected given their floral display. This demonstrates that the more showy flowers are not necessarily the most preferred by bee visitors, especially if more attractive flowers are present (but see Tuell *et al.* 2008). Interestingly, measures of node betweenness and closeness centrality were not effective for separating plant species, likely because a subset of the plants species all had a large number of connections and were thus equally central to the interaction structure (Fig. 3-3).

Although we focused on specific plant species that would be ideal for provisioning crop pollinators, the complex structure of this network demonstrates how a diversity of floral resources contributes to a diverse pollinator community. Given the

asymmetric nature of our community (and mutualistic communities in general), generalist plant species such as *V. virginicum* are visited by a large number of specialist bee species. In turn, bee diversity has been shown to result in improved crop pollination (Klein, Steffan-Dewenter, & Tschardtke 2003; Garibaldi *et al.* 2013). Thus, there is a strong relationship between diversity and functionality. Diverse communities provide more ecosystem services, and communities providing ecosystem services (i.e. pollination or floral resource provisioning) support a higher level of diversity (Kremen *et al.* 2004; Isaacs *et al.* 2009).

Our results also highlight pollinator species that might augment crop pollination. There were seven consistently generalist and abundant species that were active for most of the summer, and central to the interaction structure. Their periods of activity could overlap with multiple different crop species. Among these, *B. impatiens* stood out as more than twice as abundant and was also a very generalist bee species. It is possible that choosing plants to support *B. impatiens* might encourage its population growth. However, a different species, *C. calcarata*, was the most central when ranked by both betweenness and closeness centrality (Fig. 3-5), suggesting that it has a central role in the web of pollination services provided by these bee species and may, in fact, contribute to the stability of the community (Jordán, Benedek, & Podani 2007).

Our approach can also be used to address specific management objectives, such as to maximize provision of resources that help conserve a target group of pollinators. The results of our second illustrative case study show that three of the four *Osmia* spp. active in the floral provisioning habitat visited one plant species in particular, *P. digitalis*. Because few of the plants in our study were flowering early in the season, future studies

would benefit from including additional, early blooming species. Indeed, despite the low sample size, we have included this analysis because managers are searching for new ways to support *Osmia* species in orchard systems, and experimentally testing multiple floral provisioning species (D. Biddinger, pers. comm.). Our study suggests that an empirical test comparing scenarios with and without *P. digitalis* would help to determine whether it is a key component of floral provisioning habitat designed to support orchard crops that might benefit from visits by *Osmia* spp. *P. digitalis* may serve to increase the population size of *Osmia* spp., thereby enhancing crop pollination in future years. Our method also makes explicit how the flowering phenology of plants within the provisioning habitat synchronizes with crop phenologies. For example, *P. digitalis* flowered after orchard crops and would therefore not compete with them for pollinators; instead, our work suggests that *P. digitalis* plantings adjacent to orchards might support *Osmia* spp. at a critical time when floral resources provided by crops are absent.

Conclusions

Pollination services are critical for the production of foods necessary for a healthy human diet (Eilers *et al.* 2011). The demand for pollinator-dependent crops is increasing much more rapidly than the availability of pollination services provided by honey bees (Aizen *et al.* 2008), especially in the face of Colony Collapse Disorder. It is therefore imperative that these pollination requirements be supplemented with services provided by wild bees. However, wild bees require nesting and floral resources (Winfrey *et al.* 2007), and relatively little is known about these requirements. Providing native bee

communities with additional, well-targeted floral resources could ensure that they not only survive, but thrive; the benefits of such habitat may also support honey bees, which utilize similar floral resources, as evidenced by the visitation of honey bees to the species in our floral provisioning site. Our approach to assessing floral resources for crop pollinators integrates critical information about the community structure and phenology of relevant plant and bee species. As we illustrate, our framework can be used to inform hypotheses and design experiments, and has great flexibility for objectives intended to conserve wild bee populations and maintain the critical ecosystem service of pollination.

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Author contributions

All the authors motivated the research, discussed the results, and wrote the manuscript. L.R. performed the analyses, and wrote the initial draft of the paper, N.D. collected the data, and S.Y. assisted with the analyses.

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Tables

Table 3-1 List of twenty-five native perennial plant species used in this study, sorted by family.

SPECIES BINOMIAL	COMMON NAME
Asclepidaceae	
<i>Asclepias tuberosa</i> L.	butterfly milkweed
Asteraceae	
<i>Conoclinium coelestinum</i> (L.) DC.	blue mistflower
<i>Coreopsis tripteris</i> L.	tall tickseed
<i>Echinacea purpurea</i> (L.) Moench	eastern purple coneflower
<i>Eupatorium perfoliatum</i> L.	common boneset
<i>Eupatorium purpureum</i> L.	sweetscented joy pye weed
<i>Eurybia macrophylla</i> (L.) Cass.	bigleaf aster
<i>Liatis pycnostachya</i> Michx.	prairie blazing star
<i>Solidago rugosa</i> Mill.	wrinkleleaf goldenrod
<i>Symphyotrichum novae-angliae</i> (L.) GL. Nesom	New England aster
<i>Symphyotrichum novi-belgii</i> (L.) GL. Nesom	New York aster
<i>Vernonia gigantea</i> (Walter) Trel.	giant ironweed
Campanulaceae	
<i>Campanula rotundifolia</i> L.	bluebell bellflower
Commelinaceae	
<i>Tradescantia ohiensis</i> Raf.	Ohio spiderwort
Fabaceae	
<i>Desmodium canadense</i> (L.) DC.	showy ticktrefoil
<i>Lespedeza capitata</i> Michx.	roundhead lespedeza
<i>Senna hebecarpa</i> (Fernald) Irwin and Barneby	American senna
Lamiaceae	
<i>Monarda fistulosa</i> L.	wild bergamot
<i>Pycnanthemum tenuifolium</i> Schrad.	narrowleaf mountainmint
Polemoniaceae	
<i>Phlox divaricata</i> L.	wild blue phlox
Primulaceae	
<i>Lysimachia quadrifolia</i> L.	whorled yellow loosestrife
Ranunculaceae	
<i>Actaea racemosa</i> L.	black bugbane
<i>Aquilegia canadensis</i> L.	red columbine
Scrophulariaceae	
<i>Penstemon digitalis</i> Nutt. ex Sims	talus slope penstemon
<i>Veronicastrum virginicum</i> (L.) Farw.	Culver's root

Table 3-2 Properties of the community over time.

	Nestedness	Connectance	Number of Plant Species	Number of Insect Species	Total Size
Early	2.80	0.23	8	23	31
Middle	13.05	0.17	20	46	66
Late	7.89	0.19	19	34	53
Full	20.77	0.16	25	64	89

Table 3-3 Properties of plants in the floral provisioning habitat.

Species	Total Floral Area	Unweighted Degree	Weighted Degree	Node Duration	Betweenness Centrality	Closeness Centrality	Functional Completeness
<i>Actaea racemosa</i>	1,422	3	4	4	0.05	0.92	4
<i>Aquilegia canadensis</i>	2,201	6	10	9	0.49	0.96	7
<i>Asclepias tuberosa</i>	2,829	20	55	11	1.36	1	16
<i>Campanula rotundifolia</i>	1,332	4	4	14	0.82	0.96	2
<i>Conoclinium coelestinum</i>	1,697	12	138	5	0.49	0.96	21
<i>Coreopsis tripteris</i>	9,073	13	62	12	1.36	1	10
<i>Desmodium canadense</i>	20,877	3	5	8	0.05	0.92	5
<i>Echinacea purpurea</i>	5,519	15	44	7	0.82	0.96	11
<i>Eupatorium perfoliatum</i>	19,375	16	338	14	1.36	1	23
<i>Eupatorium purpureum</i>	5,663	9	36	9	0.49	0.96	13
<i>Eurybia macrophylla</i>	1,718	16	116	7	1.36	1	19
<i>Lespedeza capitata</i>	8,733	1	1	9	0	0.69	6
<i>Liatris pycnostachya</i>	28	11	43	3	0.49	0.96	14
<i>Lysimachia quadrifolia</i>	7,836	2	2	5	0	0.75	1
<i>Monarda fistulosa</i>	105	9	61	6	0.82	0.96	24
<i>Penstemon digitalis</i>	5,114	14	27	7	0.49	0.96	12
<i>Phlox divaricata</i>	2,113	1	1	7	0	0.89	3
<i>Pycnanthemum tenuifolium</i>	8,510	17	167	4	1.17	0.96	22
<i>Senna hebecarpa</i>	4,419	2	8	14	0.05	0.92	15
<i>Solidago rugosa</i>	2,520	11	46	7	0.49	0.96	9
<i>Symphotrichum novae-angliae</i>	47,075	14	240	8	1.36	1	24

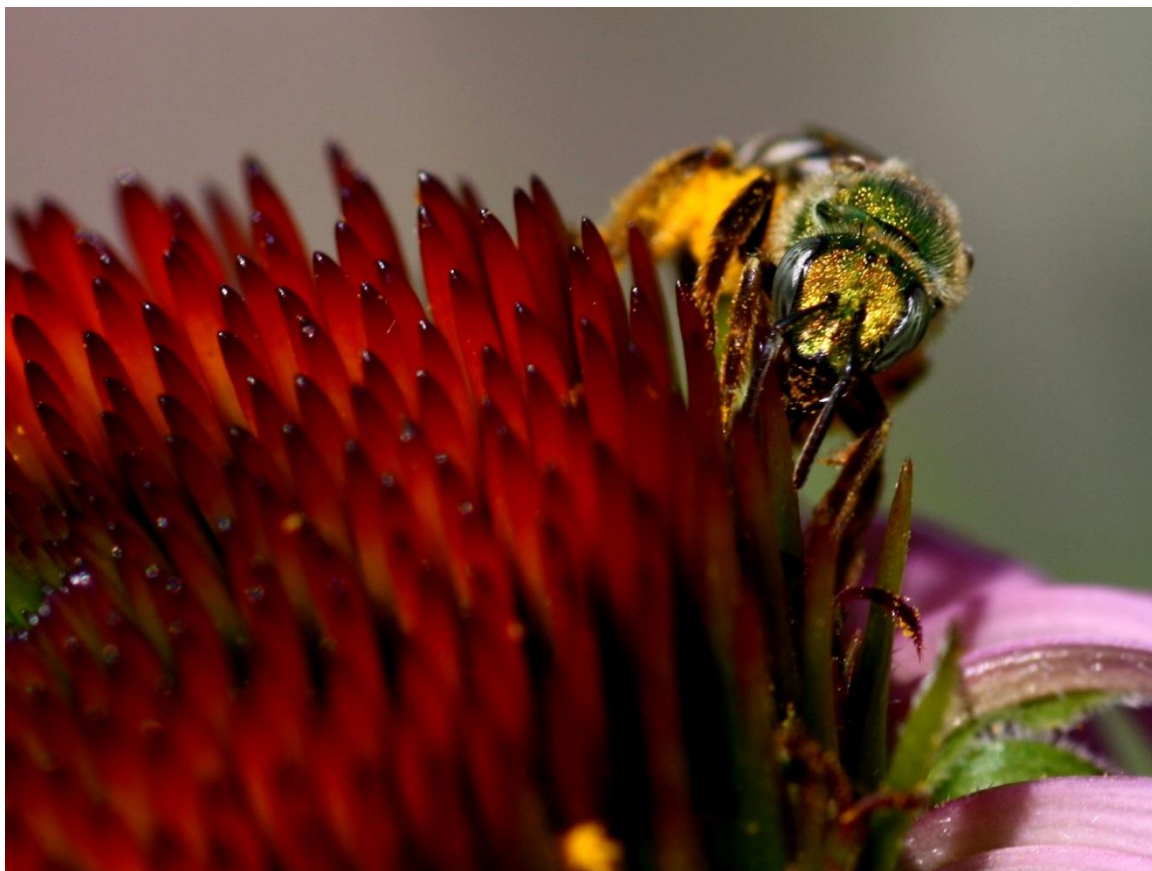
Figures

Figure 3-1 An example of an observed visitation event between a sweat bee (*Agapostemon virescens*) and one of the floral provisioning plant species (*Echinacea purpurea*). Photo by Laura Russo.

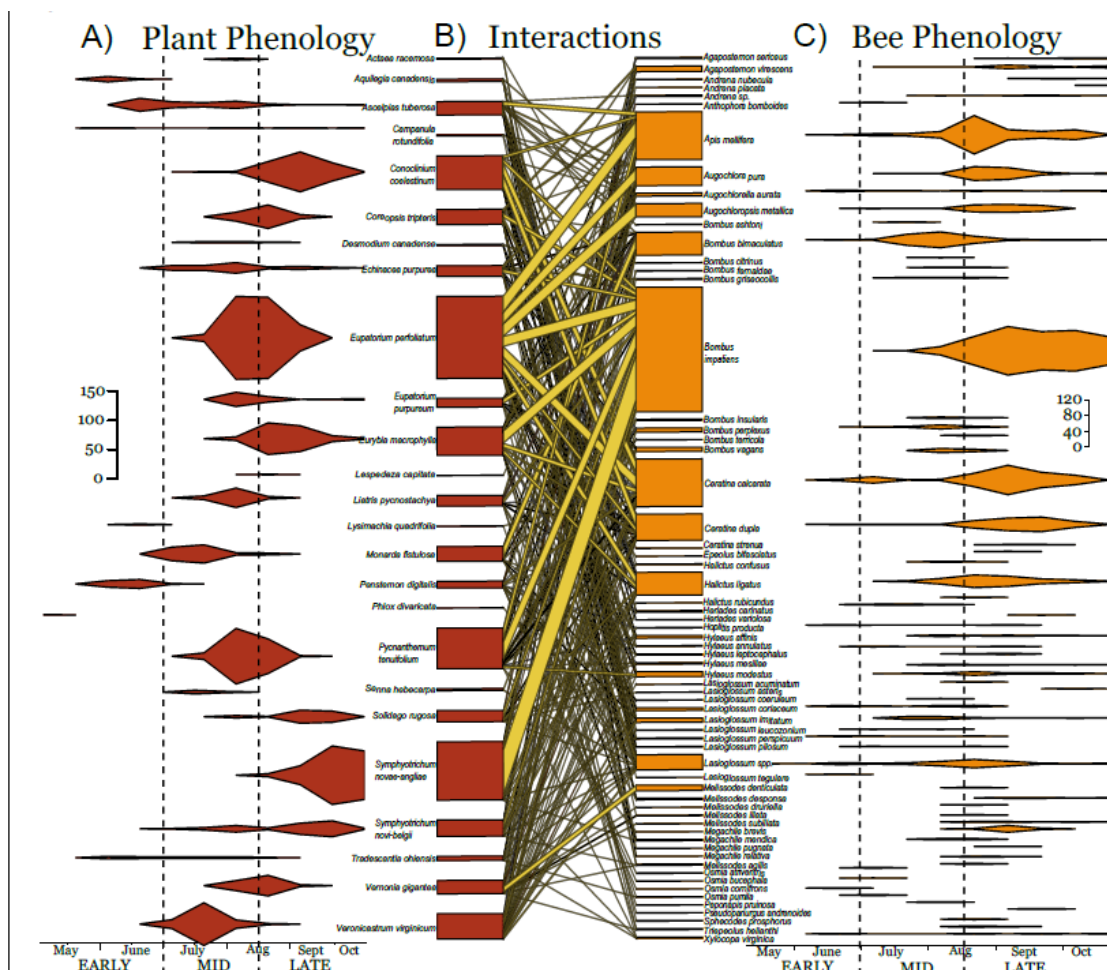


Figure 3-2 The violin-shaped plots represent the interaction phenology of plants (A) and bees (C) over the summers of 2008 and 2009. The weeks correspond to biweekly sampling dates, beginning in early May and ending in mid-October. The length of the segments demonstrates the duration of the interactive interval of that species, while the height represents the abundance of interactions and demonstrates how they fluctuate over time (plotted on the x axes). (B) This quantitative bipartite visitation network was constructed from collections of Apoidea species on flower species. The boxes (nodes) on the left represent plant species and the boxes on the right represent bee species. The height of each box is proportional to the number of interactions. Lines connecting plant and bee species represent floral visitation events and are weighted by abundance.

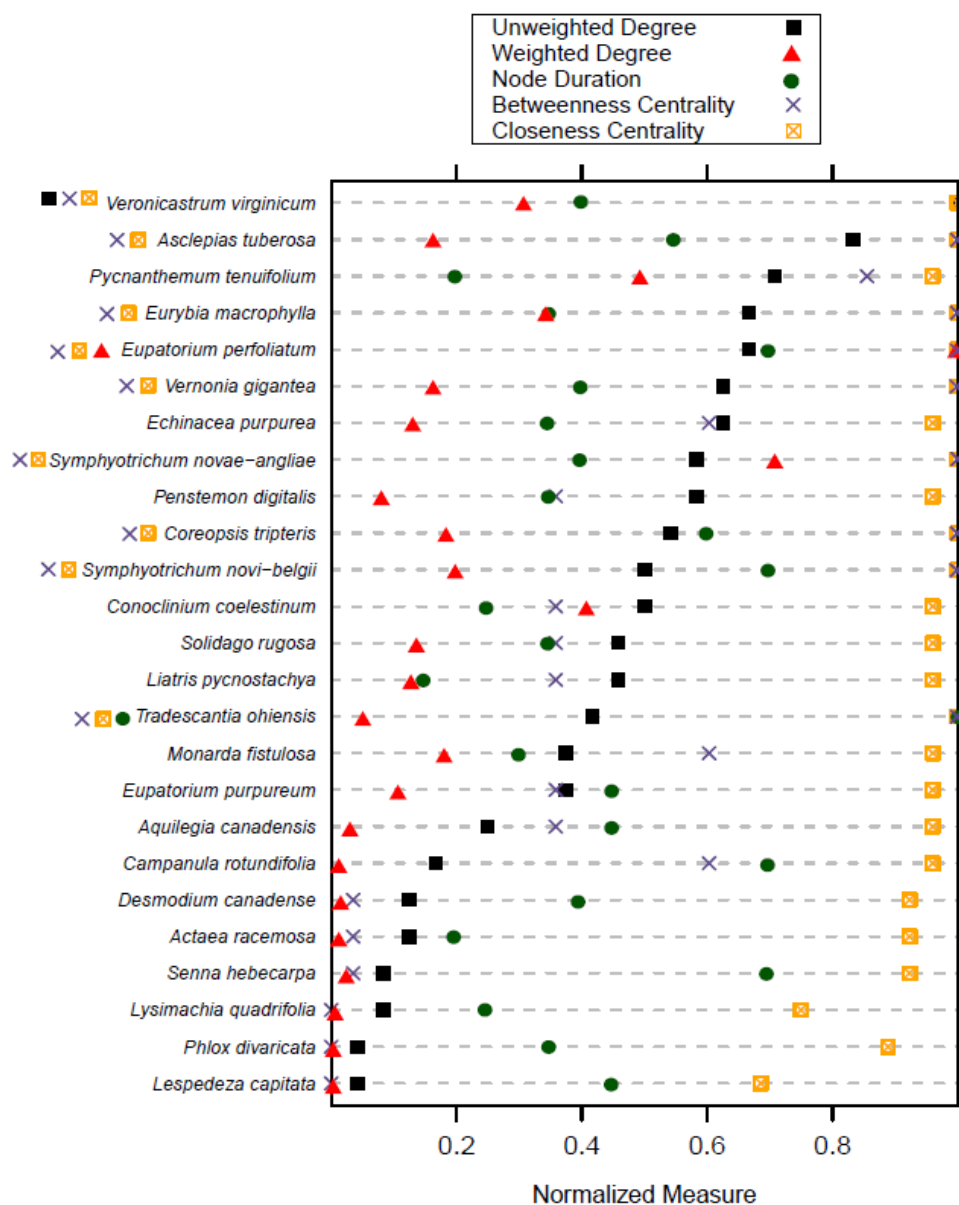


Figure 3-3 Plot of three normalized measures of importance for plants. Plant species are ranked by unweighted degree (filled square) (number of unique interactions with bees). Also shown are node duration (circle) (number of times bees were collected on the plant out of number of possible times), weighted degree (triangle) (interactions weighted by abundance of bee visitors), betweenness centrality (X), and closeness centrality (square with X). The symbols to the left of the species names indicate the highest ranking plant species for each measure.

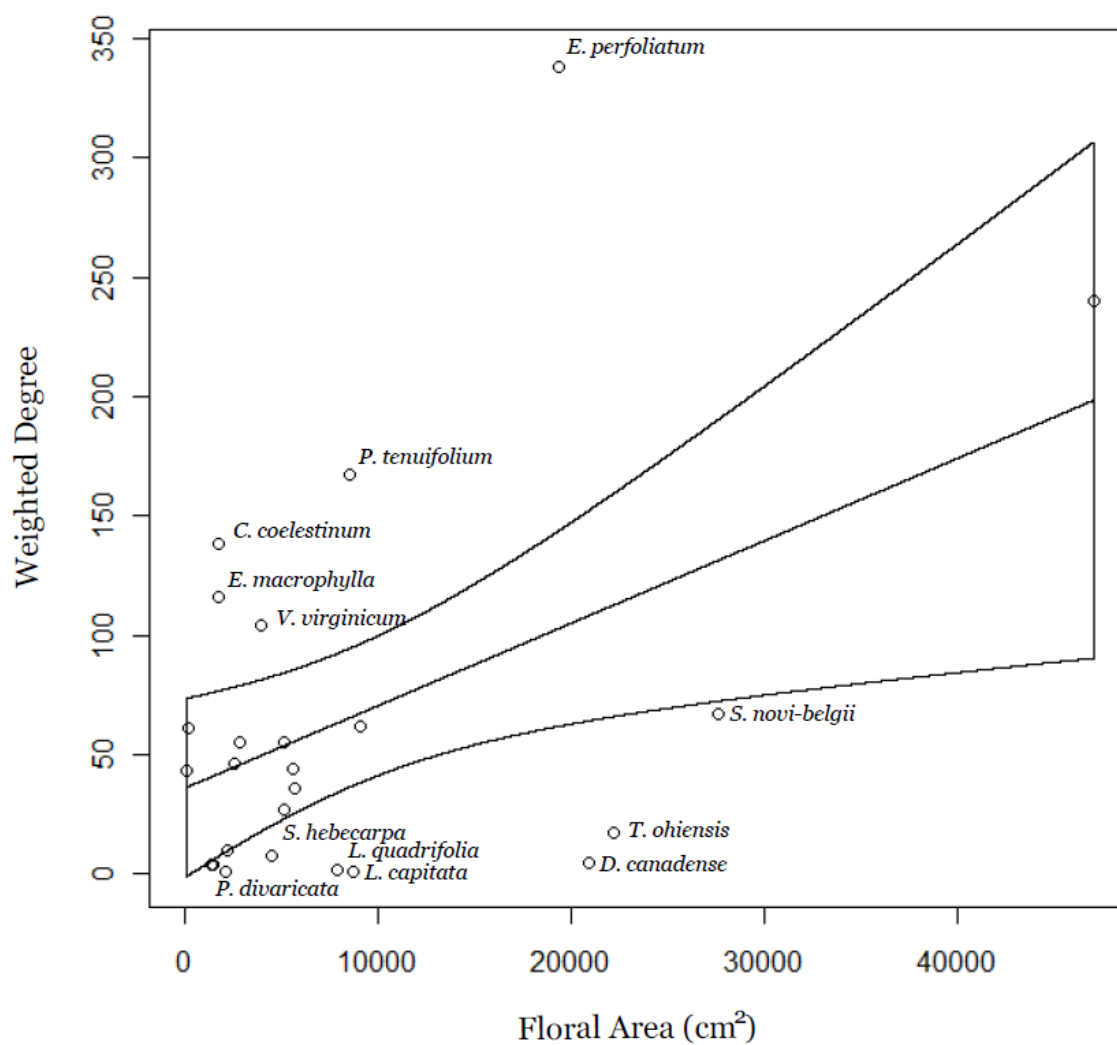


Figure 3-4 Plot of the relationship between weighted degree (visitation frequency) and floral area (summed across the summer), with 95% confidence intervals. Plant species that had more or fewer visits than would be expected given the relationship between floral area and weighted degree are labelled.

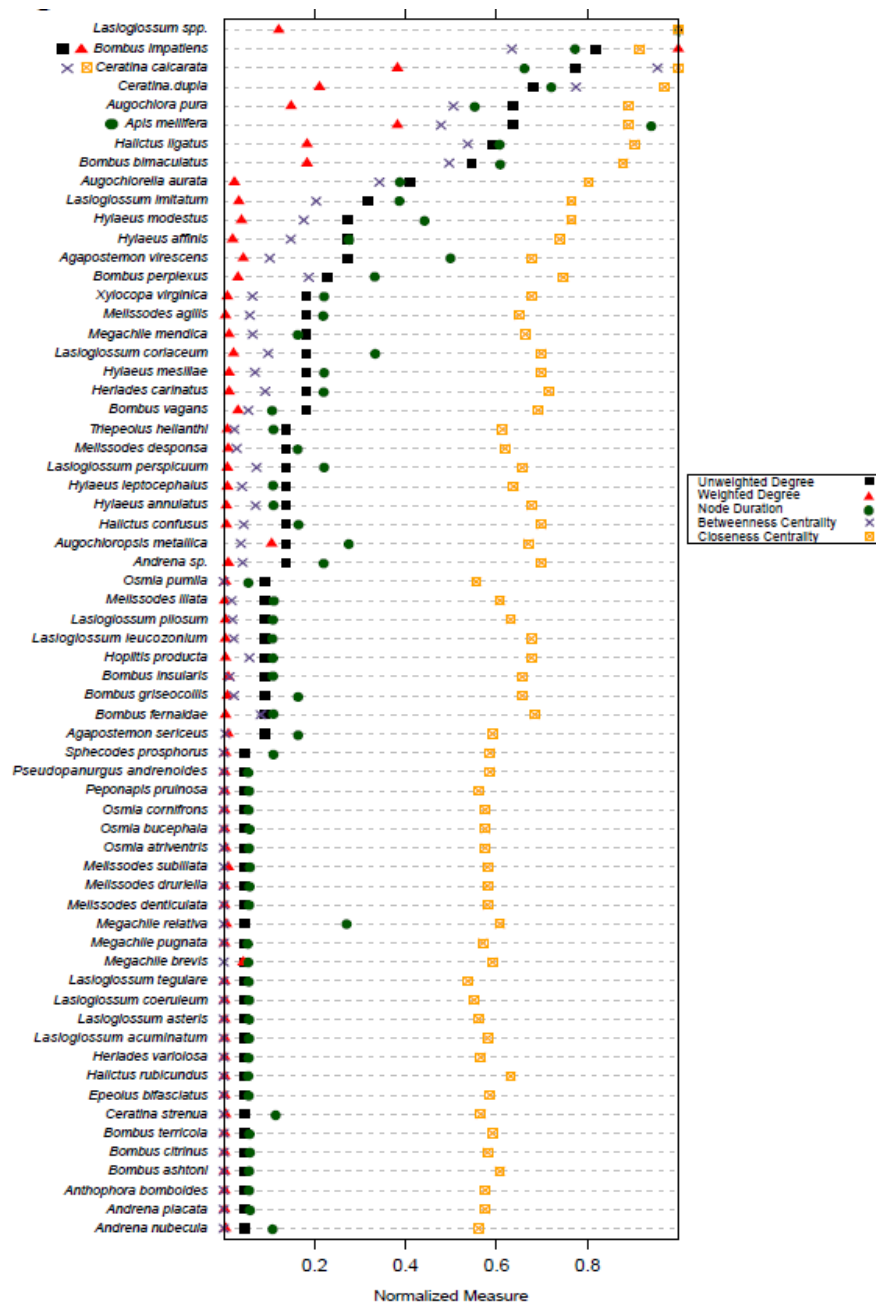


Figure 3-5 Plot of three normalized measures of importance for bees. Bee species are ranked by unweighted degree (filled square) (number of unique interactions with plants). Also shown are node duration (circle) (number of times bees were collected out of number of possible times) and weighted degree (triangle) (interactions weighted by abundance of interactions with plants), betweenness centrality (X), and closeness centrality (square with X). The symbols to the left of the species names indicate the highest ranking bee species for each measure.

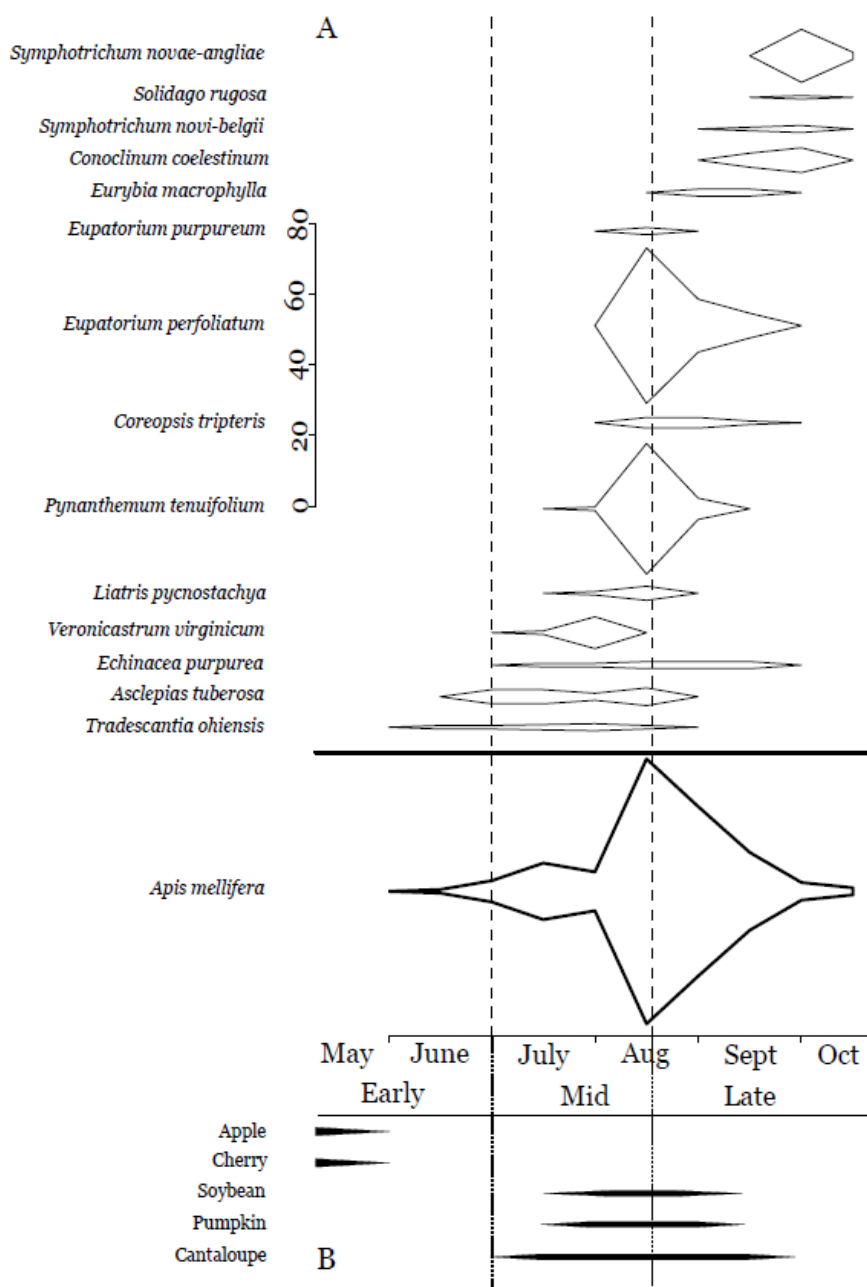


Figure 3-6 Interaction phenology of *Apis mellifera* (honey bee) across three periods of the summer. A) Phenology of plant species in the provisioning habitat that interact with *A. mellifera*. To provide continuous resources across time, a manager might select one or more plant species from early, middle, and late in the summer. B) Approximate flowering times of common pollinator-dependent crop species in the region of the study, including apple and cherry (B. Way of Way Fruit Farm, PA, pers. comm.), pumpkin (S. Sidhu, pers. comm.), cantaloupe (DeBarros 2010), and soybean (W.S. Harkcom, pers. comm.).

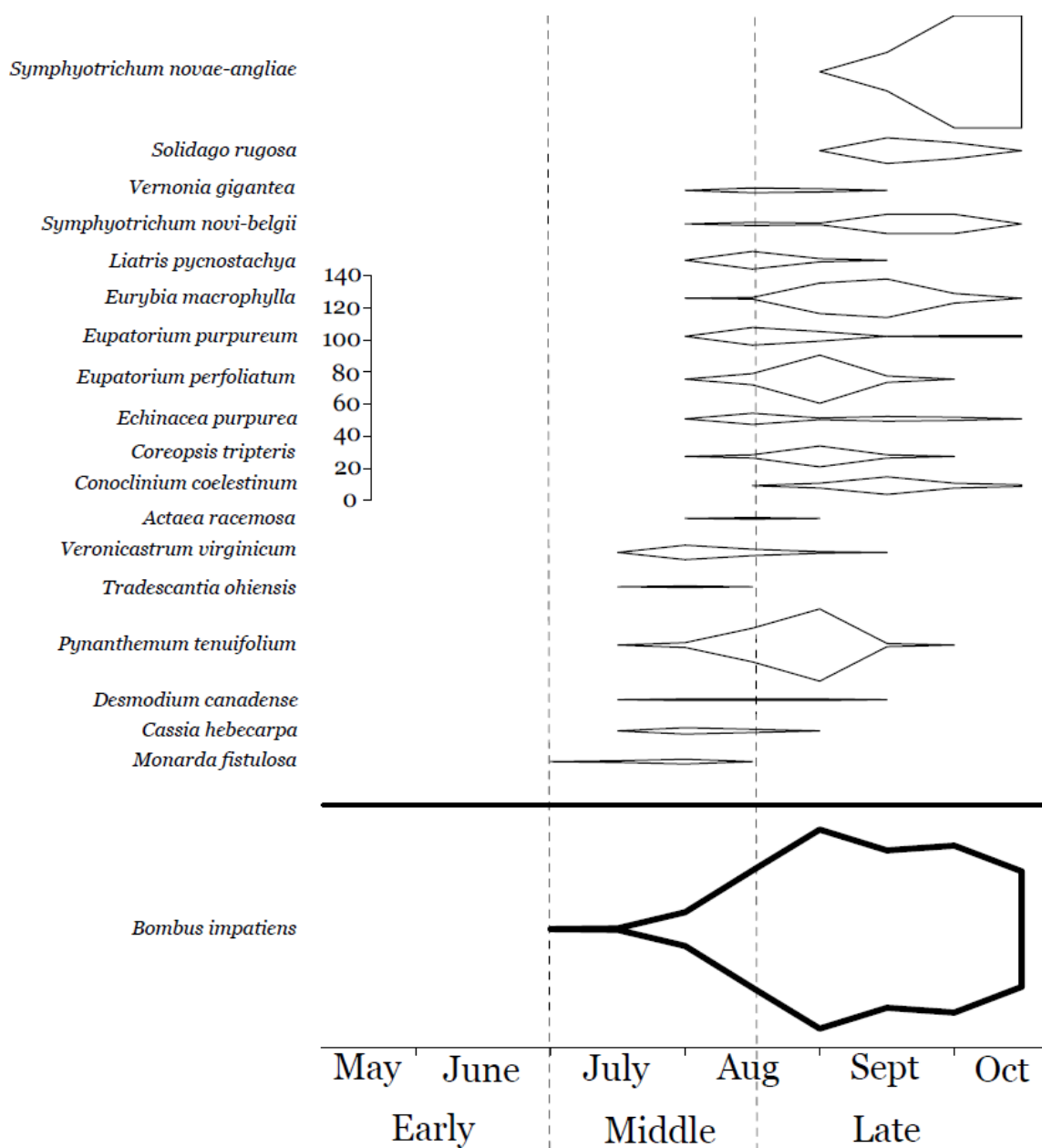


Figure 3-7 Interaction phenology of *Bombus impatiens* (bumblebee) across three periods of the summer, including phenology of plant species in the provisioning habitat that interact with *B. impatiens*. To provide continuous resources across time, a manager might select one or more plant species from middle and late in the summer.

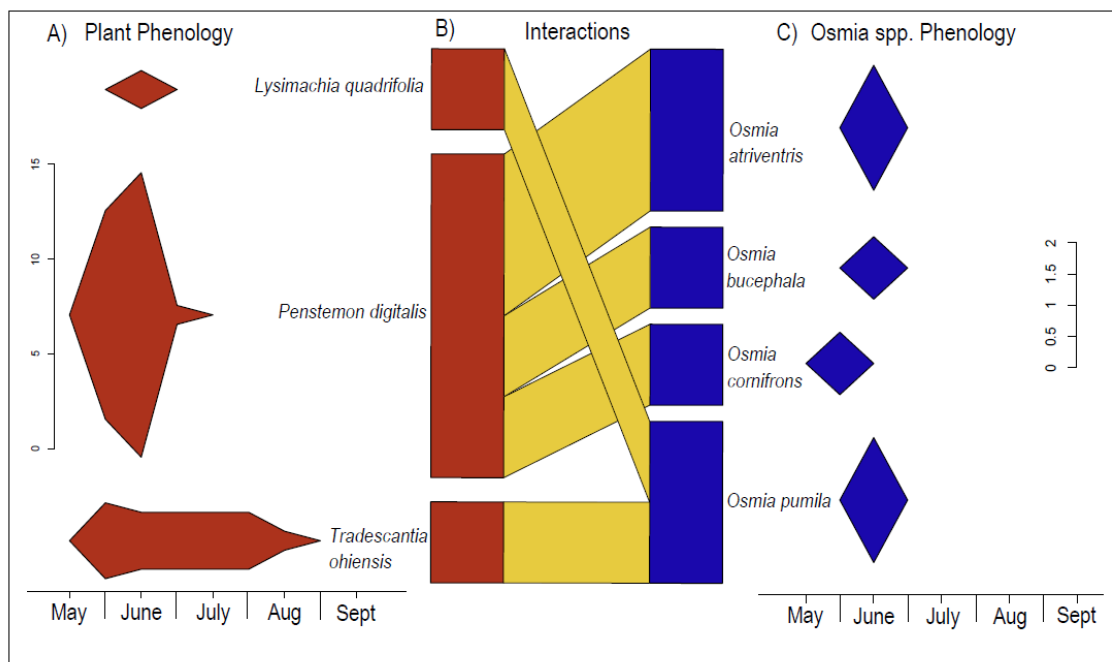


Figure 3-8 The interaction phenology (including all bee visits) of plants (A) that *Osmia* spp. visited, and of *Osmia* spp. (C) over the summers of 2008 and 2009. The weeks correspond to biweekly sampling dates, beginning in early May and ending in mid-October. The length of the segments demonstrates the interaction duration for that species, while the height represents the abundance of interactions and their fluctuations over time (plotted on the x axes). (B) This quantitative bipartite visitation network was constructed from collections of *Osmia* spp. on flower species. The boxes (nodes) on the left represent plant species and the boxes on the right represent *Osmia* spp. The height of the boxes is the proportional number of interactions. Lines connecting plant and bee species represent floral visitation events and are scaled by abundance.

Chapter 4

Patterns of novel species interaction formation affect network structure in plant-pollinator communities

In revision

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Abstract

Novel ecosystems comprise new combinations of species and their interactions. Species additions have the potential to affect the functionality and stability of ecological communities. In particular, non-native mutualists must integrate into an existing network of interactions in order to persist and may affect the structure of existing interactions. To predict the effect of novel species introduction on interaction patterns at the network level, we constructed five models of species addition which range across a hypothesized spectrum of patterns of novel species interaction formation and interaction generality. These models demonstrate that the impact of a novel species on community structure is mediated by its competitive ability, the number of interactions it forms with resident

species, and also by the additional new species it attracts. Experimental data from a community of plants and pollinators show that the invaded system was more diverse, ordered, and compartmentalized, but less complex. Our simulations reliably predict many components of this real network structure, and also demonstrate the importance of both nonrandom link formation as well as some lability in link identity. The models presented here substantially progress theory for conservation of networks of species interactions by formally outlining expected impacts of species addition in a way that can be used to direct future research and management objectives.

Introduction

In this increasingly anthropogenically-affected world (Ellis et al. 2010), the formation of novel ecosystems leads to unprecedented interactions between species, with potential implications for emergent attributes such as ecosystem stability, provision of services, and function (Hobbs et al 2006). Deliberately and accidentally introduced non-native species, species migrating in response to climate change or habitat destruction, and the assisted migration of endangered animals for conservation purposes (Hoegh-Guldberg et al. 2008) are all timely examples of how a new species might enter a community. After its introduction, a novel species will interact with those already present in the ecological community, which comprises a network of species interactions (Memmott and Waser 2002, Stokes et al. 2006, Lopezaraiza-Mikel et al. 2007, Vilá et al. 2009, Paynter et al. 2010). Because novel species form mutually beneficial as well as antagonistic (e.g.

predatory or competitive) interactions *de novo*, these interactions and their outcomes are difficult to predict.

The mutualistic interactions a non-native species forms with resident species may be particularly important (Stokes et al. 2006). Given that from 78% (in temperate communities) to 94% (in tropical communities) of plant species are dependent on insect pollinators (Ollerton et al. 2011), many introduced plant species will require integration into the existing pollination network of an ecosystem in order to establish (Chittka and Schürkens 2001, Moragues and Traveset 2005). To understand how the presence of a novel species changes the structure of interactions within the existing community, network theoretical approaches have become increasingly popular (e.g. Lopezaraiza-Mikel et al. 2007, Aizen et al 2008).

Changes in species composition can alter the emergent network-level properties of species interactions. For example, from an extinction-focused perspective, much work has been done to simulate species deletions in networks and determine effects on network structure. In general, networks are relatively robust to species loss (Memmott et al. 2004) and this robustness increases with some network level properties, such as connectance (Dunne et al. 2002), modularity (Valvodinos et al. 2009), and species richness (Dunne and Williams 2009). On the other hand, few studies model the impact of the addition of a mutualist on network level properties (but see Fedor and Vasas 2009, Romanuk et al. 2009, Devoto et al. 2012). Although invasive mutualists seem to be able to alter the network structure of resident communities, the results of comparative studies are sometimes contradictory. For example, invasive mutualists have been shown to increase

the nestedness (Bartomeus et al. 2008), but decrease the connectance (Aizen et al. 2008) of resident interaction networks. Other studies find little or no apparent impact of a species invasion on network structure (e.g. Vilá et al. 2009, Carneiro et al. 2011). It is therefore unclear how we might expect new species combinations to alter the original network of mutualistic interactions, and what the consequences of such changes would be (Hobbs et al. 2006).

Here we propose simple, yet potentially predictive, models of the impact of an introduced plant species on the network-level properties of a community of interacting plants and pollinators. We simulate introduction of a virtual plant species into a real network of plant and pollinator interactions, constructed from the replicated experimental manipulation of an invaded community (Lopezaraiza-Mikel et al. 2007). There are several plausible models for how a novel species may integrate into an existing ecological community by forging new links: a novel mutualistic species may interact with many species or few, add or replace pollination links, and/or add additional new species to the network (Fig. 4-1). We present five different models of novel species interaction formation: simple addition, competitive, supergeneralist, randomized pollinator matrix, and novel species substitution. These models form a gradient from simple to complex interactions, and demonstrate that the impact of this introduced species on network level properties relates not only to its generality (i.e. the number of species with which it interacts), but also to the ways in which it forms links, and whether it adds additional species.

Methods

We simulated the addition of a virtual plant species to plant-pollinator communities comprising interactions documented from a field experiment (Lopezaraiza-Mikel et al. 2007) using computer simulations in the program R (R Core Development Team 2008; see Appendix D for code). Each community was represented as an interaction matrix with rows of plant species, columns of insect species, and cells of the matrix the presence (1) or absence (0) of an observed interaction. This matrix represents a bipartite network of interactions represented as links between plant and insect nodes (Fig.4-1).

The simulated plant species was added as a node in the network (or a row in the matrix) with a set of interactions with pollinators determined by the interaction model type (see *Interaction Models* below). We simulated the addition of this species 1,000 times for each level of generality; each time, the interactions formed by the novel species were randomly assigned to resident species. After each simulation, network-level properties were calculated (see *Network-Level Properties* below). The results of the simulations were compared to real network structures in the presence of the invader or after its removal.

Data

The data we used for this analysis were from an experiment composed of four replicate unmanipulated sites that were paired with four replicate treatment sites where the flowers only of a single invasive species, *Impatiens glandulifera* (Himalayan Balsam), were removed from a plant community. The rest of the plant was left intact to

prevent unintended impacts from disturbance and alteration of above- and below-ground competition. Insect species that visited flower species in the sites were recorded between 2 July 2003 to 23 September 2003 and each of the eight sites was observed eight times (see Lopezaraiza-Mikel et al. 2007 for more details). The size of communities varied between sites, and the invaded sites had higher species richness for pollinators in every site, and for plants in all but one site (see Table C1 in Appendix C, also see Lopezaraiza-Mikel et al. 2007). The original study made comparisons between the community structure of the control (invaded) and experimental (*I. glandulifera* flowers removed) plots (Lopezaraiza-Mikel et al. 2007). Although data were collected on the frequency of visitation, here we only use the presence or absence of an interaction to make our models as generally applicable as possible.

The flowers of the invader were experimentally removed from manipulated sites; here we simulated the addition of a novel species back into these communities to see if they closely matched the control sites. Although our primary goal was not to perfectly recapture the behavior of this community in response to an invasion, this comparison served as a form of model validation. We believe that this is a valid comparison for two main reasons. First, this experiment was conducted to examine short term changes in community structure. The flowers of the invader were removed in the same summer as it was sampled. Thus, the insect species visiting the plots were likely foraging opportunistically, and the observed interaction structure was not a long-term community change. It is probable that, over a longer time period, the community would respond differently. However, over this short time frame, the community was likely to respond

similarly to abrupt changes in community composition (i.e. addition or removal). Second, the experimental plots were small (360-450 m²) relative to the surrounding plant community. Thus, they can be considered patches with a subset of a regional pool of species in which the pollinators can forage. Patchy distributions again lend themselves to opportunistic foraging strategies that are not strongly tied to the history of the patch community structure. Indeed, a simple removal simulation (whereby the invader was removed from control sites) shows that the community after the experimental removal of the invader was similar to the community after the simulated removal of the invader (see Lopezaraiza-Mikel 2006 for details).

Interaction Models

There are several plausible ways in which a novel species might integrate into an existing ecological community (Fig. 4-1). We generated five models of novel species interactions, each designed to simulate an introduction scenario. Together, the five models represent a gradient in the complexity of a species addition event. Each model of species addition was further run across a gradient of the network degree of the novel species (generalism), so that any interaction between degree of the novel species and the type of interaction could be observed. Because other systems may differ in the importance of interaction types (or have different novel behaviors), we have also included five other models in the supplemental materials (see Appendix C) for completeness.

The simplest model of species introduction is the **additive model** (Fig. 4-1B). In this model, a novel plant species enters the community and forges new mutualistic links with existing pollinators. We added a new row in the interaction matrix for the novel

species, and added values in the matrix to simulate its interactions with extant pollinators. In the context of the network, we added a new plant node, determined *a priori* how many interactions it would have, and then randomized these interactions with existing pollinator nodes. We simulated novel species with differing levels of generality, from specialist species that interacted with few pollinators (i.e. a plant node with few links) to a supergeneralist species that interacted with every pollinator (i.e. a fully linked node).

For the **competitive model**, the novel plant competes with resident plants for pollinator services (Fig. 4-1C). In this model, every interaction that the novel species forms with a pollinator removes one of the pollinator's existing interactions such that when the plant (added as a row) formed an interaction at random, an interaction was removed from another plant in that column (i.e. interacting with that insect). If there was more than one other interaction with that insect, the interaction removed was chosen at random.

It is also possible that the novel plant species will attract new mutualistic partners as it enters the community, thereby increasing the complexity of the addition process. In fact, there was a marked increase in the number of pollinator species in the invaded plots. For this reason, we developed two pollinator addition models. For the first, we simply added a supergeneralist pollinator with the novel plant. This **supergeneralist model** included a novel plant that interacted in the same way as in the additive model, but we also included a new column for the introduced insect (Fig. 4-1D). Because the new insect was supergeneralist, it interacted with every plant (i.e. it was a column of ones or a fully linked node). However, a more realistic approach, as informed by the data, was to add

multiple pollinator species. For the **randomized pollinator matrix model**, we added pollinators and their interactions from the invaded network to the uninvaded network (Fig. 4-1E). Specifically, we added columns to the uninvaded interaction matrix until the uninvaded community had the same number of pollinators as the invaded community. The columns that were added were randomly sampled from the invaded interaction matrix, and interactions within the added pollinator matrix were randomized during each simulation. In the pollinator matrix model, the novel plant species node was added as before, with the additional opportunity to interact with the new pollinators.

The most realistic neutral model possible for this system, which utilized all of the information available on the number of species and links in the invaded sites, was a **novel species substitution model** (Fig. 4-1F). We removed *I. glandulifera* from the networks by deleting its row from the interaction matrix and then simulated the addition of the novel plant species as a new row to replace it. We added interactions to the novel species row in a similar manner to the other models; in essence, we simulated its impact across a gradient of generality. However, it is distinct from the other models in that the interaction structure post-invasion among the resident insects and plants is kept intact. Interactions between the novel plant species and the pollinator species already present were randomized during each simulation, thus this model tested what would occur if the identity of the novel species links did not matter to the community structure.

The five other models we explored were a preferential model (novel links formed with generalist pollinators preferentially), static pollinator matrix model (similar to the randomized pollinator matrix above, but the interactions of added pollinators did not

change), novel species only pollinator matrix model (added pollinators only formed links with the novel plant species), plants and pollinators addition model (both plant and pollinator species were added until the richness of the new community was the same as the control), and randomized control model (all links in the control matrix were randomized in each simulation); they and their results are described in Appendix C.

Network-Level Properties

For each model type and degree value (i.e. number of links that the novel species formed), we ran 1,000 simulations. Each simulation generated a network of interactions between the plants and insects in our virtual communities and for each simulation we calculated six network-level properties. These properties range in complexity and provide complementary information on how the community is structured. To compare each degree/model combination with the real system, we averaged each of the six properties across the 1,000 simulations at the same number of links that the invader had in the real system.

The **average degree** demonstrates the level of interconnectedness in the community by showing how highly linked the species are, on average. It is calculated by dividing the number of links in the network by the number of nodes. We used a qualitative degree measure (i.e. an unweighted measure of degree). Although simple, the average degree gives an indication of the overall connectivity of the community. When a new plant species is added, the new system will have the same average degree if the novel species has a number of links equal to the average degree. If it has more, it will increase the average degree, if it has fewer, it will decrease the average degree (Fig. 4-1).

Because invasive plants tend to be generalists (Valvodinos et al. 2009, Tylianakis et al. 2010), the average degree can be expected to increase in an invaded system.

The **connectance** (also known as “density”) is the number of realized links divided by the number of possible links. In a sense, connectance is a normalized measure of degree. It has been suggested that connectance may relate to complexity and robustness to species loss (Dunne et al. 2002) as well as stability (Thébault and Fontaine 2010). However, connectance may be sensitive to small network sizes (Dormann et al. 2009) and so may not be useful for the smaller sites or networks smaller than fifty species. As with the average degree, if the novel species has a higher node level connectance than the number of links divided by the number of plant species, it will increase the connectance of the community after its introduction. Thus, we would expect connectance to increase after the addition of a generalist.

Compartments are subsets of nodes within the network that interact more strongly with each other than with outside nodes. The compartmentalization of a network, tied theoretically to stability and robustness (Krause et al. 2003), can be described in several ways. Here we describe the compartmentalization using **modularity (Q)**, the **number of compartments**, and the **median compartment occupancy** (i.e. the median number of species in a compartment). We measure compartments using the algorithm proposed by Newman and Girvan (2004). We hypothesized that the introduction of a new, highly connected node may reduce the number of compartments in the network, which would in turn increase the median compartment occupancy. The compartmentalization might change even when properties such as average degree and connectance remain constant. In

particular, a novel species that interacts with a broad subset of mutualistic partners would decrease the modularity, which describes how tightly the nodes within compartments interact. Higher modularity values have been theoretically shown to destabilize mutualistic networks (Thébault and Fontaine 2010), so it is possible that generalist species might be a stabilizing force. On the other hand, a larger number of compartments is thought to be stabilizing (Krause et al. 2003), so a novel species that decreases the number of compartments would destabilize the community. It is also possible for there to be a lower modularity between a higher number of compartments, resulting in a neutral effect on stability.

Nestedness is a measure of the order in a network relative to an “isocline of perfect order” and has been related to species and community persistence (Campbell et al. 2012) and stability and robustness (Thébault and Fontaine 2010, Poccock et al. 2012). It is often used because it is very stable despite environmental stochasticity (Petanidou et al. 2008) and not sensitive to sampling effort and network size (Nielsen and Bascompte 2007). We calculate nestedness using the NODF algorithm (Almeida-Neto et al. 2008). Again, in the case of an introduced invasive species, we would expect the novel species to be a strong generalist, and that the nestedness of the community would increase in response to invasion (Aizen et al. 2008). This would reinforce its overall stabilizing role in changing community structure.

Results

Invaded sites in the empirical mutualistic web (Lopezaraiza-Mikel et al. 2007) had on average a higher average degree, nestedness, number of compartments, and median compartment occupancy, but lower connectance than their invader-removed counterparts (Table 4-1). They also had higher species richness in both the plants and pollinators. Thus, the invaded system was more diverse, ordered, and compartmentalized, but less complex; the new species attracted to the invader were less well-linked on average than the original resident species. Comparing the simulations to the real system, we found that the overall best model (by sum of the absolute differences between the model predictions and observations) was the randomized pollinator matrix model, while the worst of the five was the competitive model. This suggests that the invader, in this case, was more likely to add new species and interactions than remove them. The fact that the model predictions of the randomized pollinator matrix are superior to all the other models also suggests that there is some lability in pollinator interactions that is relatively well-captured by a controlled randomization.

There was also some consensus in the predictions of the models in comparison to the real system (Table 4-2). For example, both average degree and connectance tended to be overestimated by the models (connectance was overestimated by every model) because the real invader attracted species that were more poorly linked, on average, than the simulated novel species (Fig. 4-2). The impact of the simulated novel species on average degree steadily increased as its generality increased for all five models (Fig. 4-2A), which was consistent with our expectation from the data. This trend was driven by

the high number of interactions of the novel species itself, and was thus moderated in the models where other species were also added; these other species tended to have a lower than average degree (Fig. 4-2A). The steepest slope in average degree occurred in the simple additive and supergeneralist models, while the lowest slope occurred in the competitive model, which only increased after the novel species had exhausted all extant links in the community and began adding links (Fig. 4-2A). The impact of the novel species still increased with increasing generality for connectance as we predicted (Fig. 4-2B), but in a much less linear fashion. In our simulations, the novel species substitution model exactly recaptured the average degree and connectance, as expected (Table 4-2), because the identity of the links was unimportant for these measures. Among the other models, the randomized pollinator matrix model best predicted the average degree of the real invaded system, while the competitive model better predicted the connectance (Table 4-2).

The impact of the novel species on nestedness tended to increase with increasing generality, but the responses of the supergeneralist and simple additive models (which behaved very similarly) were nonlinear (Fig. 4-3A). The sudden change in slope in these models was likely due to the fact that the novel species had become so highly linked that the nested subgroups became less apparent. In other words, the novel species, if a moderate generalist, acted to create a more ordered system but, if a supergeneralist, created a less ordered system unless its impact was mediated by additional species. Nestedness was well-represented by the novel species substitution model (Table 4-2).

In contrast, simply replacing the invader from the control communities with a node whose interactions were randomized (novel species substitution model) did not recapture the compartmentalization of the real invaded system (Table 4-2). Indeed, the compartmentalization of the real invaded network was greater than predicted by any model because the novel species in the simulations interacted at random with resident species, generating a more diffuse community, whereas the real invader formed links in a nonrandom pattern as evidenced by a more compartmentalized structure. It is notable that, in the real system, the modularity remained fairly constant (Table 4-1). Thus, all five models underestimated the change in modularity (Fig. 4-3C) and all but one underestimated the median compartment occupancy at the real number of invader links (i.e. the number of interactions the invader had in the real system, Fig. 4-3D). Similarly, the majority of the models underestimated the change in the number of compartments (Fig. 4-3B, except for two supplemental models, see Table C2). The three measures of compartmentalization (modularity, number of compartments, and mean compartment occupancy) were best predicted by the competitive, randomized pollinator matrix, and supergeneralist models, respectively (Table 4-2).

Among the different network level measures, the largest difference between the overall model predictions and the real system was in the median compartment occupancy (Fig. 4-3D), while the best predicted measure was connectance (Fig 4-2B). In all measures, the size of the community mediated the impact of the added species for all models, leading to the jagged appearance of the model responses as smaller communities were saturated with novel species links (Figs. 4-2 and 4-3). However, there were some

consistent trends across sites. For example, the median compartment occupancy (Fig. 4-3D), connectance (Fig. 4-2B), and number of compartments (Fig. 4-3B) remained fairly constant across a gradient of novel species degree and between different model types, while nestedness (Fig. 4-3A) and average degree (Fig. 4-2A) generally increased linearly with generality and the modularity (Fig. 4-3C) slightly decreased. Though the number (Fig. 4-3B) and size of distinct groups (Fig. 4-3D) of interacting species remained fairly constant in response to species additions, the interactions between separate groups became more diffuse (Fig. 4-3C). Both the simulated and real systems were more ordered when the novel species was present (Fig. 4-3A).

Discussion

As species expand their ranges, naturally or due to anthropogenic causes (Hoegh-Guldberg et al. 2008), they begin to interact with resident species, forming novel ecosystems (Hobbs et al. 2006). Here, we argue that formal theoretical expectations of species addition substantially advance our ability to make predictions about how introduced mutualists will interact in novel ecosystems. Our models demonstrate that degree (or the number of interactions) of the novel species is important, but not the only factor, for determining a novel species' network level impact. The way a novel species forms new mutualistic links (e.g. competitive or additive) and the other species it attracts also determine how the network structure will change. Neutral models of species interactions (where interactions are randomized) recapture simple measures of network

structure well, but show limitations when recapturing complex network structures that are informed by link identity or nonrandom interactions.

These models are limited in that (1) they do not incorporate any interaction dynamics (see Olesen et al. 2008; Romanuk et al. 2009), (2) we use the presence or absence of an interaction, as opposed to interaction frequency, and (3) the validation is done with only one system. Overcoming these limitations would improve our understanding of the process of species addition. For example, the incorporation of dynamics would allow for exploring the persistence of novel species through time, and would show how their impacts on the interaction structure are strengthened or reduced as the system equilibrates to their presence. The invader in this system, *I. glandulifera*, also had a much higher interaction frequency than any other plant species, so the incorporation of weighted interactions could provide new insights into its impact. Unfortunately, experiments like this one, which include not only detailed species-level interactions, but also experimental manipulation and replication, are uncommon. However, it would be very interesting to compare the behavior of these models in a larger number of empirical communities. Different results would suggest not only the importance of novel species behavior to mediate its impact, but may also suggest whether some communities are more or less resistant to invasion based on their interaction structure.

These models also have many advantages. They are simple and intuitive, provide a range of different interaction types and novel species behaviors to explore, do not require extensive knowledge of the system *a priori*, and there are many promising ways

to extend them. For example, with information about invader traits (i.e. corolla depth or proboscis length), these models could be used to make predictions about link identity as well (e.g. Campbell et al. 2011, Eklöf et al. 2013). We accurately predict simple descriptors of network structure, such as average degree and connectance, with relatively little information about the novel species. Because most invasive species are generalists (Valvodinos et al. 2009, Tylianakis et al. 2010), our expectations are that the overall number of interactions in the community would increase after invasion. However, it is also possible to design models of species addition where this is not the case. Even highly generalist novel species fail to increase the average degree and connectance of a network if they attract other new, poorly linked, species (as in the randomized pollinator matrix model) or compete for interactions (as in the competitive model).

We hypothesized that systems where a strong generalist is introduced would become more nested, but less compartmentalized. If the novel species interacts at random with mutualistic partners, the links will be diffusely distributed among the compartments, leading to a lower modularity. However, our models generally underestimate these measures of compartmentalization. Because of the interaction between modularity and number of compartments, one might expect a relatively neutral effect on stability (Krause et al. 2003, Thébault and Fontaine 2010). These results show that neutral link formation and deterministic behaviors may jointly shape the network structure of invaded communities, as has been seen in with previous research on the architecture of arthropod communities (Ellwood et al. 2009).

In many cases, the properties of the networks respond linearly to increasing novel species degree. This implies that the more generalist the introduced species, the larger the impact it will have on the community. For example, the average degree and nestedness of the community tend to increase linearly, while modularity tends to decrease linearly. On the other hand, some network level properties are relatively constant across a broad range of novel species generality. For connectance, number of compartments, and median compartment occupancy, the addition of new species is more important than the number of links that the focal species forms. The models show very different outcomes if the invader is competitive as opposed to adding links; the complexity of the interactions and the capacity to attract other new species strongly affect the ultimate network structure.

Notably, just because we do not detect strong changes in network-level properties does not imply that the novel species is not changing the interaction patterns of the community. For example, in the competitive model, the novel plant species removes links from other plant species in the community, yet nearly all of the network-level properties remain constant across a range of novel species generality. The exception, in this case, is modularity, which decreases even in the competitive model. Some properties, such as median compartment occupancy, remain fairly constant even after a suite of both plants and pollinators are added (Fig. 4-3). This suggests that studies that detect no or little invader impacts on a network level (e.g. Vilá et al. 2009, Kaiser-Bunbury et al. 2011) may be missing some competitive interactions, and highlights the importance of measuring multiple aspects of network structure. There are several ways in which one

property of a network might remain constant after the introduction of a species, while other properties show the effect.

Among the five models we tested here, the model that performed the best across all measures (relative to the real system) was the randomized pollinator matrix model. This model incorporated some information about the reality of the system (i.e. new pollinators are attracted to invaded sites), but it did not contain information about link identity, as interactions with these new pollinators and the invader were randomized for each simulation. It is promising that such a simple model can predict some measures very closely. In contrast, the model that performed the worst was a simple randomization of all links in the actual data comprising the invaded network (Table C2 in Appendix C). This demonstrates the importance of nonrandom link formation among the resident species present in the network and also shows that the novel species, as a mutualist, interacts differently than the resident plants.

These five simple models generate predictions of how a novel species will change the structure of an ecological community through both direct and indirect effects on mutualistic interactions. To the best of our knowledge, these are the first models to simulate the addition of a virtual species to real data collected through experimental manipulation. Using a gradient of model complexity and novel species generalism, we demonstrate that the impact of even highly generalist novel species can be strongly mediated by the way it forms interactions with resident species. A few basic hypotheses about how a novel species will interact can change our predictions. For managers of systems that are facing imminent species introductions or for those that seek to conserve

species interactions in novel ecosystems, these predictions might help direct conservation objectives. More broadly, these models have the potential to provide insight into how ecosystems respond to the invasion of species on a community level, and how these impacts might cascade through mutualistic interactions to directly compete for mutualistic partners, or indirectly shift the structure of the entire network. Our models suggest that the addition of a new species could alter the stability of the community, and that the impacts of the novel species might be concealed by compensating changes in the interactions of other species.

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Author contributions

All the authors motivated the research, discussed the results, and wrote the manuscript. L.R. performed the analyses, and wrote the initial draft of the paper, J.M. provided the data, and Y.B. assisted with the analyses.

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Tables

Table 4-1 Properties of the real data for experimental and control plots averaged across four sites.

	Average Degree	Connectance	NODF	Modularity	Number of Compartments	Median Compartment Occupancy	Number of Pollinator Species	Number of Plant Species
Invaded Invader	1.26	0.16	11.9	0.39	8	7.63	61	10
Removed	1.01	0.23	6.93	0.34	5.75	3.63	33.25	6.5

Table 4-2 Difference values (directionality indicated in parentheses) for the model outputs for the first order properties at the precise number of links that the actual invader had in the real system (21.75). The difference between the size (number of plant and pollinator species) of the different models and the control plots is also included. The lowest differences are highlighted in bold.

	Average Degree	Connectance	NODF	Modularity	Number of Compartments	Median Compartment Occupancy	Number of Pollinator Species	Number of Plant Species
Additive	0.28	0.14	2.90	(-) 0.27	(-) 2.61	(-) 4.42	(-) 27.75	(-) 2.5
Competitive	(-) 0.28	0.02	(-) 5.57	(-) 0.02	(-) 1.55	(-) 4.62	(-) 27.75	(-) 2.5
Supergeneralist	0.41	0.16	2.48	(-) 0.29	(-) 3.23	(-) 1.94	(-) 26.75	(-) 2.5
Randomized Pollinator Matrix	0.22	0.08	(-) 1.89	(-) 0.21	(-) 0.81	1.95	0	(-) 2.5
Substitution	0	0	(-) 0.75	(-) 0.11	5.84	(-) 6.13	0	0

Figures

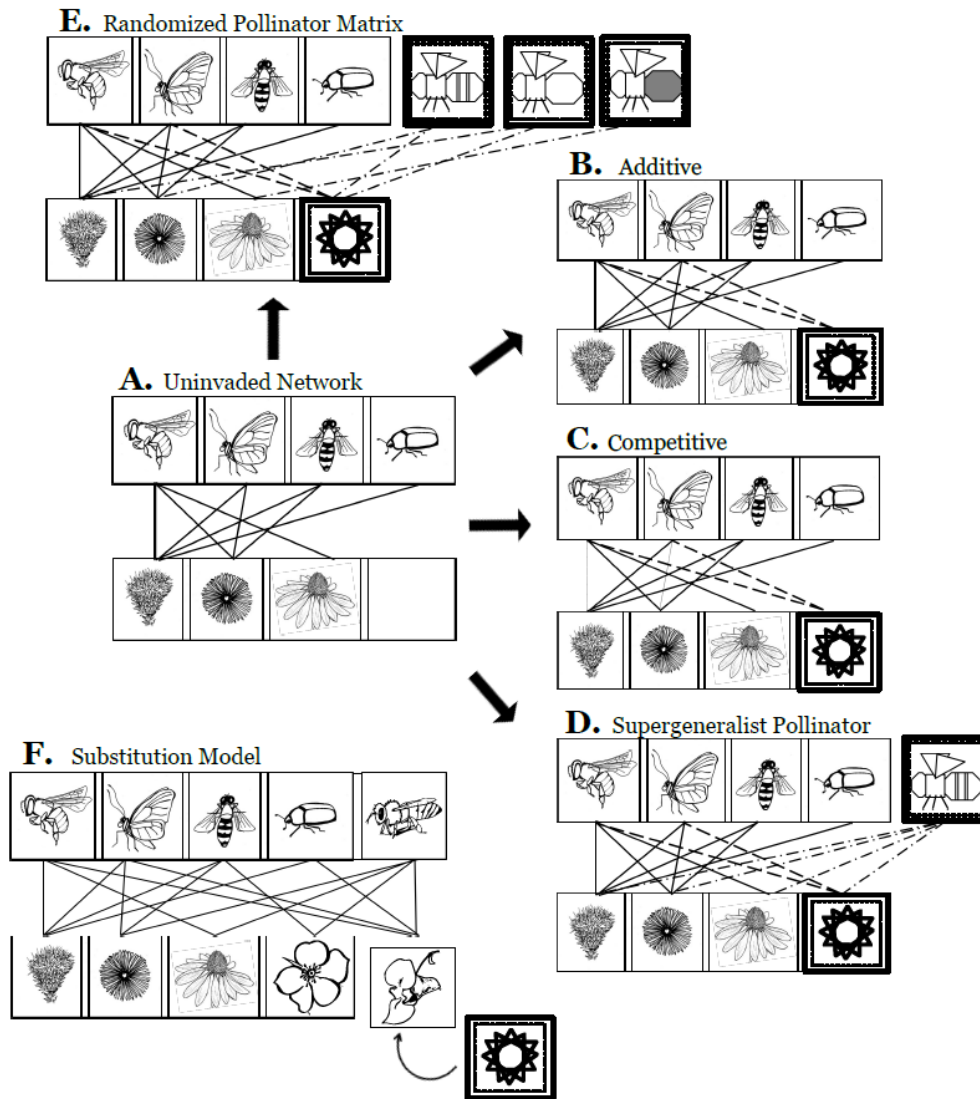


Figure 4-1 A heuristic figure to demonstrate the simulated addition of a novel species node to an uninvasive plant-pollinator network (A), according to different models: an additive model (B) where the novel species forms new links (dashed lines) with existing species, a competitive model (C) where the novel species removes one link (dotted lines) from each species with which it forms a new link (dashed lines), a supergeneralist pollinator model (D) where the novel species attracts a supergeneralist pollinator that interacts with all resident plant species, a randomized pollinator matrix (E) where the novel species attracts a matrix of pollinator species that interact randomly with resident species. Additionally, an invader substitution model (F) replaces the real invader in the invaded (more species rich) sites with a simulated novel species.

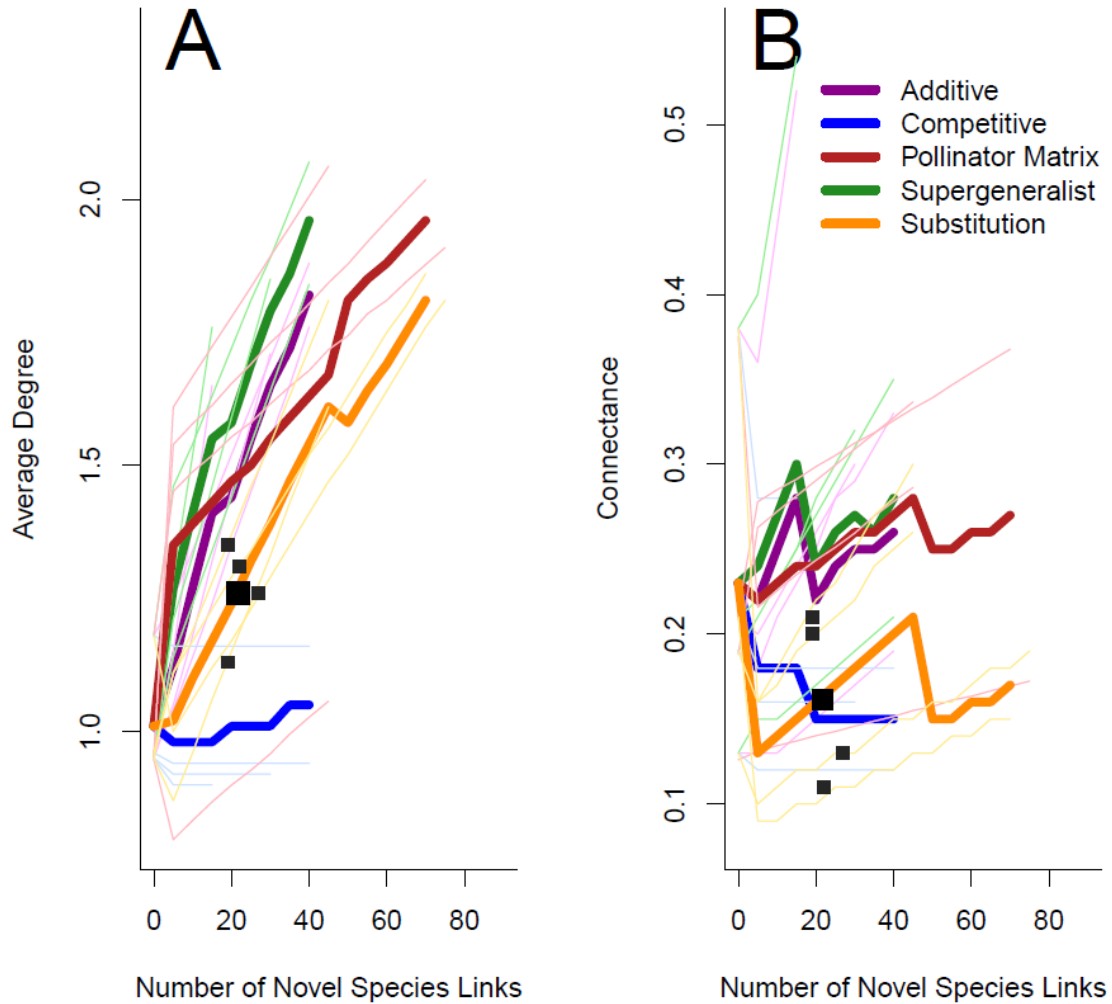


Figure 4-2 The impact of simulated species addition on the average degree (A) and connectance (B) of the community, across increasing novel species degree. The five models of simulated novel species behavior are represented as follows: additive is a purple line, competitive a blue line, supergeneralist a green line, randomized pollinator matrix a red line, and novel species substitution an orange line. The thick lines represent the average model response across the four sites, while the thin lines represent the individual model responses of the four sites. The novel species saturates the links available in the smaller of the sites sooner, leading to the jagged appearance of the response. The black squares (■) represent the average degree (A) and connectance (B) of the control community. The large squares represent the averages across four sites, and the smaller squares represent the properties of the individual sites.

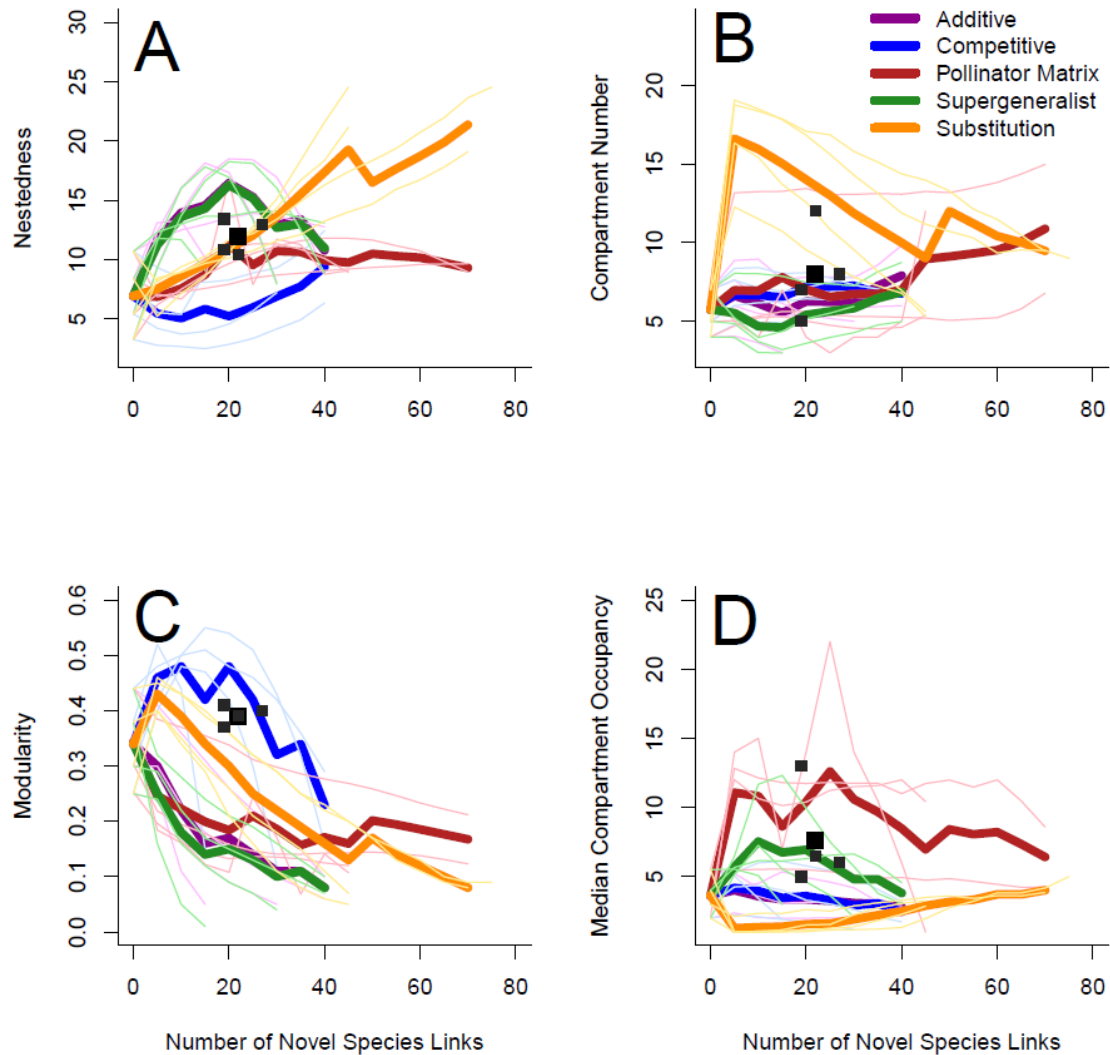


Figure 4-3 The impact of the simulated species addition on nestedness (A), compartment number (B), modularity (C), and median compartment occupancy (D). As before, the additive model response is a purple line, competitive a blue line, supergeneralist a green line, randomized pollinator matrix a red line, and novel species substitution an orange line. The thick lines represent the average model response across the four sites, while the thin lines represent the individual model responses of the four sites. The novel species saturates the links available in the smaller of the sites sooner, leading to the jagged appearance of the response. The black squares (■) represent the nestedness (A), compartment number (B), modularity (C), and median compartment occupancy (D) of the control community. The large squares represent the averages across four sites, and the smaller squares represent the properties of the individual sites.

Chapter 5

The Composite Insect Trap: an innovative combination trap for biologically diverse sampling

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Abstract

Documentation of insect diversity is an important component of the study of biodiversity, community dynamics, and global change. Accurate identification of insects usually requires catching individuals for close inspection. However, because insects are so diverse, most trapping methods are specifically tailored to a particular taxonomic group. For scientists interested in the broadest possible spectrum of insect taxa, whether for long term monitoring of an ecosystem or for a species inventory, the use of several different trapping methods is usually necessary. We describe a novel composite method for capturing a diverse spectrum of insect taxa. The Composite Insect Trap incorporates elements from four different existing trapping methods: the cone trap, malaise trap, pan trap, and flight intercept trap. It is affordable, resistant, easy to assemble and disassemble, and collects a wide variety of insect taxa. Here we describe the design,

construction, and effectiveness of the Composite Insect Trap tested during a study of insect diversity. The trap catches a broad array of insects and can eliminate the need to use multiple trap types in biodiversity studies. We propose that the Composite Insect Trap is a useful addition to the trapping methods currently available to ecologists, and will be extremely effective for monitoring community level dynamics, biodiversity assessment, and conservation and restoration work. In addition, the Composite Insect Trap will be of use to other insect specialists, such as taxonomists, that are interested in describing the insect taxa in a given area.

Introduction

There are many methods of insect collection, both active and passive. However, most specialize on one type of insect and depend on the insect's behavioral response to stimuli. This is ideal for studies that focus on a single taxon or guild of interest, but these traps do not collect a representative sample of all insects present in a given ecosystem. In fact, many scientists recommend using multiple trap types to ensure a complete collection, even for just one taxon [1-5]. However, it is time consuming and expensive to implement multiple trap types.

Because we were interested in the diversity of the flying insect community rather than in one particular taxon, none of the traditional trap types were sufficient by themselves, and it would have been prohibitively expensive to use multiple trap types. For these reasons, we designed a nonspecific insect trap, the Composite Insect Trap. To construct this passive trap, we incorporated the design of components from other, more

targeted trap designs. In addition to the collection of a diversity of insect taxa, we designed the Composite Insect Trap to be cost-efficient and easy to assemble and disassemble, making it ideal for rapid biodiversity assessment, investigative pilot studies, large-scale censuses, low-budget research, and educational purposes. We also designed the Composite Insect Trap to be easy to transport so that we could move it long distances between trapping locations, and robust so that it would withstand inclement weather conditions.

Our trap was inspired by four other widely used and well recognized passive trapping devices for catching flying insects: the malaise trap, cone trap, pan trap, and flight intercept trap [1,6-9]. Each of these trap types has advantages and disadvantages and there are many cases where one of these traps excels while another is deficient (e.g. [10]). The deficiencies of a trap are particularly restrictive when insect biodiversity is of interest, because they will fail to catch select groups of insects. Here we briefly describe each of these traps and how they influenced the construction of the Composite Insect Trap.

Malaise traps catch those insects that fly upward to avoid an obstruction in their flight path [11]. They are very widely used and typically involve a mesh netting canopy which slopes up, forcing insects into a collecting jar filled with a killing agent. Malaise traps have been shown to be consistent and reliable in the species they catch over a summer [12].

Cone traps (specifically “Texas” cone traps) consist of a wire mesh cone on a pole with a collection container on top and also catch insects that fly upward. However, the cone trap differs from the malaise trap in that it employs a bait to attract insects [13].

Although there are many studies touting the effectiveness of the cone trap, it is inherently species-specific because the bait is often pheromone based, and is thus unlikely to attract nonfocal species. Cone traps are usually used to monitor Lepidopteran pests, such as the tobacco budworm [13,14].

Flight intercept traps consist of a vertical mesh barrier above a collection dish filled with a killing agent. The flight intercept trap catches those insects that drop down to avoid obstacles. Although one study found that malaise traps caught a greater diversity and abundance of beetles than flight intercept traps [12], most studies agree that beetles drop when encountering an obstacle and are more likely to be caught in a flight intercept trap [1,15,16]. As with malaise traps, the mesh barrier intercepts not only local arthropods, but also those that are dispersing through an area and is thus an important component of the trap, allowing it to capture more than the collection dish would alone [3]. This trap is sometimes constructed with plastic or glass instead of mesh netting, but these materials not only make the trap more expensive, but also heavier, bulkier, and more susceptible to damage [15].

Pan traps consist of a shallow dish filled with soapy water. The soap acts as a surfactant and breaks the surface tension so that the insects drown and can be collected later. Pan traps are often painted to be attractive to various kinds of insects. In particular, yellow pan traps [6] are broadly attractive to pollinators, aphids, and parasitoid wasps [3]. Although certain groups are more attracted to blue pan traps, yellow seems to attract a greater diversity of insects [17,18]. Other insects are attracted to the pan trap because of the water. In some cases, pan traps have been shown to collect groups that are poorly

represented by malaise trap collections and sometimes are more effective at catching pollinators [2,19].

The Composite Insect Trap has components that resemble each of these standard trap types (Fig. 5-1). The cone portion of the Composite Insect Trap combines elements from the malaise and cone traps. Although it superficially resembles the cone trap in appearance, it is functionally more closely related to the malaise trap because it relies on the malaise method of forcing the insects upward to avoid a mesh obstruction below and has a collecting container filled with a killing agent instead of a pheromone bait. The cone component of the Composite Insect Trap serves as the capturing mechanism and is set on top of the middle portion of the Composite Insect Trap, a flight intercept trap that captures insects that fly downward as well as upward. As a collection dish below the flight intercept trap, there is a yellow pan trap filled with soapy water that collects insects attracted both by the yellow color and by the water, as well as those that drop down when encountering the mesh of the flight intercept component.

By combining these designs, we aim to maximize the advantages of each trap type and minimize their disadvantages without requiring the collector to set up multiple traps at every sampling site. Our Composite Insect Trap is designed so that the deficiencies of each component are covered by the others. Although the Composite Insect Trap also has biases, as dictated by its components, it is designed to capture the broadest diversity of insects, and to be as nonspecific as possible. The Composite Insect Trap is not tailored to collect any one taxon and provides a nonspecialized method to collect a diversity of insects, thus providing an alternative that reduces the cost in a sampling design targeted at biodiversity assessment.

Materials and Methods

In the Composite Insect Trap, elements of the malaise trap design are used in the cone and flight intercept trap components, but there is not a separate malaise component. Thus, the trap has three main components: cone, flight intercept, and pan traps (Fig. 5-1A). Each of these components is constructed separately and then assembled at the field site.

Cone trap construction

We used readily available components to build the Composite Insect Trap (Table 5-1). To prepare the collection chamber of the cone portion of the trap, we cut the top of a two-liter bottle (PET plastic) approximately 6 cm down (where the curve began) (Fig. 5-1C). The lid of the two-liter bottle also needed modification. We removed the top of the lid so that only the threads remained, thus creating a tube which could connect the collection chamber to the cone section. Although the lid of the two-liter bottle and the plastic container became one unit, it was still possible to attach the lid to the top of the bottle, while leaving an opening for insects to fly through.

Next, we modified a plastic container (Fig. 5-1B). We cut a hole in the center of the bottom of the container and inserted the lid of the two-liter bottle until the bottom of the lid and the bottom of the container were level. The top of the lid now protruded into the empty container (Fig. 5-1B). The lid was held in place by a sealant applied around the edge of the lid/container connection. When filled with alcohol and attached to the severed top of the two-liter bottle, this formed the collection chamber.

The collection chamber was then attached to a cone constructed from a square piece of mesh netting, approximately 80 cm to a side. We sewed canvas strips (approximately 50 cm) onto the mesh using a sewing machine to create pockets that held the PVC flag stems (Fig. 5-2A). A hole was cut out of the center of the mesh to create a narrow opening at the top, which was attached with silicone sealant onto the collection chamber.

In use, the container at the top of the trap was filled with 70% ethanol to just below the rim of the bottle cap. Insects flew in through the hole in the bottle cap and were simultaneously killed and preserved in the ethanol.

Flight intercept construction

To build the flight intercept portion of the trap (Table 5-1), we first drilled one hole through each PVC pipe, approximately one centimeter from the top, and a second hole another centimeter below and offset by 90 degrees to the first. In the field, we inserted bamboo rods through these holes to stabilize the trap. We also drilled a small hole approximately 75 cm from the bottom of the pipe.

The flight intercept trap required two rectangular pieces of mesh netting (approximately 120 cm long and 90 cm wide), which were attached along the center in an “X” pattern. Using a sewing machine, we sewed hem tape along the longitudinal center line of each piece of mesh and then sewed the hem tape strips together to connect the pieces. Next, we created pockets large enough to hold the PVC pipes by folding over the side of the mesh and sealing it with hem tape to avoid tearing (Fig. 5-2B).

After placing the pipes in the mesh pockets, we threaded the string through the hole 75 cm from the bottom of the pipe. We then tied the other end of the string to the garden stake and repeated this procedure for the three other pipes.

To set the trap up at a site, we stood the PVC pipes on top of the four plastic rebar stakes, which were set approximately a half meter apart in the ground. We then pulled the string taut so that the pipes stood upright at each of the four corners, and hammered the garden stakes into the ground. Finally, we threaded the bamboo rods through the holes at the top of the pipes to stabilize the trap (Fig. 5-1D).

Pan trap construction

The pan trap was simple to construct (Table 5-1). We sprayed yellow paint (“Rust-oleum” gloss protective enamel, yellow) on the inside of an aluminum pan 34 by 46 and 9 cm deep. In use, we filled the pan with soapy water and placed it beneath the flight intercept trap (Fig. 5-1E).

Field procedure

We designed, constructed, and utilized six Composite Insect Traps during the summer of 2009. The traps were in continuous use from June through August and were set up, taken down, and transported between field sites on a daily basis. Over the summer, these six traps were set up a total of 134 times; 90 times in an agricultural system and 44 times in weedy fallow fields in central Pennsylvania, USA. Traps were left up for 24 hours for each collection event.

During collection, the chamber of the cone trap with its ethanol and insect specimens was emptied into a labeled scintillation vial. The pan trap was emptied

through a funnel into a mesh bag, which was then stored in the freezer until it could be processed.

Processing procedure

We identified the insect specimens collected in the traps by following the dichotomous keys provided in Borror and DeLong's *Introduction to the Study of Insects*, 7th edition [20]. We tested the differences in mean abundance of insects caught per trap per day and mean number of orders captured per trap per day between the cone and pan traps with a two-tailed *t* test. Voucher specimens of the insects collected will be stored at the Pennsylvania State University.

Results and Discussion

Insects are a hyperdiverse taxonomic group, probably more diverse than any other terrestrial metazoan group [21,22]. There is a need for new collection methods that are cost effective, precise, and reliable in order to document the taxonomic and ecological value of this important class of animals. We know very little about insect diversity; it is estimated that less than 20% of the species on Earth have been identified and described [23], but this estimate is based entirely on the diversity of small samples of tropical arthropods [24,25]. Equipment similar to the Composite Insect Trap would be useful for consistent sampling across multiple taxa and for documenting and understanding the diversity of insects. Toward this goal, we designed the Composite Insect Trap to be nonspecific and to collect a range of taxa. It is a composite of four commonly used trap types, and collects insects that avoid obstacles by flying upward, as well as those that

avoid obstacles by dropping down. To the best of our knowledge, no one else has published a description of a similar trap, although unpublished designs may exist.

The Composite Insect Trap is a useful tool for sampling at multiple locations, as it can be assembled and disassembled rapidly and transported easily. It is light weight but withstands weather well and can be used for multiple years. A complete trap weighs approximately 4.5 kg, excluding the alcohol and soapy water required to fill the cone and pan components, respectively. One person was able to set up the trap alone and required approximately 15 min to assemble or disassemble a Composite Insect Trap at a sampling location. Despite multiple thunderstorms and high winds, the traps never collapsed or were destroyed. Minor damage, such as tearing, was easily repaired with patches of mesh netting. If a trap sustains more severe damage, its individual components are affordable and easy to replace. In terms of durability, the Composite Insect Trap is similar to malaise traps in that the fabric becomes more fragile with time due to exposure to UV light (J. Tooker, personal communication). However, at the end of the summer, the traps were in good condition for use in future field seasons.

We collected almost 15,000 specimens of 21 different orders with the Composite Insect Trap over a period of three months during the summer of 2009. All of these specimens were identified to the order level. At this resolution, we found great diversity. The majority of the insects in the traps were Diptera (56%), Hemiptera (26%), Coleoptera (7%), and Hymenoptera (7%), but there were representatives from the insect orders Blattodea, Collembola, Dermaptera, Ephemeroptera, Lepidoptera, Mecoptera, Neuroptera, Odonata, Orthoptera, Plecoptera, Psocoptera, Thysanoptera, Trichoptera, and non insect arthropods such as Acari (mites), Araneae, Opiliones, and Diplopoda.

Because the pan and cone components of the Composite Insect Trap were collected in separate chambers, their captures could be evaluated separately. However, their captures were not independent of each other because they shared the trapping mesh of the flight intercept component. The pan component caught a greater abundance of insects and a greater diversity of insect orders (abundance per trap day [mean, SE]: cone [4.34, 0.50], pan [106.03, 5.56], $P < 0.001$; number of orders: cone [1.57, 0.10], pan [5.44, 0.13], $P < 0.001$). Despite the efficacy of the pan trap, Neuroptera was only found in the cone component and, at a higher taxonomic resolution, it may be found that the cone component selectively catches some taxa the pan does not. Compared to standard “Texas” style cone traps, which are designed to catch a particular pest organism, the cone component of the Composite Insect Trap nonetheless catches a much greater variety of insects [13].

In order to fully test the efficacy of the Composite Insect Trap, we would ideally have deployed a full suite of other insect traps at the same sampling sites for comparative purposes. Unfortunately, this was financially and logistically prohibitive. Instead, we compared our collection to published studies using a range of other trap types. The Composite Insect Trap appears to compare well with these studies in terms of the number of taxa collected [3,4,16,18]. Even though these studies took place in a broad range of habitats, from salt marsh [18] to tropical forests [4] to agricultural landscapes [3], the Composite Insect Trap lacked only 3 insect orders that were caught in these other studies: Mantodea, Isoptera, and Archaeognatha. It is unknown whether the absence of these orders was due to their actual distribution or to a bias of the trap itself.

For a researcher interested in the biases of the trap, it would be possible to test the Composite Insect Trap against the other trap types. It would also be possible to achieve a greater understanding of the sampling biases of the Composite Insect Trap by comparing catches with and without different components. For example, one could compare the catch in the pan with and without the yellow paint. Finally, it would be possible to test different colors or sizes of mesh to understand the visibility of the material used in the flight intercept and cone components. All traps have intrinsic biases, however, and the biases of the Composite Insect Trap should not prevent it from being a useful tool in studies focusing on insect diversity.

Although it is difficult to compare the results of our study directly with those in published studies because of differences in climate, habitat, and sampling design, the diversity collected by the Composite Insect Trap appears to compare favorably at the level of insect order with that collected by each of the other four trap types. The diversity of insects we collected in one summer of trapping suggests that the Composite Insect Trap could conveniently be used as a part of biodiversity assessment or a species inventory at a given location (e.g. [23]). With the number and diversity of insect types it collects, it could provide a broad overview of the insects that live within that environment. Because the use of multiple species in several different taxa would be a more reliable indicator of ecosystem health than a single indicator, the Composite Insect Trap could be used in conservation work where the ecosystem must be assessed for overall health [26]. Similarly, insects that act as ecological indicators could help in the assessment of restoration areas [4].

In addition to evaluating the relative effectiveness of the Composite Insect Trap, we collected information from major biological retailers to compare the cost of the Composite Insect Trap to other trap types (Table 2). This simple evaluation demonstrates the relative affordability of the Composite Insect Trap. It appears that that only malaise and flight intercept traps can be ordered prefabricated, although a smaller fabric cone trap is also sold (Table 5-2). The “Texas” style cone trap must be built locally as it is neither commercially available, nor easily shipped. The nets for a flight intercept trap are sold without a pan, but pan traps can be made by painting any aluminum or plastic container, and their price is minimal. The pan component of flight intercept traps and stand alone pan traps are similar to the pan component of the Composite Insect Trap.

For a study that requires multiple trap types, the supplies for the Composite Insect Trap (approximately \$25.00 US) would represent a savings of up to 90% - 97% (Table 5-2). Even after adding in the time spent constructing the insect trap, it compares favorably. Approximately 42 hours were spent constructing 6 Composite Insect Traps: an average of seven hours per trap. At a cost of \$7.50 US per hour for labor, this equates to an additional \$52.50 US per trap, or a price of \$77.50 US per Composite Insect Trap. At this cost, the Composite Insect Trap represents a savings of up to between 70% - 90%.

To purchase and employ each of the four trap types used in the design of the Composite Insect Trap would be both time consuming and expensive. Our review of some common traps available through major biological retailers suggests that the Composite Insect Trap is a much more affordable option than either the malaise trap or cone trap alone, and that its relative value is much greater when multiple trap types are considered.

If long-term monitoring is of interest, the Composite Insect Trap has the potential to simplify the sampling protocol because it combines multiple trap methods into one. In this way, it reduces the variation due to trap type and will be more easily standardized. For example, it could be incorporated into a design where the long-term monitoring of multiple pest species is an objective [13]. Eliminating the necessity for multiple traps may facilitate cross-study comparisons as well as reduce the cost and time required to implement a sampling regime.

To the best of our knowledge, the Composite Insect Trap is the only trap of its kind to combine multiple different methods to capture many different insect taxa. The Composite Insect Trap is flexible in its usage because individuals using the trap may choose to construct it to their own desired size specifications to address different ecological questions. However, its greatest utility lies in its capacity to catch a large diversity of insects. In addition, it is ideal for pilot studies, studies on a restricted budget, educational collections, and for those interested in diversity as opposed to a single taxon or guild. Because it is affordable to construct, simple to assemble, robust and easy to transport, more traps can be built within a restricted budget or time frame. The Composite Insect Trap has the potential to eliminate the need to use multiple trap types in studies of biodiversity and in the assessment of conservation and restoration areas where insects are ecological indicators.

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Author contributions

All the authors motivated the research, discussed the results, and wrote the manuscript. L.R. designed the trap and wrote the initial draft of the paper; M.H., R.S., and L.R. constructed and implemented the traps and identified the insect specimens.

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Tables

Table 5-1 Components required to build the three portions of the Composite Insect Trap.

Materials Needed:	Cone	Flight Intercept	Pan
Strips of canvas fabric	X		
4 PVC flags (with flags removed)	X		
1 plastic container with lid (~11.5 cm diameter and ~5.5 cm deep) for holding alcohol	X		
1 two-liter soda bottle (PET plastic) with lid	X		
Silicone sealant (GE Silicone II Kitchen and Bath)	X		
70% ethanol	X		
Mesh netting (bridal tulle)	X	X	
String	X	X	
Hem tape		X	
4 thin bamboo rods		X	
4 PVC pipes 2 cm diameter, 1.5 m length		X	
4 plastic garden stakes		X	
1 drill with drill bits		X	
4 plastic rebar stakes		X	
Soap			X
Water			X
1 aluminum pan (34 X 46 cm, ~9 cm deep)			X
Yellow spray paint, "Rust-oleum" gloss protective enamel			X

Table 5-2 Prices for the Composite Insect Trap and other commonly used trap types collected from major biological retailers. (Websites accessed April 2010.)

Trap Name	Brief Description	Example Source	Estimated Cost
Composite Insect Trap	Flight intercept/ Malaise/ Cone/ Pan trap composite for diversity studies	_____	\$77.50 US
Malaise Trap	Flight trap with mesh barrier and killing reagent in collection chamber above	BugDorm Store: http://bugdorm.megaview.com.tw/ or John W. Hock Company: http://www.johnwhock.com/	\$184.00- \$574.00 US
Flight Intercept Trap	Flight trap with mesh barrier and killing reagent in collection chamber below	Alana Ecology: http://www.alanaecology.com/ or Sante Traps: http://www.santetraps.com/	\$30.00 US
Pan Trap	Dish on ground filled with killing reagent	Fleischer 2010, pers. comm	\$2.00- \$10.00 US
“Texas” Cone Trap	Large wire cone trap with pheromone bait in collection chamber above	Fleischer 2010, pers. comm	\$225.00- \$275.00 US
Trap Name	Brief Description	Example Source	Estimated Cost
Maryland Wire Cone Trap	Smaller “Texas” type trap	S. Fleischer, personal communication	\$180.00 US
Scentry Heliothis Trap	Nylon mesh cone with pheromone to attract members of the <i>Heliothis</i> genus	Gempler's: http://www.gemplers.com/	\$80.00 US

Figures

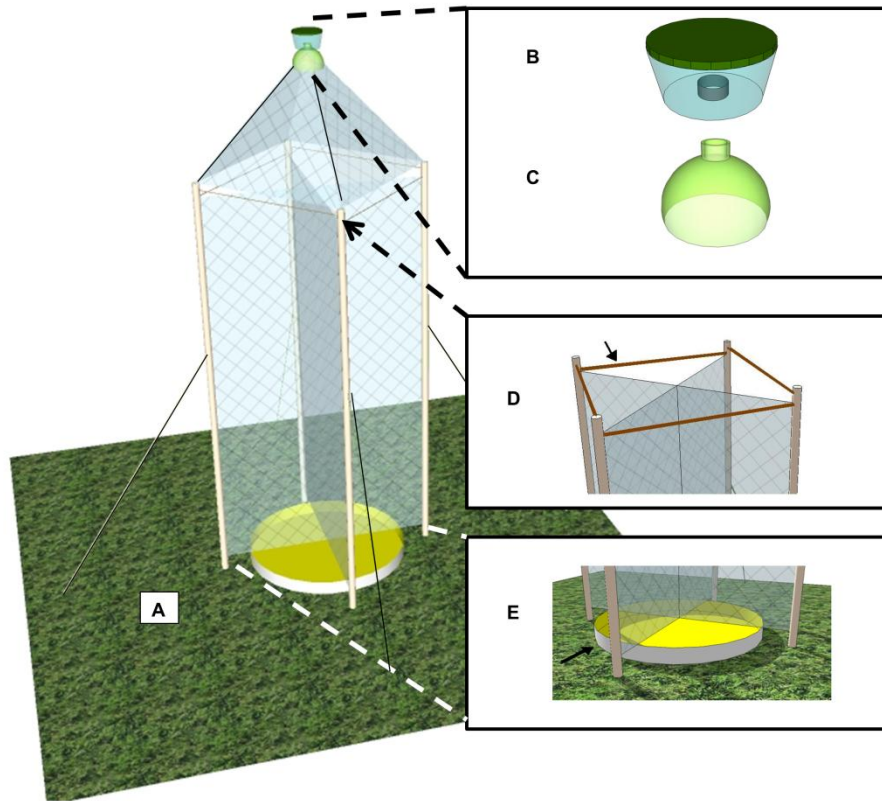


Figure 5-1 A) The Composite Insect Trap is a passive trap designed to collect as broad a spectrum of insects as possible, utilizing ideas from other, more specialized trap designs in a novel construction. The plastic components of the cone form the collection chamber (B and C). B) The modified plastic container with embedded bottle top will hold the alcohol for killing and storing insects. C) The top of the two-liter bottle will be attached to the mesh fabric of the cone. D) The bamboo rods stabilize the top part of the flight intercept trap. E) The yellow pan trap sits below the flight intercept trap and is filled with soapy water to act simultaneously as a killing and collecting dish.

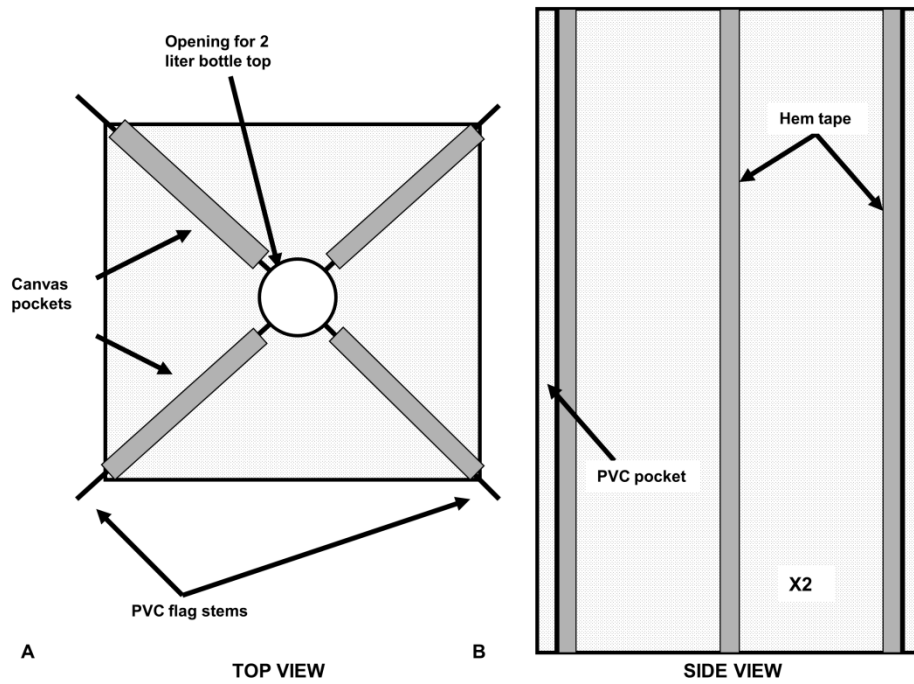


Figure 5-2 Mesh fabric (bridal tulle) is represented by the dotted areas. A) The mesh cut out for a cone trap. Four canvas pockets (solid) are sewn onto the diagonals and contain the PVC flag stems. B) The mesh cut out for a flight intercept trap. Two of these fabric sections are sewn together along the center line, which is reinforced with hem tape. The pockets for the PVC pipes are along the sides and are also reinforced with hem tape.

Chapter 6

Pollinator floral provisioning by a plant invader: quantifying beneficial effects of detrimental species

Submitted

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Abstract

Though the negative effects of invasive species are globally recognized, little is known about the potential positive impacts they can have on other species in the ecosystems they invade. However, the persistence of invaders in a wide range of ecological communities may mean that they provide resources and refuge for threatened native species, or supplement ecosystem services. With a two year field experiment, we demonstrate that an invasive thistle (*Carduus acanthoides*) is both highly visited and strongly preferred by bees relative to other flowering species. Bee abundance increased by 302% and bee species richness by 35% in habitat patches where the thistle was present. In addition, the abundance of *Bombus* species, a native group recently found to be in decline, increased by 479% when the thistle was present. Our results suggest that, despite causing significant problems, the invasion of this non-native species may also provide crucial benefits to pollinators.

Introduction

The negative impacts of non-native invasive species are well documented. Indeed, invasive species are known to have many detrimental effects on ecosystems (e.g. Elton 1958, Charles and Dukes 2007, Vilá et al. 2011), and are considered by some to be one of the major threats to biodiversity (Williamson 1998, Parker et al. 1999, Hejda et al. 2009). The costs of controlling the populations of non-native species, in addition to the costs from losses and damages, are estimated to be billions of dollars per annum (Pimentel et al. 2005). Particularly in agricultural ecosystems, management of unwanted weeds can be a costly burden, with estimates of \$8 billion US in control costs and \$25 billion US in damages and losses per year for crop and pasture weeds (Pimentel et al. 2005). Given that approximately 40% of the land surface is dedicated to agriculture (Foley et al. 2009), the management of these ecosystems may have far-reaching impacts.

Considerably less is known about the potential beneficial aspects of these invaders, in part because studies of invader impact often focus on competitive effects with native species. However, invader impacts are not always negative (Gurevitch and Padilla 2004, Rodriguez 2006, Sax and Gaines 2008, Davis et al. 2011). In some cases, invasive species can become integral parts of the ecosystem, even to the point of providing refuge for endangered native species (Zavaleta et al. 2001) or replacing lost pollination services (Junker et al. 2010). Invasive species may have facilitative effects on native species, for example, by providing limited resources or habitat (Zavaleta et al. 2001, Rodriguez 2006, Stout and Morales 2009, Gleditsch and Carlo 2010, Davis et al.

2011). The opposing negative and positive effects of invaders may lead to significant controversy (e.g. Cullen and Delfosse 1984, Davis et al. 2011, Simberloff 2011).

One important way in which invasive species may have unrecognized beneficial effects is in providing floral resources for pollinators (Rodriguez 2006, Stout and Morales 2009). Pollination services in agroecosystems are valued in the billions of dollars (e.g. Gallai et al. 2009) and to ensure sufficient pollination for crop species dependent on insect vectors, many land managers must expend resources to buy or rent hives of honeybees and colonies of other domesticated bees. At the same time, they often attempt to encourage the populations of wild bee communities by establishing patches of floral provisioning habitat (Isaacs et al. 2009). Many recent studies suggest that fragments of remnant habitat are also critical refuges for wild bee populations (Garibaldi et al. 2011). These fragments, though not always thoroughly described in the literature, are likely composed of mostly hardy weed species that can persist in the face of intense agricultural practices (D. Mortensen, *pers. comm.*). Weed diversity has been found to enhance pollinator diversity within sunflower fields (Carvalho et al 2011) and invasive weeds can be disproportionately well-visited in agricultural systems and therefore important components of the plant-pollinator community structure (Pocock et al. 2012); indeed, pollen from crop species has been found to be a relatively small component of the pollen found in honeybee hives (Pettis et al. 2013). This suggests that agricultural weeds have the potential to provide resources or habitat which support valuable crop pollinators.

We selected a noxious invader, the plumeless thistle (*Carduus acanthoides*), which is notoriously difficult to eradicate (Zhang et al. 2011), for our study. Our goal was to quantify the impacts of the thistle on the insect community, and whether such changes

might have beneficial implications for those managing agroecosystems. Specifically, the thistle has a showy floral display and can produce hundreds of large purple-red flower heads over a summer. Its attraction for pollinators is noticeable (K. Shea, L. Russo, pers. obs.) and, for this reason, we hypothesized that the largest changes would be in the community of insects that are anthophilic, especially the bees (members of the superfamily Apoidea). Using both active and passive sampling methods, we monitored the insect communities at experimental plots composed of a background community of flowering annuals with and without this thistle over two years. Our hypothesis was well supported by our results.

Methods

Study Species

The plumeless thistle (*Carduus acanthoides* L. Asteraceae), native to Eurasia, was first recorded in the United States in 1879 (Desrochers et al. 1988) and is invasive in North and South America, Australia, and New Zealand. The thistle is widely distributed in the United States, and is considered a pest in agricultural areas and roadsides (Desrochers et al. 1988, Allen and Shea 2006); in agricultural systems, it can reach extremely high densities of more than 15,000 individuals per hectare (Tipping 1992). The thistle is actively managed, but notoriously difficult to eradicate, requiring continuous reapplication of both mechanical and chemical control methods (Desrochers et al. 1988). It does not compete well in natural systems, but does persist in the face of extreme disturbances, for example, frequent mowing by farmers (Zhang et al. 2011).

C. acanthoides is a monocarpic perennial and reproduces strictly by wind-dispersed seed (Skarpaas and Shea 2007). *C. acanthoides* is self-compatible (Warwick and Thompson 1989), but depends on insect pollinators for outcrossing pollination (Yang et al. 2011). It produces hundreds of composite flower heads across its flowering season and an individual plant can have more than 200 flower heads at a given time (Yang et al. 2011); each flower head can have more than 100 florets (Giurfa and Nuñez 1993) and produces up to 80 mg of nectar in a day (Giurfa and Nuñez 1992). In addition to these nectar resources, a single flower head can produce more than 125,000 grains of pollen (Costa and Yang 2009).

Experimental Design

Eighteen paired 2 x 2 meter plots were established in March 2009 at the Russell E. Larson Agricultural Research farm, Pennsylvania USA. All were spatially separated by more than 100 meters along the edges of agricultural fields and sprayed with the herbicide glyphosate (“Round-up”™) in early April, then seeded with 3.5 g each of 10 flowering annual species (Table 6-1). The flowering species in the background community were selected based on the following criteria: a) they were annuals and would flower in the same summer in which they were planted b) they had a showy floral display attractive to pollinators c) we could acquire them from a local source d) they were not considered weedy or invasive (though some were non-native). The background species presented a variety of different floral displays in terms of color, size, and morphology (Table 6-1).

Four second-year *C. acanthoides* rosettes (greater than 10cm in diameter to ensure bolting) were transplanted into each of the four corners of one randomly selected plot

from each pair; this density falls within the naturally occurring range. After being seeded, plots were subsequently layered with mulch and watered. Maintenance of the plots included watering and weeding out any plant material that had not been planted. Eighteen new plots were established 10 meters away in 2010; they were prepared in exactly the same way except that the thistle rosettes were planted in the corners in the previous fall to allow them to overwinter in the plots and avoid transplant stress. In 2010, three additional plots were established with the same background community of annual plants and four thistles. In these additional plots, all flower heads were removed from the thistles every other day throughout the summer, while other thistle biomass was left intact. The purpose of these floral removal plots was to allow us to distinguish whether changes in the insect community might be solely attributed to the green biomass or to the flower heads of the thistle and also whether there were any competitive effects on the other plants in the community due to the presence of the thistle root and shoot biomass.

Data Collection

We completed three plant censuses each year by recording the stem number, tallest stem, and number of flowering stems of each species in each plot. We terminated the experiment in early September of each year by collecting, drying, and weighing all above ground plant biomass. Flower number of each species was counted for every sampling period. In both years, plots were sampled for insects every other week from late May through early September. At each sampling period, plots were observed for 5 minutes to record floral visitors, vacuumed for 1.5 minutes with a Bioquip insect vacuum, and passively sampled with a composite insect trap (Russo et al. 2011). Each plot was

sampled six times in each summer, for a total of 19.5 observation hours and 5.85 vacuum hours.

The observations have the advantage of recording interactions on particular plant species, but lose resolution in insect identification to groupings of solitary bee (SB), bumblebee (BB), honeybee (HB), Syrphid fly (SF), carpenter bee (CB), and Lepidoptera (Lep). In contrast, we identified all bee specimens collected in the vacuum to the species level, but do not have information about the specific plants they were visiting when they were captured.

Insect Identification

All collected insect specimens were identified to the order level. All hymenopteran specimens were identified to the family level (voucher specimens at the Pennsylvania State University). Within Hymenoptera, all members of the superfamily Apoidea were identified to the species level (voucher specimens for each species at the Pennsylvania State University) and all identifications were confirmed by Leo R. Donovall at the Pennsylvania Department of Agriculture.

Data Analysis

Differences in plant biomass, insect counts at the order, family, and species level between thistle and non-thistle plots were tested for significance using Generalized Linear Regression Models using the R package lme4 (R Development Core Team 2011). The count data were assumed to be Poisson distributed, while the individual bee genera and species were assumed to follow a zero-inflated Poisson distribution. Random effects included sampling date, plot location, and year, while the treatment was the presence of the invasive thistle. Full details on each model are included in the supporting information

(Appendix E). To compare the relative proportion of bee species and genera found only in thistle plots, only in non-thistle plots, or found in both, we used a single sample chi-square test. We also used a two-way chi-square test to compare the proportion of specimens in thistle and non-thistle plots for hymenopteran families that include natural enemies of agricultural pests. The two-way test was used in this case because we were interested in the association between many independent variables (hymenopteran families) in thistle versus non-thistle plots, while we used a single sample chi-square for the first test because we were only interested in the response of one independent variable (i.e. bee species or genera richness) in thistle versus non-thistle plots.

Results

There were no significant differences between non-thistle plant biomass or flower counts between the plots with and without thistles in either year ($P > 0.05$, Table E1 in Appendix E). The composite insect trap collected a total of 24,852 insect specimens over the two years, but did not detect significant differences in the number of insects between thistle and non-thistle plots for any taxonomic group and is therefore described further only in the supporting information (Appendix E). Rather than targeting insects specifically visiting small habitat patches, the composite traps are more likely to capture a background community of insects in the landscape (Cane et al. 2000, Russo et al. 2011); thus, it is not surprising that these traps did not detect a difference between our small plots.

Observational Data

We observed a total of 1,903 insect visitors to flowers in the plots. There was a higher average number of observed pollinator visits at the plot level in thistle plots across both years ($P < 0.01$, Fig. 6-1A). However, there was no significant impact on the visitation rates to other plants in the plots (i.e. no significant difference between the visitation to plots without thistles as compared to thistle plots, excluding visits to thistles, $P > 0.05$, Fig. 6-1A). Bumblebees, solitary bees, and lepidopterans were found in significantly higher numbers when the thistle was present ($P < 0.01$, Fig. 6-1B). Syrphid flies, honeybees, and carpenter bees did not respond significantly to the thistle, although each of these groups was slightly more abundant in thistle plots ($P > 0.05$, Fig. 6-1B). The presence of the thistle did not affect the frequency of visits by these groups to other plants in the plots (i.e. there was no difference between the number of visits received by non-thistle plants in plots without thistles and plots with thistles, $P > 0.05$, Fig. 6-1B).

Vacuum collections

We collected 3,008 insects, of which 432 were bees, with the vacuum over the two years. These collections included twenty-eight bee species (Fig. 6-2, Table E2). The vacuum collections follow a similar pattern to the observations. There were more than 4 times as many bee specimens collected in thistle plots relative to non-thistle plots over the two years (346 vs. 86), and significant increases in both bee abundance ($P < 0.01$, average number of bee specimens collected per plot-sample, Fig. 6-3A) and species richness ($P < 0.05$, average number of bee species collected per plot-sample; Fig. 6-3B). The genera *Bombus* and *Ceratina* responded especially strongly, with almost 6 and 12 times as many specimens, respectively, collected in thistle plots over the two years (110 vs. 19 and 83 vs. 7, $P < 0.01$). The genus *Lasioglossum* ($P < 0.05$, Fig. 6-2) and the

species *Augochlora pura*, *Bombus bimaculatus*, *Bombus impatiens*, *Ceratina calcarata*, and *Ceratina dupla* ($P < 0.01$, Fig. 6-2) were also more abundant in thistle plots. There were no bee species that were in significantly lower abundance in thistle plots.

Seventy percent of all bee species and 77% of all bee genera were more common in thistle plots relative to non-thistle plots (Table 6-2, $P < 0.01$). Ten bee species (including two species of *Bombus*) were only found in thistle plots, while 3 species were only found in non-thistle plots (Fig. 6-2).

Other insect groups

At the order level, we collected fewer Diptera in thistle plots, but more Hymenoptera overall ($P < 0.05$, Fig. E1). We did not detect a difference in any other insect order collected ($P > 0.05$, Fig. E1). Within the Hymenoptera, we did not detect significant differences in the abundance of any non-Apoidean family, or in the number of families collected between thistle and non-thistle plots ($P > 0.05$, Table E3). We looked specifically at 18 families of Hymenoptera that contain natural enemies of agricultural pest species, but did not detect a significant difference in their abundance in thistle plots relative to non-thistle plots ($P > 0.05$, Table E4).

Discussion

Recent research increasingly highlights the beneficial impacts that invaders can have on native species (Rodriguez 2006; Davis et al. 2011). For example, invasive species can provide resources in disturbed and managed ecosystems (Gleditsch and Carlo 2010) and habitat for threatened species (Zavaleta et al. 2001). As we demonstrate here,

an invasive species may impact the resident community as a whole, and may have significant positive effects on key native species, with important implications for the conservation of bee species in decline. Indeed, non-native species that integrate into ecological communities may become a key component of the ecosystem services those communities provide.

Our study quantifies the changes in the insect community of an agroecosystem in response to the presence of an invasive thistle, demonstrating that non-native plants can significantly increase the abundance and species richness of resident pollinator species. Our results show that this noxious invader is highly attractive to bee species relative to a selection of ten other flowering annuals. The strong response of the bee community is distinct from the rest of the insect community, which suggests that bees as a group are attracted to thistles in a way that other insects are not. This information, coupled with the fact that bees were not in higher abundance in plots where thistle flowers had been removed, suggests that bees are primarily responding to the floral resources (i.e. nectar and pollen) provided by thistles. Given the value of crop pollination services (Gallai et al. 2009), and the relationship between pollinator diversity and crop yields (Garibaldi et al. 2013), the presence of a plant attractive to pollinators may be advantageous to land managers. Thus, there is added complexity to the impact of an invasion: an undesirable species may also have desirable effects.

In otherwise identical plots, we found the abundance of bees to be four times higher in plots where the thistle (*C. acanthoides*) was present. Some bee species responded particularly strongly to the presence of the thistle. On the other hand, we also found that the presence of the thistle did not alter visitation rates of insects to other plant

species present, suggesting that the thistle has neither a “magnet species” effect (i.e. pollinator visitation rates to nearby species are enhanced by the presence of a species with large floral rewards, Molina-Montenegro et al. 2008) nor a competitive effect in terms of pollinator visitation. In the short term, the thistle is likely attracting bees from elsewhere in the landscape. An important future direction will be to assess whether the floral resources provided by the thistle will lead to an increase in the size of the populations of some bee species in the longer term,

We collected almost 6 times as many bees of the genus *Bombus* in plots where the thistle was present. Two of the bumblebee species in our study were only detected in plots where the thistle was present and the other two species were both significantly more abundant in thistle plots. Our observational data corroborate this result; we observed much higher visitation rates of *Bombus* specifically to thistles in thistle plots, without affecting their visitation to other plant species in the plots. This result may be of interest because bumblebees have recently been shown to be in decline and therefore of conservation concern (Bartomeus et al. 2013). Although the species found in our study are not among the most threatened, this larger bodied genus has been shown to be more vulnerable overall, relative to other bee species (Bartomeus et al. 2013).

The order Hymenoptera as a whole was significantly more abundant in thistle plots, though there was no significant difference in the non-apoidean families collected, suggesting that this effect was largely driven by the increased abundance of the bee species. The order Diptera (flies) was significantly less abundant in thistle plots. In contrast, we did not detect significant differences at the order level of Hemiptera (true bugs), Coleoptera (beetles), or Lepidoptera (butterflies and moths), though it is possible

that the level of taxonomic resolution in these groups was too low for us to detect significant differences. We did not detect a significant difference in any insect group by using passive trapping methods (see Appendix E), in agreement with studies that show such traps collect the wider background community of insects rather than those actively visiting flowers or foraging in small patches of habitat (Cane et al. 2000).

In contrast to the negative impacts of invasive species, which, in agricultural systems largely seem to manifest via resource competition with crop species or through reduction of available forage in pasturelands (Pimentel et al. 2005), their potential benefits may be seen in the trophic subsidy they provide to resident species (Rodriguez 2006). This trophic subsidy may result in the provisioning of floral resources for bees, as our study suggests, but it also has the potential to provide resources for natural enemies of pests (Landis et al. 2000). Although we did not detect significant differences in hymenopteran families of natural enemies between thistle and non-thistle plots, other agricultural weeds might be more attractive to them. Indeed, there is the potential for many non-native species to provide resources to insects (Carvalho et al. 2011, Pocock et al. 2012). Many other species may also provide resources for agricultural pollinators (Pettis et al. 2013), especially those with a phenology complementary to the crop species requiring pollination (Russo et al. 2013).

The invasive thistle *C. acanthoides* is already ubiquitous in agricultural regions throughout the United States (as well as other invaded countries). It is a persistent, noxious weed, yet the very fact that it is difficult or impossible to eradicate may mean that it also provides resources and habitat in regions of high intensity agriculture. We do not suggest that the thistles (or any other invader) be planted deliberately, only that, in the

calculation of the negative impacts of non-native species, the potential indirect and facilitative effects of their presence also be accounted for.

Our work shows that native pollinators, some of which are in decline, are strongly associated with invaded habitat, and that the entire pollinator community is positively impacted by invader presence. Thus, our results suggest that agricultural weeds may provide undocumented resources for a broad range of crucial crop pollinators, in turn buffering a key ecosystem service. Indeed, it is possible that some pollinators may depend on the weedy habitat fragments that pervade agroecosystems. More general insights also arise; though the potential beneficial services provided by invaders do not counter their negative impacts, non-native species often become integrated into ecological communities and may have important implications for a wide range of conservation, restoration, and land management issues. Only with clear quantification of beneficial, as well as negative, effects in a diversity of different invaded systems can the true impacts of invasive species on resident communities be assessed fairly.

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Author contributions

All the authors motivated the research, discussed the results, and wrote the manuscript. L.R. performed the analyses and wrote the initial draft of the paper, C.N. assisted with the analyses.

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Tables

Table 6-1 List of ten flowering annual plant species, as well as the invasive thistle (in bold), used as the background community in this study, sorted by family.

Species Binomial	Flower Color	Bloom Diameter (cm)†	Plant Height (cm) ††
Asteraceae			
<i>Carduus acanthoides</i>	pink/purple	2.5 - 3	89.4 ± 1.4
<i>Centaurea cyanus</i>	blue/purple	1.5 - 3	45.4 ± 1.7
<i>Coreopsis tinctoria</i> *	yellow/maroon	2.5 - 4	42.9 ± 1.4
<i>Cosmos bipinnatus</i>	pink/purple	7.5 - 8.9	107.3 ± 6.1
<i>Gaillardia pulchella</i> *	red/yellow	4 - 6	16.4 ± 3.7
<i>Helianthus annuus</i> *	yellow	7.5 - 12.7	137 ± 1.6
<i>Rudbeckia hirta</i> *	yellow	5 - 7.5	40.3 ± 1.9
Fabaceae			
<i>Chamaecrista fasciculata</i> *	yellow	1.5 - 2	52.1 ± 0.6
<i>Melilotus albus</i>	white	0.4 - 0.5	2.5 ± 1.8
<i>Melilotus officinalis</i>	yellow	0.5 - 0.7	2.5 ± 1.8
Malvaceae			
<i>Lavatera trimestris</i>	pink	6 - 7	28.5 ± 1.8

*Species native to the study area.

†Estimated diameter range of individual flower or flower heads on the plant.

††Height of tallest flowering stem averaged across each plot and year at the time of the destructive census (± standard error).

Table 6-2 Number and percent of species/genera and their distribution in thistle/non-thistle plots, with one way χ^2 comparison and two degrees of freedom.

	Percent of total	Species			Percent of Total	Genera		
		Number	χ^2	P value		Number	χ^2	P value
More in thistle plots	0.7	21	12.1	--	0.77	10	7.41	--
Fewer in thistle plots	0.17	5	2.5	--	0.15	2	1.26	--
Same in thistle/non-thistle plots	0.13	4	3.6	--	0.08	1	2.56	--
Total	--	30	18.2	< 0.01	--	13	11.23	< 0.01

Figures

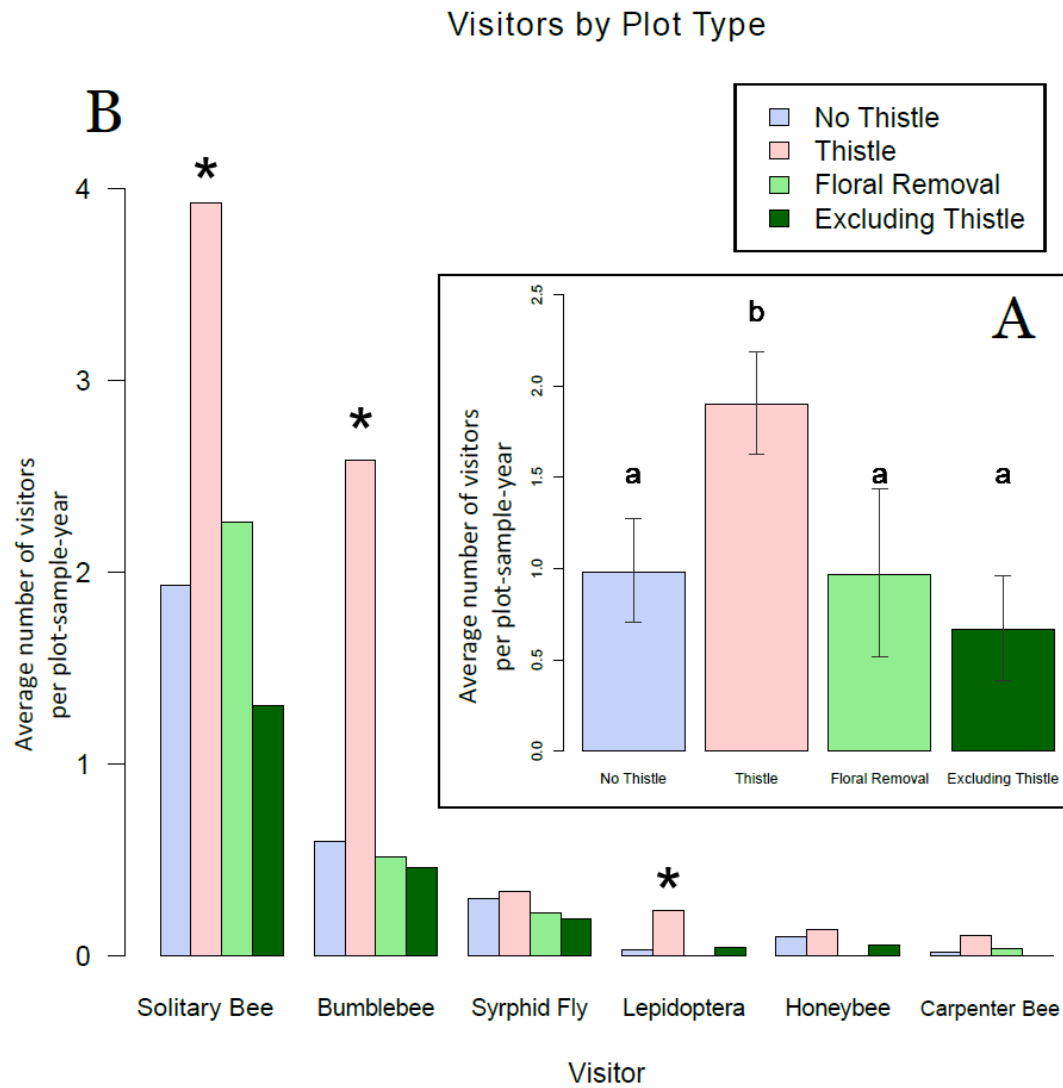


Figure 6-1 The average number of insect visitors per plot-sample day-year observed in experimental plots, organized by plot type. The average sum (A) of all visitors to plots with no thistle (blue), thistle present (red), and floral removal plots (light green), as well as the average sum of visitors in thistle plots excluding visits to the thistle (dark green), with error bars representing the standard error. The thistle plot has a significantly higher number of visitors on average, while the other plot types are not significantly different from each other. The average number of visits sorted by insect group (B) shows that there were significantly more bumblebees, solitary bees, and lepidopterans in thistle plots, while the other plot types are not significantly different from each other. The asterisk (*) represents statistical significance ($P < 0.01$).

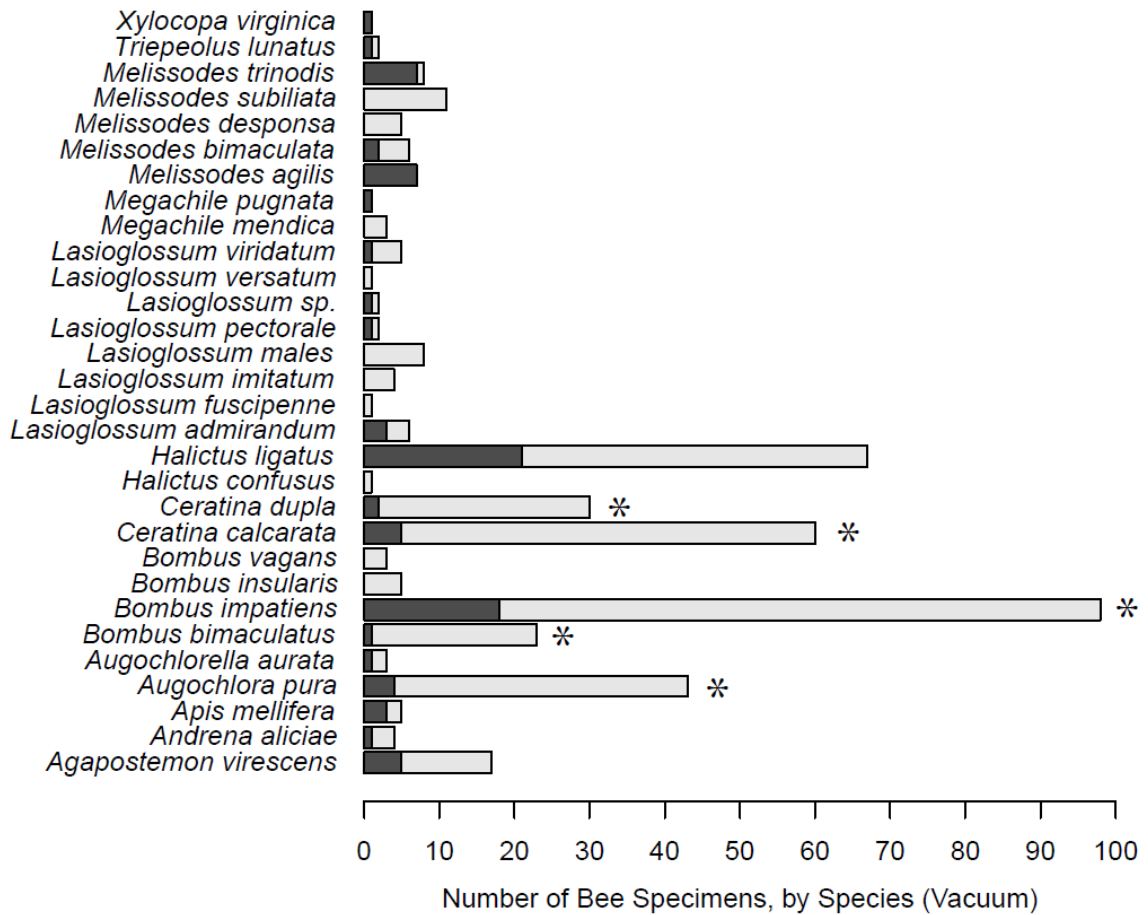


Figure 6-2 The total number of bee specimens collected in vacuum traps, arranged by bee species. The number of specimens is separated by those that were collected in non-thistle (light grey) and thistle (dark grey) plots, with significant ($P < 0.01$) differences between the treatments indicated by an asterisk (*). See Table S2 for numbers.

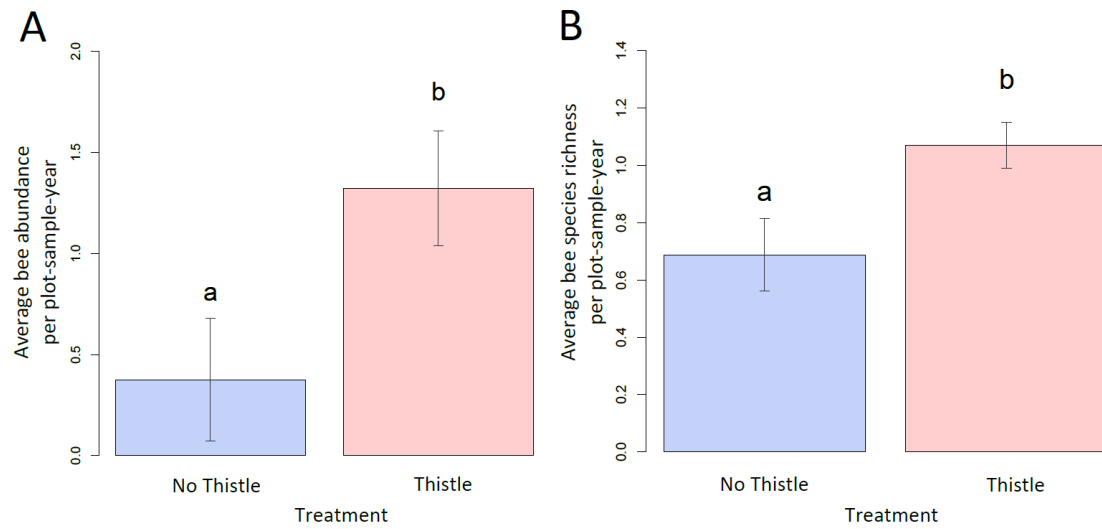


Figure 6-3 The average abundance (A) and species richness (B) of bee species collected by vacuum traps in thistle (red) and non-thistle (blue) plots. The error bars represent the standard error. The thistle plots had both significantly higher species richness ($P < 0.05$) and abundance ($P < 0.01$).

Chapter 7

Experimentally increasing the network connectance of a simple bee-plant community

In preparation

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Abstract

The structural properties of ecological networks have been theoretically tied to the stability and function of biological communities, but empirical support for this theory is based on relatively few, usually observational studies. Despite recent calls for interaction network measures to be targets of conservation and management efforts, it has yet to be determined whether they can be deliberately manipulated, and whether these manipulations will have the desired effects on community function. We designed a field experiment to increase the connectance of a mutualistic community. By manipulating the attractiveness of plant species in a simple bee-plant interaction network, we demonstrate that the network-level property of connectance can indeed be manipulated with a straightforward management action. This manipulation leads to a significant increase in both the diversity and abundance of pollinators, suggesting that there is an association between community structure and function, with positive implications for important ecosystem services.

Introduction

It has become increasingly common to represent ecological communities as complex interaction networks (Vázquez et al. 2009) and such networks are easily modified to specifically represent mutualistic interactions between species (Ings et al. 2009). There is a large body of observational work detailing the structural properties of mutualistic networks (Bascompte and Jordano 2007), and much theory addressing the potential ecological importance of the architecture of these communities (Bastolla et al. 2009, Thébault and Fontaine 2010). Properties of mutualistic network structure have been theoretically tied to stability (e.g. Campbell et al. 2012, LaBar et al. 2013), but the observational data collected in ecological communities rarely support and sometimes contradict theory (Heleno et al. 2012). In addition, the relationship between these network properties and community function remains unclear (Macfadyen et al. 2009). Though there are many studies that evaluate network structure as an emergent property of manipulated communities (e.g. Lopezaraiza-Mikel et al. 2007), there are few experiments designed to directly address the role of structural characters in the stability and dynamics of mutualistic communities. Although network-level properties have frequently been recommended as management targets (Macfadyen et al. 2009, Tylianakis et al. 2010, Heleno et al. 2012, Hagen et al. 2012) it has not been demonstrated that managers can take direct action deliberately to manipulate them (Hagen et al. 2012). Indeed, it is

difficult to conceptualize how many of these properties, which are emergent attributes of complex webs of dynamic and labile interactions, might be manipulated.

One challenge to directly manipulating network-level properties is to do so without changing the size of the network. Many network properties strongly depend on the number of species in the community (or nodes in the network) (Dormann et al. 2009) and the interaction structure of species within the community can be sensitive to the presence or absence of a single species (Brosi and Briggs 2013). Eradicating a species from a community can be challenging and potentially detrimental (Zavaleta et al. 2001, Kaiser-Bunbury et al. 2010, Brosi and Briggs 2013), while there are many risks to introducing new species (e.g. biocontrol agents (Howarth 1991, Simberloff and Stiling 1996). Connectance (also known as “density”), however, is a relatively simple network-level property and can be expressed as the number of realized links (i.e. species-species interactions) divided by the number of possible links. It summarizes the complexity of the interactions in a community and has been tied theoretically to community stability (Thébault and Fontaine 2010) and robustness to species loss (Dunne et al. 2002). As such, it has been suggested as a possible valuable management target (Tylianakis et al. 2010; Hagen et al. 2012). Furthermore, it can hypothetically be manipulated without adding or removing species from the community by changing the number of realized links.

One area where networks have the potential to provide useful recommendations for management and conservation is the mutualistic interactions between plants and their pollinators (Forup et al. 2008). This area is of particular interest in contemporary work because of the valuable service bees provide pollinating crops in agricultural systems

(Klein et al. 2007). The number of pollinator-dependent crop species has increased over time (Aizen et al. 2008a), but the domesticated crop pollinators, honey bees, on which we would traditionally rely are in decline (Pettis and Delaplane 2010). For this reason, many land managers are turning to communities of native bees to supplement or replace the honey bee as primary crop pollinators (Garibaldi et al. 2013) and strategies to ensure such communities thrive are being developed. For example, land managers might plant habitat along crop field margins to provide floral resources that the bees are lacking (Isaacs et al. 2008), especially when the crops are not in flower (Russo et al. 2013). The effectiveness of the pollination services provided by wild pollinators has been strongly tied to the species richness of the bee community (Fontaine et al. 2006, Steffan-Dewenter and Westphal 2008, Hoehn et al. 2008, Garibaldi et al. 2013). Management of pollination services, however, focuses not only the number of bee species in the community (Klein et al. 2003, Garibaldi et al. 2013, Kennedy et al. 2013), but also on increasing the stability and efficacy of the community (Kremen 2008); in essence, the objectives are to improve and manage community structure (Klein et al. 2003, Steffan-Dewenter and Westphal 2008, Hoehn et al. 2008, Kennedy et al. 2013, Garibaldi et al. 2013).

Motivated by a theoretical interest in testing whether deliberate manipulation of a network measure is logistically feasible, and if so, whether such a manipulation could be used to achieve applied goals in a real field system, we designed an experiment with the goal of increasing the connectance of a simple bee-plant network. We address two questions: (1) can connectance be experimentally increased and (2) does an increase in connectance relate to some measurable increase in the function of the community? Our experimental treatment was the application of a chemical fertilizer to increase the floral

resources provided by four plant species, a management action that does not change the number of species or individuals of plants. To the best of our knowledge, this is the first experiment to deliberately attempt to manipulate a network-level property. Our results show that we can indeed alter the complexity of mutualistic networks, and that network level properties can thus be suitable for directing management objectives in the field.

Materials and Methods

In the spring of 2012, we planted individuals of each of four native perennial plant species in ten plots established 100m apart along a transect in the Arboretum at the Pennsylvania State University, in a matrix consisting largely of grasses, but with weedy components such as thistles (*Carduus acanthoides* and *Cirsium arvense*), horsenettle (*Solanum carolinense*), teasel (*Dipsacus* spp.), knapweed (*Centaurea maculosa*), crown vetch (*Securigara varia*), bird's foot trefoil (*Lotus corniculatus*), and clovers (*Trifolium repens* and *T. pretense*). The four plant species we used in this study were *Veronicastrum virginicum* (L.) Farw. (culver's root), *Eupatorium perfoliatum* L. (common boneset), *Pycnanthemum tenuifolium* Schrad. (mountain Mint), and *Monarda fistulosa* L. (bergamot). We chose these species based on prior research addressing their potential for floral resource provisioning in agroecosystems (DeBarros 2010; Russo et al. 2013); among the 25 species evaluated, *V. virginicum*, *E. perfoliatum*, and *M. fistulosa* were the three most highly visited species, while *P. tenuifolium* was a less well-visited species and thus had the greatest potential for an increase in linkage (DeBarros 2010).

We paired the plots spatially, and one plot of each pair was randomly selected to receive a fertilization treatment consisting of 14.3 grams NPK 11-35-15 dissolved in 7.6 liters of water applied every three to four days as recommended by the manufacturer, depending on rain, while control plots received 7.6 liters of water at the same time. In all, there were five fertilized and five control plots along the transect. All plots received 7.6 liters of water every non-treatment day unless there was a substantial rainfall.

Fertilization treatment began in early May and continued through the duration of the experiment, which we terminated in the first week of August. We grew the individual experimental plants in pots, and the surrounding vegetation did not receive water or fertilizer. We counted the number of florets (*P. tenuifolium*), racemes (*V. veronicastrum*), or flower heads (*M. fistulosa* and *E. perfoliatum*) for each plant species for every sampling period (see below). For species with flower heads or racemes comprising many florets (i.e. *V. veronicastrum*, *M. fistulosa*, and *E. perfoliatum*), we selected a subset of the heads or racemes and counted the florets, obtaining an estimate of the average number of florets per head or raceme. We used this estimate of number of florets per individual over the entire summer as a proxy for total floral display.

We sampled each plot with a Bioquip Insect Vacuum (a modified hand-held vacuum) (Tuell et al. 2008) for 2 minutes 9 times throughout the summer, for a total of 3 hours of sampling time. In addition to the vacuum sampling, we also collected insects in bee bowls (Droege et al. 2010). Three bowls (one each of yellow, white, and blue) were situated adjacent to the plots during a sampling period, and filled with soapy water. We collected them after 24 hours. There were 4 bee bowl collections during the summer. The vacuum and pan sampling began after first anthesis of the plant species with the earliest

phenology (*M. fistulosa*) in mid-June and continued until the termination of the experiment in early August. We washed bee specimens (superfamily Apoidea) collected from the bee bowls and pinned and identified all bee specimens to the species level. Species level identifications were confirmed by L. R. Donovall at the Pennsylvania Department of Agriculture in Harrisburg, PA and voucher specimens are available at the Pennsylvania State University. The bee bowls collect a substantially different community of bees than active sampling of bees on flowers, and the two methods are often considered complementary to each other (Roulston et al. 2007). Here, by using both an active and a passive trap, we were able to better characterize both the species present in the wider bee community and the subset that actually visit flowers.

We used General Linearized Models (specifically in the package lme4 in R (R Development Core Team 2011)) to test for differences in the number of florets and destructive biomass of each plant species, as well as the abundance of bee specimens and bee species richness in vacuum and pan traps, with fertilizer as the fixed effect and sampling date and plot as random effects. We used a paired t-test to determine whether the proportion of realized links increased for any of the plant species individually. We also performed a two sample chi-square comparison to test the association of all bee species with fertilized versus unfertilized plots and a one way chi-square analysis to test whether individual species were more likely to be found in fertilized plots than one would expect given random (50%) chance. In the one way chi-square analysis, we used a Yates correction for 1 degree of freedom (Yates 1934).

To determine whether there was a significant change in network connectance, we generated interaction matrices for each plot, comprising the sum of all interactions

between bee species and plant species over the summer. For each interaction matrix, we calculated the number of unique interactions, the interaction frequency (abundance of a particular species on a particular plant), and connectance. Connectance is measured as the number of realized links divided by the number of possible links, which in a bipartite network such as this one, constitutes the number of possible interacting pairs of species (Jordano 1987). The number of realized links was obtained from the vacuum trap data of actual visitation by bee individuals to flower species. The number of possible links (i.e. possible interactions) was defined as the total number of bee species collected in any trap multiplied by the number of plant species in flower at any plot at any time during the summer.

Results

Fertilization resulted in a significant increase in the biomass of three of the four plant species ($p < 0.01$, Table 7-1). However, there were no differences in the floral display of any of the species in terms of number of florets produced in fertilized versus unfertilized plots ($p > 0.05$, Table 7-1). We selected *P. tenuifolium* for our study because it was previously found to be poorly visited relative to the other three plant species (Russo et al., 2013); thus, we expected it to experience the largest increase in visitation. However, we did not see a significant increase in its proportion of realized links; instead, there was a significant increase in the visitation to *V. virginicum*, the species that had the highest diversity of bee visitors in previous research ($p < 0.05$, Russo et al. 2013) as well

as the highest abundance of bee visitors in this study. The proportion of realized links was consistently higher in fertilized plots for all plant species.

A total of 24 bee species were collected on the plants using the vacuum trap, and 21 species in the bee bowls. Overall, 388 bee specimens were collected, comprising 34 species (Table 7-2). Among the bee species, there were significantly more individuals of *Augochlorella aurata*, *Halictus ligatus*, *Lasioglossum viridatum*, and *Bombus impatiens* ($p < 0.01$) as well as *Hylaeus mesillae* ($p < 0.05$) caught in fertilized plots than unfertilized plots and no species were negatively associated with the fertilized plots ($p > 0.05$, Table 7-2).

In addition to a significant increase in the average abundance of bees in both pan and vacuum traps ($p < 0.05$, Fig. 7-1 and Table 7-3), there was also a significant difference in the bee species composition associated with fertilized versus unfertilized plots (two sample chi-square 51.98, df 33, $p < 0.01$). For a given treatment (i.e. fertilized or unfertilized) there was no significant difference in the abundance of bees collected by pan versus vacuum traps ($p > 0.05$, Fig. 7-1). On the other hand, while the vacuum traps detected significantly more bee species on average in fertilized plots ($p = 0.001$, Table 7-3), the pan traps did not ($p > 0.05$, Table 7-2).

We measure connectance as the total number of realized links divided by the total number of possible links; here, the possible links are defined by the bee species collected by any method in any plot over the summer (i.e. the bees present in the community and available to visit plants). The number of possible links is therefore the same for both fertilized and unfertilized plots. In contrast, the number of realized links, defined as the documented interactions between bee visitors and plant species (i.e. vacuum collected

specimens), increased significantly with fertilization (Fig. 7-2, Table 7-3). Thus, the proportion of bee species captured visiting flowers relative to the total number of available bee species was greater in fertilized plots than in unfertilized flowers. We also found a significant relationship between the summed interaction frequency (bee abundance) and connectance ($p = 0.0005$, Fig. 7-2a) and between connectance and the plot-level species richness of pollinators ($p = 0.0121$, Fig. 7-2b). In other words, more bee species were found to visit more plant species in fertilized plots, and in greater numbers.

Discussion

While there has been much theoretical and empirical work addressing the importance of the structure of ecological networks, we are not aware of any experiments that deliberately attempt to manipulate network properties in real ecological systems. There have been many calls for empirical tests of properties of ecological networks (Carlo and Yang 2011), however, experiments involving deliberate manipulations of such structural properties are rare, primarily because they are both conceptually and logistically challenging to alter. We specifically designed our study to determine whether we could in fact intentionally manipulate a network-level property and, if so, whether it would have the desired (and hypothesized) impact on the functionality of the community. Our work suggests that network-level properties can indeed be manipulated, with repercussions for community function.

We focused on one structural property, connectance, thought to be an important indicator of the stability and robustness of ecological communities (Dunne et al. 2002, Thébault and Fontaine 2010). We were able to experimentally increase the connectance of a bee-plant community (via a greater proportion of realized interactions). The connectance of ecological networks, though sensitive to community size and ecosystem context, is believed to be relatively robust within a given ecosystem between years (Petanidou et al. 2008). Indeed, it remains unperturbed even in the face of experimental warming in food webs (Woodward et al. 2012) and species invasions in plant-pollinator interactions (Aizen et al. 2008b, Vilà et al. 2009, Kaiser-Bunbury et al. 2011). Thus, it is interesting that we were able to increase connectance experimentally by improving soil quality with a fertilization treatment. This suggests that it may also be possible to manipulate other network properties with a straightforward management action.

Not only did we increase connectance; our manipulation also led to higher species richness and abundance in the bee species collected. The preponderance of evidence suggests that diversity is important for maximizing the stability and efficacy of ecosystem services, particularly in the case of pollinator mutualisms, where it seems that pollination services provided by communities of wild bees are strongly tied to the diversity of bee species available (Klein et al. 2003, Steffan-Dewenter and Westphal 2008, Hoehn et al. 2008, Garibaldi et al. 2013). In addition, the structural properties of networks representing these interactions are thought to be critical indicators of the health and function of the community (Bascompte and Jordano 2007, Bastolla et al. 2009, Thébault and Fontaine 2010, Tylianakis et al. 2010). The relationships we show here, between

interaction frequency, bee species richness, and connectance, suggest that, at least on this small scale, connectance does relate to the ecosystem service of pollination.

Our results provide an interesting contrast to prior empirical (Jordano 1987) and theoretical (Winemiller 1989) research showing that connectance decreases with increasing species richness because the number of possible links increases more rapidly than the number of realized links. When comparing two communities of different sizes and a similar probability of interaction, the connectance will perforce be lower in a community with more species. In our experiment, we carefully controlled for community size (i.e. the number of possible links) by constraining the number of plant species and measuring the background community of possible bee species. Given the controlled community size, higher species richness was positively correlated with connectance; in other words, plots that had a higher diversity of bee visitors also had a higher connectance (Fig. 7-3b).

An alternative explanation for this contrast is that the change in species richness, though statistically significant, was very small relative to the differences recorded in observational reviews and meta-analyses. At this much smaller scale, the increase in species richness may relate directly to the increased attractiveness of the fertilized plants, even though they did not have larger floral displays. Plants fertilized with vermicompost have been shown to have a higher quality nectar, which is attractive to bumblebees in greenhouse experiments (Cardoza et al. 2012); a similar change in nectar quality may be responsible for the increased attractiveness of our experimental plants. It is also possible that the fertilization resulted in a higher pollen quality. The significant increase in

biomass may also be related to the increased soil quality, and may indicate that the plants were healthier in general.

In addition to species richness, the frequency of interactions (or abundance of bee individuals collected) was also significantly related to connectance. The association between frequency and connectance has been suggested by prior research, which showed a predictive relationship between the relative abundance of plants and insect visitors and the connectance of the community (Vázquez et al. 2009). On the other hand, it may also be related to sampling error. Interactions that occur with higher frequency are more likely to be observed (Blüthgen 2010), thus, increasing the frequency of rare interactions would make them more observable. Regardless, the increased attractiveness and potentially higher nectar quality of fertilized plants may have implications for the stability of bee populations. For example, bumblebees visiting plants with a higher nectar quality have been shown to have increased fitness, suggesting possible population-level effects (Cardoza et al. 2012).

One of the fundamental problems hampering conservation of ecological systems arises from a poor understanding of how to manage the complex interactions that lead to emergent ecosystem services. Here, we demonstrate the effects of an experimental manipulation designed to change a community-level property and assert that similar manipulations will be essential to future studies applying network theory to ecological communities. Not only were we able to successfully increase the proportion of realized links in this simple bee-plant community with a relatively modest perturbation, we also demonstrate that this increased connectance was significantly associated with higher bee species richness and abundance. Unfortunately, this suggests that other perturbations,

such as the elimination of plants on field margins (Egan and Mortensen 2012) or application of herbicides (Mortensen et al. 2012) could potentially have dramatic impacts on community function, even without reducing the species richness of the plants, by changing the quality of the floral resources available to pollinators. Managing communities of interacting species remains a significant challenge, but our results suggest that network theoretical predictions can be experimentally tested in the field, and that relatively subtle manipulations could have significant conservation benefits. Such experiments have the potential to provide insight into the complexity of ecological systems, as well as the fundamental dynamics of species interaction networks.

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Author contributions

All the authors motivated the research, discussed the results, and wrote the manuscript. L.R. performed the analyses, and wrote the initial draft of the paper.

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Tables

Table 7-1 The number of florets averaged across the season (rounded to the nearest integer) and the final biomass (in grams) for each of the four plant species in the study, with standard errors. There was no significant effect on the number of florets for any species ($p > 0.05$), but there was a significant increase in the biomass of *E. perfoliatum*, *P. tenuifolium*, and *V. virginicum* ($p < 0.01$).

Species	Florets		Biomass (g)	
	Fertilized	Unfertilized	Fertilized	Unfertilized
<i>Eupatorium perfoliatum</i>	52 ± 36	40 ± 13	193.6 ± 29.4	83.4 ± 20.1
<i>Monarda fistulosa</i>	960 ± 230	662 ± 194	98.5 ± 3.5	90 ± 14.6
<i>Pycnanthemum tenuifolium</i>	253 ± 99	269 ± 65	133.2 ± 10.9	48.3 ± 15.2
<i>Veronicastrum virginicum</i>	712 ± 253	418 ± 197	174.8 ± 26.3	102.8 ± 7.1

Table 7-2 Bee species collected and their abundance in fertilized and unfertilized plots, combining both vacuum and pan traps across the season. The one-way Chi square values test whether each species associates with a fertilized or unfertilized plot more than would be expected, given a random (i.e. 50%) chance; there is a Yates correction for 1 degree of freedom. Significance of $p < 0.05$ is indicated by one asterisk (*), while significance of $p < 0.01$ is indicated by two asterisks (**).

Species	Fertilized	Unfertilized	Sum	X2	Significance
<i>Agapostemon virescens</i>	2	1	3	0.00	
<i>Anthidellum notatum</i>	3	2	5	0.00	
<i>Apis mellifera</i>	27	33	60	0.42	
<i>Augochlora pura</i>	11	6	17	0.94	
<i>Augochlorella aurata</i>	15	3	18	6.72	**
<i>Bombus bimaculatus</i>	8	9	17	0.00	
<i>Bombus impatiens</i>	48	24	72	7.35	**
<i>Bombus ternarius</i>	0	3	3	1.33	
<i>Calligesis andreniformis</i>	7	2	9	1.78	
<i>Ceratina calcarata</i>	1	0	1	0.00	
<i>Ceratina dupla</i>	1	1	2	0.50	
<i>Halictus confusus</i>	1	1	2	0.50	
<i>Halictus ligatus</i>	38	13	51	11.29	**
<i>Halictus rubicundus</i>	7	4	11	0.36	
<i>Holcopasites calliopsidis</i>	2	0	2	0.50	
<i>Hoplitis pilosifrons</i>	1	0	1	0.00	
<i>Hylaeus affinis</i>	22	20	42	0.02	
<i>Hylaeus mesillae</i>	6	0	6	4.17	*
<i>Hylaeus modestus</i>	3	1	4	0.25	
<i>Lasioglossum admirandum</i>	10	3	13	2.77	
<i>Lasioglossum coriaceum</i>	0	1	1	0.00	
<i>Lasioglossum imitatum</i>	7	5	12	0.08	
<i>Lasioglossum males</i>	1	2	3	0.00	
<i>Lasioglossum pectorale</i>	1	0	1	0.00	
<i>Lasioglossum pilosum</i>	1	0	1	0.00	
<i>Lasioglossum tegulare</i>	4	0	4	2.25	
<i>Lasioglossum versatum</i>	1	0	1	0.00	
<i>Lasioglossum viridatum</i>	12	1	13	7.69	**
<i>Lasioglossum zonulum</i>	1	0	1	0.00	
<i>Megachile pugnata</i>	1	0	1	0.00	
<i>Megachile rotundata</i>	0	1	1	0.00	
<i>Melissodes bimaculata</i>	3	0	3	1.33	
<i>Sphecodes illinoensis</i>	1	0	1	0.00	
<i>Xylocopa virginica</i>	2	4	6	0.17	

Table 7-3 The average pollinator species richness, abundance, and connectance in fertilized and unfertilized plots, separated by trap type. Because species-species interactions were not recorded in pan collections, there is no measure of connectance for the pan trap. Standard errors are included for each measure.

		Fertilized	Unfertilized
Pan	<i>Pollinator Species Richness</i>	6.2 ± 1.59	3.4 ± 1.29
	<i>Abundance</i>	9.8 ± 2.87	4.4 ± 1.75
Vacuum	<i>Pollinator Species Richness</i>	11.6 ± 0.81	8.4 ± 0.93
	<i>Abundance</i>	39.8 ± 5.31	23.6 ± 4.88
	<i>Connectance</i>	0.127 ± 0.011	0.089 ± 0.007

Figures

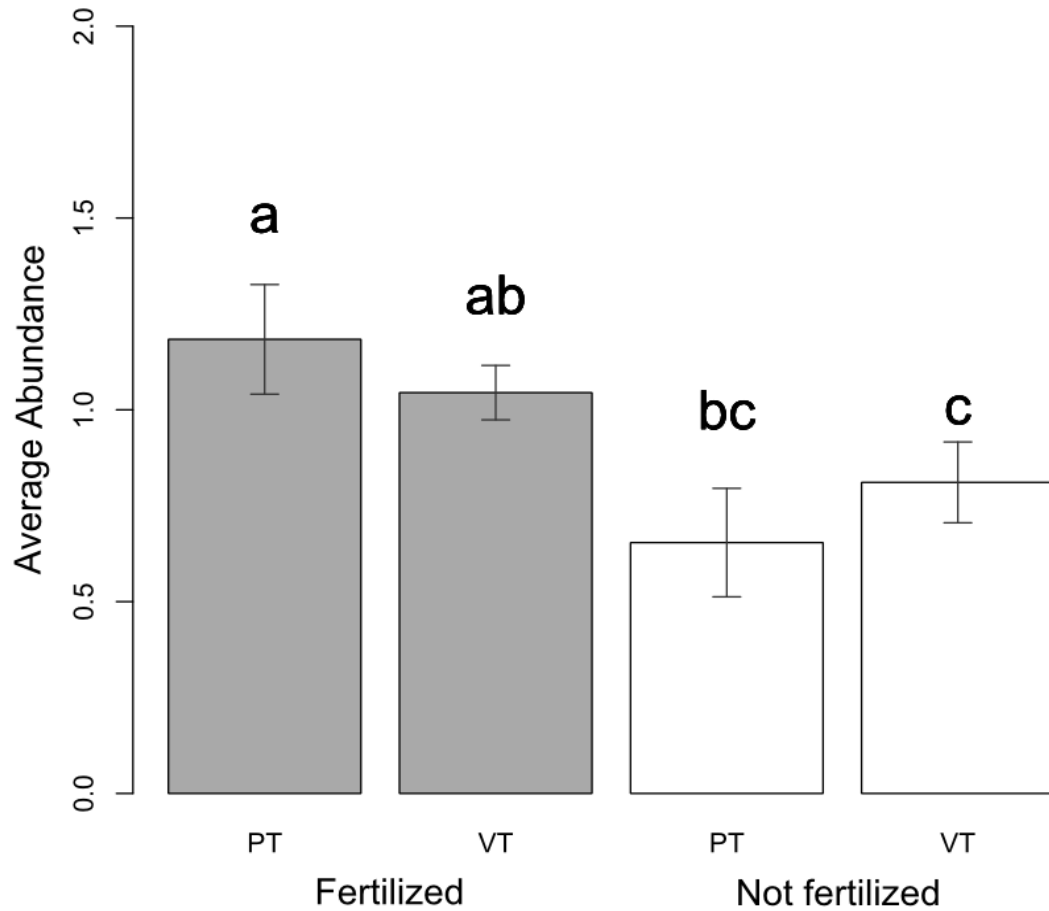


Figure 7-1 The relationship between the abundance of all bees collected in plots (averaged over sampling period and plot) for pan (PT) and vacuum (VT) traps. There was no significant difference between vacuum and pan traps within a given treatment type ($p > 0.05$). Fertilized plots (dark grey) had a significantly higher average abundance of bees than unfertilized (white) within a trap type ($p < 0.05$).

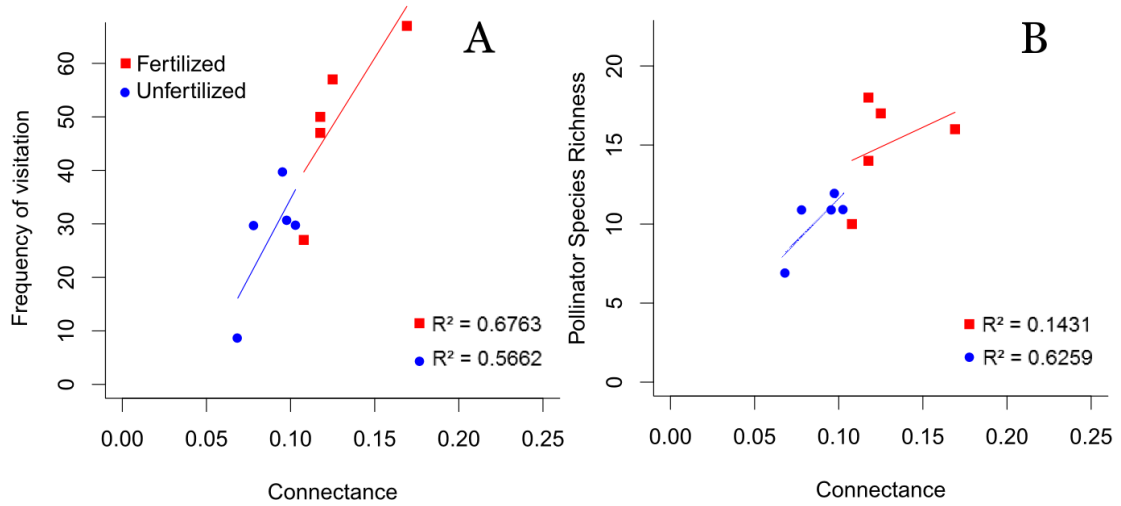


Figure 7-2 The relationship between connectance and the frequency of visitation (A) and species richness of pollinators (B) in fertilized (red square) and unfertilized (blue circle) plots. This relationship is significant ($p = 0.0005$, $R^2 = 0.799$; and $p = 0.012$, $R^2 = 0.566$ respectively). R^2 values for the fertilized and unfertilized treatments separately are provided on the graphs.

Chapter 8

Conclusion

Ecological communities can be defined by the interactions between member species. This interaction structure provides useful insights into emergent community functions and ecosystem services. In addition, analyzing the structure of extant interactions can provide information about the past drivers of the formation of communities and interactions, and insight into how the community will respond to species introductions and other perturbations in the future. As I have demonstrated, studying multispecies interaction structure can be a useful way of coping with the inherent complexity of ecological communities, by exposing synergy at the community level while preserving the identity of individual species. In this dissertation, I have focused on mutualisms, specifically on the interactions between plants and their insect visitors. This focus was driven both by the relative lack of knowledge about the role of mutualisms in structuring ecological communities, but also by several pressing contemporary ecological challenges. As a result, my findings are not only significant for the study of ecological communities in general, but also provide recommendations that can be applied in a management context.

On a broad ecological scale, I have shown that plant-pollinator mutualisms may be important for the assemblage, diversity, and functioning of ecosystems as a whole. Studies of these mobile mutualisms have, in the past, often been limited by the level of complexity in large groups of species interacting in a diffuse and generalized fashion

(Janzen 1980, Waser et al. 1996, Johnson and Steiner 2000, Inouye and Stinchcombe 2001, Strauss and Irwin 2004). However, by utilizing an exceptionally large legacy dataset (Robertson 1929, Marlin and LaBerge 2001), I was able to demonstrate that the interactions between plants and pollinators are taxonomically conserved and lineage specific on an extremely large scale, suggesting that they are important to the fitness of one or both interacting partners (Chapter 2). In addition, I found that many groups of insects that are not typically considered pollinators have highly conserved interactions with plants, suggesting that they may have a greater dependence on the resources provided by flowers than previously suspected (Faegri & Van der Pijl 1979, Jervis et al. 1993). Indeed, though much of this dissertation focuses specifically on bee communities, I also demonstrated that other taxa may contain important floral visitors; their relatively unstudied association with plants may be a promising avenue for future research. This highlights the importance of sampling beyond the groups already suspected to be important (Chapter 5).

Much remains to be learned about the drivers of community structure. One potential avenue for future exploration is in the application of the methods I developed in Chapter 2 to groups of interacting species where molecular phylogenies have been resolved. This would allow one to determine whether groups of plants and insect visitors that have correlated taxonomic hierarchies also have complementary speciation times (Klassen 1992). Similarly, morphological data (e.g. proboscis lengths and corolla depths) would be suggestive of trait matching in these interacting partners, and phenological information would elucidate forbidden links between species. The interplay between all four factors (taxonomy, interaction structure, morphology, and phenology) may

illuminate the relative importance of these mutualistic interactions for different groups of insects and plants (Quek et al. 2004). Such work could ultimately lead to evidence for or against diffuse and/or pairwise coevolution in plant-pollinator mutualisms on a broad scale (Iwao and Rausher 1997).

I have also shown that relatively modest changes to the number, identity, and quality of plant species in these communities can have great implications for the responses of their mobile partners, with potential impacts on population dynamics, leading to long-term and indirect effects (Chapters 3, 4, 6, and 7). On a fundamental level, the identity of individual species determines their role in the community; for example, if their interactions are constrained by their phenology (Chapter 3). From the perspective of the pollinators, plants vary in attractiveness and quality, while from the perspective of the plants, the pollinators vary in their fidelity and efficacy (Chapter 3). Changing the composition of the community therefore constitutes a perturbation, one that is particularly ubiquitous (Ellis et al. 2010). For example, the introduction and subsequent integration of non-native species into mutualistic interactions can directly alter the structure of the plant-pollinator community, leading to novel ecosystems (Hobbs et al. 2006, Aizen et al. 2008b, Chapter 4). However, this change is not necessarily negative; I show that the experimental addition of a plant invader has strong positive effects on the species richness and abundance of threatened pollinators (Chapter 6). The impact of the removal of single species has also been examined, with apparently dramatic effects on the structure of mutualistic communities (Brosi and Briggs 2013).

The experimental addition or removal of species from ecological communities is logistically challenging and, at times, ethically dubious. However, while carefully

preventing its establishment, I experimentally added an invasive species to a plant community, resulting in subsequent responses in the insect community (Chapter 6). Experimental manipulations such as the one presented here have the potential to tell us much about the dynamics of ecological communities, their resilience to change, and the importance of species identity in interaction structure. The experiment I describe in Chapter 6 is likely the first of its kind and more experiments of a similar nature may broaden our understanding of the cascading implications of species invasions. Similarly, the theoretical models I present in Chapter 4 to investigate the introduction of a mutualist are a starting point for empirical tests of species additions as well as more complex theoretical models that incorporate population dynamics and labile interactions. It may also be necessary to conduct further investigations into the experimental manipulation of network-level properties. Though our experiment was successful in increasing the connectance of the community, I did not discover the mechanism driving the change (Chapter 7). The plants in my study were more attractive to pollinators when fertilized, but my measure of floral display did not capture this increased attractiveness. An experiment that quantifies the sugar concentration or volume of nectar might demonstrate there is an increase in the quality of the floral resources provided by fertilized plants (Cardoza et al. 2012). It is also possible that the fertilization had an effect on the quality of the pollen produced by the experimental plants; further studies could demonstrate whether this contributed to changes in bee visitation.

This work also addresses more specific applied challenges. For example, it may be particularly useful in the agricultural setting, where the potential undocumented ecosystem services of communities of wild bees may become essential (or at least cost-

effective relative to renting honeybee hives) in the near future (Klein et al. 2003, Losey and Vaughan 2006, Greenleaf and Kremen 2006, Chapter 3). In fact, this is already occurring. In Adams County, Pennsylvania's largest apple producing county, some 30% of the orchards are no longer supplemented with commercial hives; rather, many orchard managers are relying on wild bees and spillover of commercial bees and feral *A. mellifera* to pollinate their crops (D. Biddinger, *pers. comm.*). Viewing these services from a community perspective, rather than focusing on one species of domesticated pollinator, may become more important if the honeybee continues to decline (Aizen et al. 2008a). Indeed, the understanding of interactions between many species of plants and insect visitors may be key to future conservation efforts, as entire communities of pollinators have been shown to be in decline (Bartomeus et al. 2013). Results and recommendations from the work presented in Chapter 3 are already the focus of experimental tests in apple orchards. Given that agricultural systems now constitute approximately 40% of the land surface (Foley et al. 2005), focusing on maintaining the underlying mechanisms driving these systems is critical for their long-term sustainability. For example, the indirect impacts of pesticide usage on bees could be very important (Pettis et al. 2013).

Much of my work has been directed toward the floral resources available to bees in agroecosystems, including those that are already provided by agricultural weeds (Chapter 6). There is evidence to suggest that weedy field edges in agroecosystems are very important to bees; for example, pollen from crop species often represents only a small proportion of the total pollen collected by honeybees (Pettis et al. 2013). Given the strong attractiveness of weeds like the thistle (Chapter 6), herbicide spraying (either direct or drifting from crops) could have a very negative effect on both domesticated and

wild bee populations, if only via the removal of resources. Moreover, insecticide contamination of pollen via drift could also be extremely damaging (Pettis et al. 2013).

The development of holistic management strategies for supporting communities of wild bees in agroecosystems is already well underway (Kennedy et al. 2013). The work presented here hopefully contributes to this effort, and provides several new avenues for investigation; for example, using phenology and interaction structure to match floral resource provisioning habitat with particular crops (Chapter 3). This idea could be investigated more thoroughly by identifying the species that are effective crop pollinators, and subsequently targeting floral provisioning habitat specifically for their needs when crops are not in flower. For conservation purposes, the ideal floral provisioning habitat would have a suite of species that provide resources throughout the active period of the pollinator species.

In many ways, this work supports and builds on previous research, both on mutualistic communities in general and on plant-pollinator networks specifically. Though much observational and theoretical work has been done on the importance of network-level properties in plant-pollinator communities (Bascompte et al. 2006, Bastolla et al. 2009, Thébault and Fontaine 2010, Campbell et al. 2012, LaBar et al. 2013), here I attempt to rigorously test these hypotheses in the field. For example, the work here shows that network level measures of a community can be used for predicting the impacts of perturbations (Chapter 4), but also that it is possible to manipulate these measures in a meaningful way (Chapter 7), and that they can be used to provide useful management recommendations (Chapter 3). These three chapters have the potential to greatly increase

the applicability of network theory to real and pressing ecological problems (Hagen et al. 2012).

However, there is still much to explore before network level properties are identified as management objectives in real systems. The application of network theory to ecological systems does have some limitations. Most network level properties are sensitive to the size of the community, as well as to sampling effort and the taxonomic resolution of the interacting species. In addition, as they are currently applied, networks are static representations of a dynamic system, where both the species available to interact and the identity of interactions are constantly changing. As such, networks represent an incomplete subset of all possible interactions. The fact that direct interactions between nodes of a certain class (i.e. plant nodes or insect nodes) are forbidden also means that we do not directly account for competition, predation, and facilitation between species. Recent work suggests that such interactions may in fact be extremely important to the structure of plant-pollinator communities (Brosi and Briggs 2013). Networks also remain time-intensive in terms of both sampling and analysis and it has yet to be demonstrated that the properties of these communities on the whole are more useful than other community level measures, such as species richness and evenness, that do not require detailed species-species interactions (Blüthgen 2010). Thus, they must still be validated in the field and with comparative modeling efforts.

Another potential limitation of my research is that I constructed networks within this thesis with a plant-biased perspective; the insects were observed or sampled as they visited plant species. It is much more difficult to construct networks from the perspective of the mobile partner. Thus, the behavior and dynamics of pollinators and other floral

visitors outside of their interactions with plants are poorly understood. However, work is being done to address this bias toward the sessile partner by incorporating pollen carried by floral visitors into interaction networks (Ackerman and Roubik 2012). This thesis is also restricted in its focus on the, admittedly critically important, ecosystem service of pollination. Other ecosystem services and community functions could also be addressed with the methods applied here. For example, integrated pest management in agroecosystems could benefit from a community level perspective that addresses the floral resources utilized by other beneficial insects (e.g. predators and parasites of pest species). Moreover, integrating the evaluation of multiple ecosystem services at one time is an important new area of research (Nelson et al. 2009).

The findings included in this dissertation are strongly scale-dependent. For example, we can contrast the results of Chapters 2, 4, and 6: while Chapter 2 seems to suggest that the interactions between insects and host plants are strongly influenced by a shared evolutionary history, Chapters 4 and 6 demonstrate that species can rapidly and opportunistically take advantage of novel resources. However, these studies are dramatically different in terms of their spatial and temporal scale. The legacy dataset used in Chapter 2 captures interactions over a very long time span (i.e. 33 years) and across a very large spatial scale (i.e. 225,000 hectares). In contrast, the interactions in the other chapters of this thesis represent experiments occurring over one (Chapters 4 and 7) or two (Chapters 3 and 6) summers and within a relatively small spatial area. Short term responses are likely drowned out in the legacy dataset, but dominate the other studies; the long-term scale addressed by the Robertson dataset elucidates only patterns that are sustained (e.g. phylogenetic patterns of interaction), while the short-term observational

work demonstrates behavioral responses to experimental stimuli. Within these chapters, I do not address the intermediate scale, which might inform more about population dynamics. For example, Chapter 6 shows that there is no effect on pollinator visitation to nearby plant species in the presence of a highly attractive invasive thistle. However, at the short time scale that the sampling occurred, an increase in visitation locally likely means that floral visitors are being drawn from elsewhere in the landscape. Thus, to truly have positive implications for other plant species in the long-term, the resources provided by the thistle would have to lead to higher fitness or an increase in the population size of the pollinators.

This thesis combines both theoretical and experimental methods to address the importance of community structure in plant-pollinator interactions. These two approaches are complementary to each other; for example, Chapter 4 applied theoretical models to extend the conclusions drawn in Chapter 6, while Chapter 7 was an empirical test of some of the hypotheses put forward in Chapter 3. In other words, the experiment often motivates future theoretical models, and the theoretical models provide testable hypotheses that are the foundation of the experiments. The two approaches are stronger in combination than alone.

The interactions between plants and pollinators are taxonomically structured and conserved within lineages on broad ecological scales, but pollinators are also opportunistic. Because of this, novel species can become integrated into mutualistic communities, resulting in changes in community structure and composition. As properties of the interaction structure can be indicators of the function of the community, it is possible to manipulate them while nevertheless preserving information about the

behavior of individual species. The information stored in networks of species interactions can thus be used to direct conservation and management objectives.

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Appendix A

Chapter 2 Appendix: Taxonomic structuring in mutualisms

```
#####MANTEL TESTS FOR CORRELATION BETWEEN TAXONOMIC DISTANCE AND INTERACTION
STRUCTURE
```

```
require(prabclus)
require(ape)
require(vegan)
require(ade4)
```

```
#TAXONOMIC DISTANCE MATRIX
```

```
#If two species belong to the same GENUS, their distance is 0
#If they belong to the same FAMILY but are from different GENERA, their distance is 1
#If they belong to the same ORDER but are from different FAMILIES, their distance is 2
#And so on...
```

```
#####
```

```
int=read.table("interactionmatrix.txt",header=T) #binary interaction matrix for plants (or insects)
mat=as.matrix(int) #coerce to matrix
jac=jaccard(mat) #use jaccard algorithm to make distance matrix
```

```
taxonomies <-read.table("taxonomies.txt",header=TRUE) #taxonomic hierarchy of plants (or insects)
attach(taxonomies) #a three column dataframe of the family, subfamily, and genus each species belongs to
dO<-weight.taxo(Order)
dF <- weight.taxo(Family)
dG <- weight.taxo(Genus)
dS <- weight.taxo(Species)
Dist <- dO+ dF + dG + dS
taxo <- (max(Dist)-Dist) #taxo is the matrix of taxonomic distances
detach(taxonomies)
```

```
mantel(taxo,jac) #the mantel test prints a correlation statistic and significance
#default is 999 permutations
```

```
#This is a one way test, so it can be done to compare the relationship between
#the interaction structure of the insects and their taxonomies, or the plants and
#their taxonomies.
```

```
#####FOURTH CORNER ALGORITHM FOR CORRELATIONS BETWEEN TAXONOMIES OF INTERACTING
#####PARTNERS
```

```
tabL<-read.table('interactionmatrix.txt',header=T)
tabR<-read.table('orddist.txt',header=T) #matrix of insect species in order
tabQ<-read.table('orddist_plants.txt',header=T) #matrix of plant species in order
```

```
ordfourth=fourthcorner(tabR,tabL,tabQ,nrepet=999)
```

```
save(ordfourth,file="fourth_ord.RData")
```

```
sumfour=summary(ordfourth)

write(sumfour,file="sumfour_ord.txt")

tabL<-read.table('interactionmatrix.txt',header=T)
tabR<-read.table('famdist.txt',header=T)      #matrix of insect species in families
tabQ<-read.table('famdist_plants.txt',header=T) #matrix of plant species in families

famfourth=fourthcorner(tabR,tabL,tabQ,nrepet=999)

save(famfourth,file="fourth_fam.RData")

sumfour=summary(famfourth)

write(sumfour,file="sumfour_fam.txt")
```

Appendix B

Chapter 3 Appendix: Supporting native pollinators

Table B1. A matrix of correlation coefficients between different node level measures for the plants. Significant ($p \ll 0.01$ except floral area and node duration, for which $p \sim 0.02$) correlations are highlighted in grey. (Correlations are symmetric.)

	Betweenness Centrality	Closeness Centrality	Unweighted Degree	Weighted Degree	Node Duration	Floral Area
Closeness Centrality	0.72					
Unweighted Degree	0.82	0.67				
Weighted Degree	0.57	0.41	0.58			
Node Duration	0.38	0.25	-0.01	0.02		
Floral Area	0.35	0.15	0.10	0.46	0.37	
Functional Complementarity	0.63	0.54	0.68	0.74	-0.06	0.25

Table B2. A matrix of correlation coefficients between different node level measures for the insects. Significant ($p \ll 0.01$) correlations are highlighted in grey. (Correlations are symmetric.)

	Betweenness Centrality	Closeness Centrality	Unweighted Degree	Weighted Degree
Closeness Centrality	0.94			
Unweighted Degree	0.97	0.95		
Weighted Degree	0.67	0.64	0.72	
Node Duration	0.91	0.92	0.95	0.68

Appendix C

Chapter 4 Appendix: Novel mutualistic species interactions

Other models of novel species interactions

In addition to the models presented in the manuscript, we also investigated five additional ways in which the novel species could form links in the community. In the same fashion as the manuscript, the novel species is modeled as an added node whose interactions are randomized in each simulation. The additional models are as follows:

Preferential

The preferential model is similar to the simple additive model presented in the manuscript. The introduced species enters the community and forges new mutualistic links without altering existing links. However, in this case it forms links preferentially with more generalist species of pollinator (i.e. those that have a high degree). In practice, this model differs little from the additive model, and was thus excluded from the main results.

Static Pollinator Matrix

This model used the same pollinator matrix as the manuscripts “randomized pollinator matrix” model, but the interactions within that matrix are not randomized for each simulation. In other words, the interactions of the new pollinator species remain static.

Novel Species Only Pollinator Matrix

In this case, multiple pollinators are added, but they can interact only with the novel species.

Plants and Pollinators Added

In this model, we add both plant and pollinator species so that the species richness in the invader-removed community equals that of the invaded community. However, one site did not have higher plant species richness in the invaded site, and the number of replicates was lower from this model.

Randomized Control Matrix

We also randomized the interactions within the control (invaded) communities, and measured the network properties after randomization. We performed 1,000 simulations of the randomization and then averaged the properties across all simulations. We were interested to see whether the identity of the links mattered for these network level measures, and whether the link identity had the same importance for different measures. The randomized control matrix also exactly recaptured the average degree and connectance (similar to the novel species substitution model), although it was the worst performing model for the other properties (Table C2).

Table C1. Sizes of the real communities in control and treatment (*I. glandulifera* flowers removed) sites (Lopezaraiza-Mikel et al. 2007).

Site	Treatment	Plant Species	Insect Species	Total
AC	Control	8	49	57
AC	Treatment	6	31	37
CD	Control	15	72	87
CD	Treatment	5	43	48
RA	Control	11	75	86
RA	Treatment	12	43	55
SA	Control	6	48	54
SA	Treatment	3	16	19

Table C2. Values for all model outputs for network properties at the precise number of links that the actual invader had in the real system (21.75). The real data is highlighted in dark grey; the models from the main manuscript are highlighted in light grey.

Model	Average Degree	Connectance	NODF	Modularity	Number of Compartments	Median Compartment Occupancy
Preferential Plants and Pollinators Added	1.54	0.30	15.37	0.11	5.25	2.75
Novel Species Only Pollinator Matrix	1.66	0.21	22.8	0.03	6.26	2.24
Randomized Control Matrix	0.94	0.16	10.6	0.27	22.9	1.01
Static Pollinator Matrix	1.26	0.16	6.2	0.33	19.97	1.23
Invaded	1.51	0.25	15.89	0.17	7.2	2.72
Invader Removed	1.26	0.16	11.9	0.39	8	7.63
Additive	1.01	0.23	6.93	0.34	5.75	3.63
Competitive	1.54	0.3	14.8	0.12	5.39	3.20
Supergeneralist Static Pollinator Matrix	0.98	0.18	6.33	0.37	6.45	3.01
Randomized Pollinator Matrix	1.67	0.32	14.38	0.10	4.77	5.69
	1.51	0.25	15.89	0.17	7.2	2.72
	1.48	0.24	10.01	0.18	7.2	9.57

Table C3. Absolute difference values for the model outputs for network properties at the precise number of links that the actual invader had in the real system (21.75). Lowest values are bolded (where the randomized control matrix is the lowest, the best alternative model is also bolded).

Model	Average Degree	Connectance	NODF	Modularity	Number of Compartments	Median Compartment Occupancy
Plants and Pollinators Added	0.28	0.14	3.47	0.28	2.75	4.88
Preferential Randomized Pollinator Matrix	0.40	0.05	10.90	0.36	1.74	5.38
Novel Species Only Pollinator Matrix	0.22	0.08	1.89	0.21	0.80	1.95
Randomized Control Matrix	0.32	0.00	1.30	0.13	14.90	6.62
	0.00	0.00	5.70	0.06	11.97	6.39

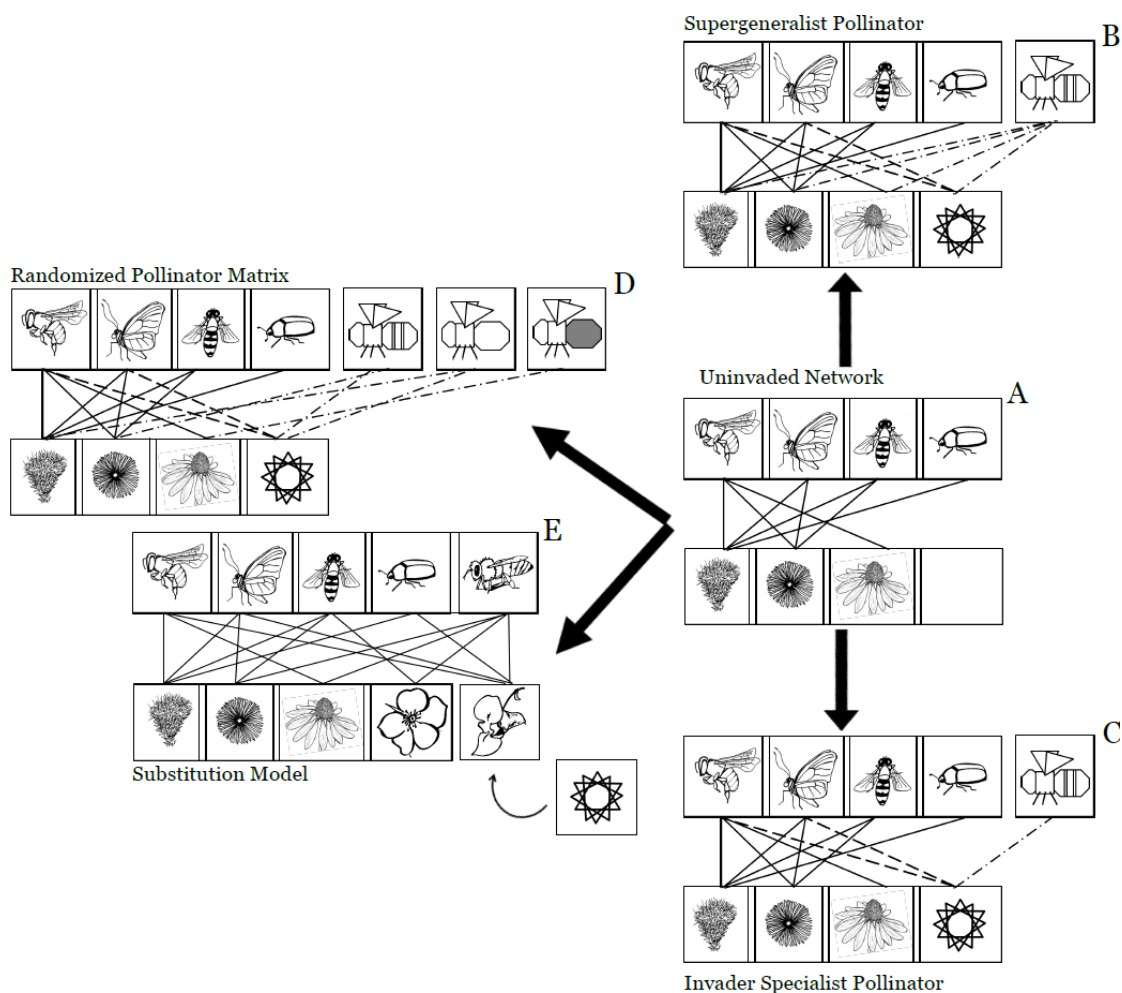
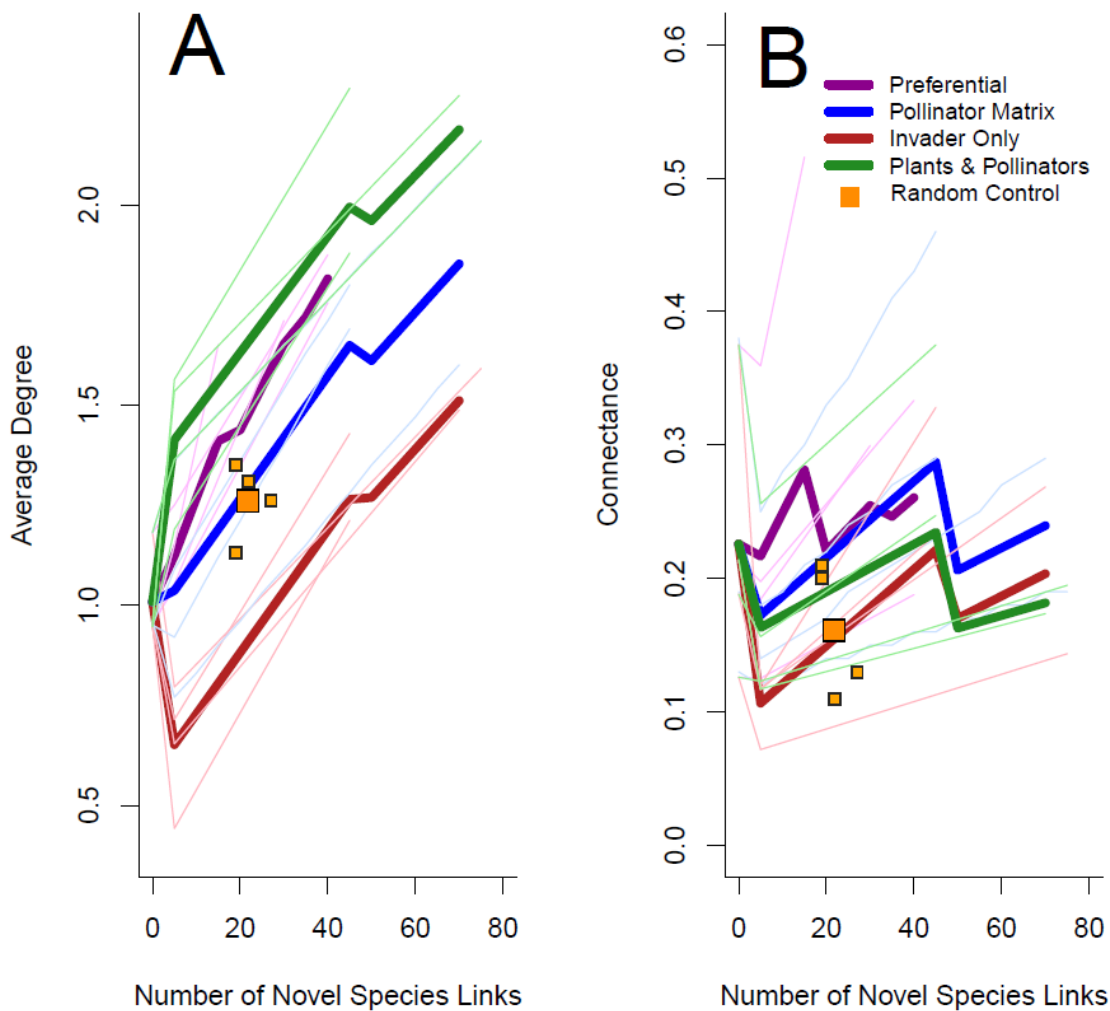


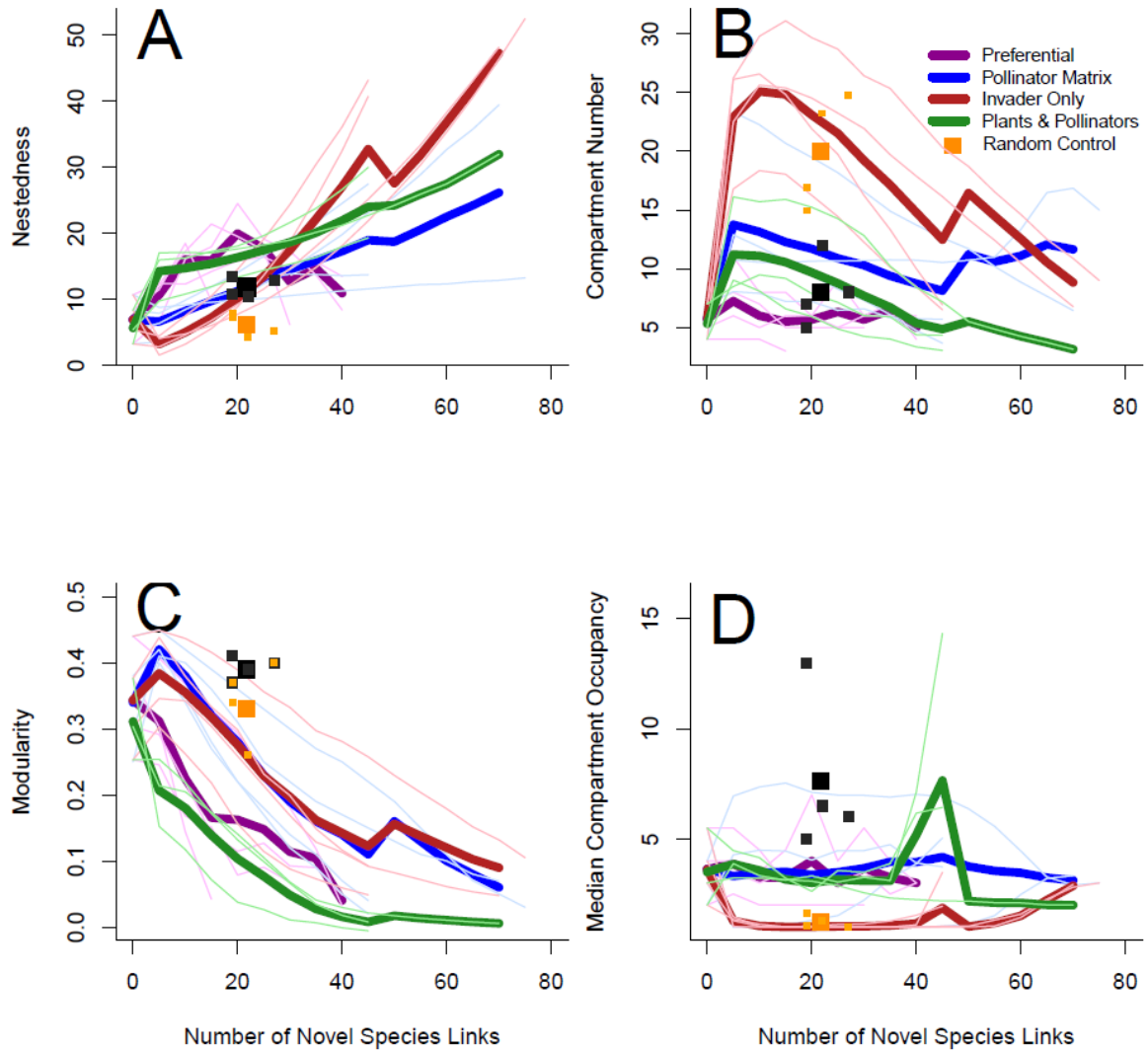
Figure C1 A heuristic figure demonstrating four models of species addition to an uninvaded network (A). The novel species may attract a specialist (C) or a supergeneralist (B) pollinator, or it may attract a matrix of pollinators whose interactions may be randomized (main text) (D) or static (supplemental). Also shown here is the substitution model (E), where the real invader is replaced by the simulated novel species.

Figure C2



The impact of a simulated novel species on the average degree (A) and the connectance (B) of the community, across increasing novel species degree. The four models included here are preferential (purple line), static pollinator matrix (blue line), pollinator matrix that interacts only with novel species (green line), and plant and pollinator matrix added (red line). The thick lines represent the average model response across four sites, while the thin lines represent the individual model response of each site. The black squares (■) represent the average degree and connectance of the control community, averaged across the four sites. The orange squares represent the properties of the randomized control community. In both cases, the larger squares represent the averages across four sites, and the smaller squares represent the properties of the individual sites.

Figure C3



The impact of the simulated species addition on second order properties of nestedness (A), compartment number (B), modularity (C), and median compartment occupancy (D). The four models are represented by the same colors as the previous figure.

Appendix D

Chapter 4 Appendix: R Code for species additions models

```
##### APPENDIX 2, R CODE FOR SIMULATIONS for "Characteristics of novel species interactions determine
network level impacts in plant-pollinator communities"
##### by Russo, L., J. Memmott, D. Montoya, K. Shea, and Y. Buckley
### SPECIES ADDITIONS SIMULATIONS
##required packages
require(bipartite)
require(vegan)
require(ggplot2)
require(igraph)

## Functions required

#to calculate average degree and connectance
firstorder<-function(x) {      #x must be an interaction matrix
  a<-(sum(x)/(ncol(x)+nrow(x)))  #average degree
  b<-(sum(x)/(ncol(x)*nrow(x)))  #connectance
  c<-sum(x)                      #total number of interactions
  answer<-c(a,b,c)
  return(answer)}

# this is the competition function for the Competitive model
foo <- function(mat, vec) {      #a function of the interaction matrix and invader vector
  nr <- nrow(mat)
  nc <- ncol(mat)
  cols <- which(vec == 1L)      #where the invader has an interaction
  rows <- sapply(seq_along(cols), #apply this function to the columns with interactions
  function(x, mat, cols) {
    ones <- which(mat[,cols[x]] == 1L) #pick the ones with interactions
    out <- if(length(ones) == 1L) {
      ones
    } else {                    #if there is more than one interaction
      sample(ones, 1)          #randomly pick the one to remove
    }
    out
  }, mat = mat, cols = cols)
  ind <- (nr*(cols-1)) + rows
  mat[ind] <- 0                 #remove that interaction you picked
  mat <- rbind(mat, vec)        #bind the invader which has stolen the interaction
  rownames(mat) <- NULL
  mat
}

#this compartments function is modeled after Newman and Girvan 2004
#compartments function requires igraph object
newmancommunities=function(x){ #x should be an igraph object
  graph2<-x                    #rename the original graph
  out=data.frame("V1"=0)       #generate an empty data frame to put Q scores in
```

```

out2=list("")          #generate an empty list to store communities in

for(l in 1:ecount(graph2)){
  betcen=(edge.betweenness(graph2,e=E(graph2),directed=F))*(2/(vcount(graph2)*(vcount(graph2)-1)))
  #the edge betweenness centrality, as calculated by igraph
  t=which(betcen==max(betcen))
  tempgraph=delete.edges(graph2,(t[[1]]-1))
  #delete the edge with the largest betweenness centrality
  clu=clusters(tempgraph)
  mem=clu$membership
  edges=E(x)
  edg.mat=get.edges(x,edges) #two column matrix of edge destinations

  Q=vector(length=clu$no)

  for(i in 1:clu$no){

    e_ii=vector(length=nrow(edg.mat))
    for(k in 1:nrow(edg.mat)){
      e_ii[k]=ifelse(mem[edg.mat[k,1]+1]==(i-1) & mem[edg.mat[k,2]+1]==(i-1),1,0)    #x is the edge matrix
    }
    e_ii<-sum(e_ii)/ecount(x)

    a_i=vector(length=nrow(edg.mat))
    for(j in 1:nrow(edg.mat)){
      a_i[j]=ifelse(mem[edg.mat[j,1]+1]==(i-1) | mem[edg.mat[j,2]+1]==(i-1),1,0)
    }
    a_i<-sum(a_i)/ecount(x)

    Q[i]=e_ii-(a_i^2)
  }

  out[l]=sum(Q)
  out2[[l]]=list(get.edgelist(tempgraph))
  graph2=tempgraph
}

best=which(out==max(out,na.rm=T))
final=graph.edgelist(as.matrix(as.data.frame(out2[[best[[1]]]]))) #the graph itself
cluf=clusters(final)      #membership, size and number of clusters/compartments in the final graph
comp=cluf$no
medsize=median(cluf$size)
avsize=mean(cluf$size)
mod=max(out,na.rm=T)      #max Q, this is our measure of modularity/compartmentalization

est<-list(best2=best, final2=final, cluf2=cluf, comp2=comp, mod2=mod, medsize2=medsize, avsize2=avsize)
return(est)
}

# to calculate compartmentalization and nestedness
secondorder<-function(x, z) {      #x is interaction matrix, z is igraph object
  a<-nested(x,method="NODF")      #nestedness: in NODF, 0 indicates absence of nested
                                #pattern, whereas 100 indicates the perfect nestedness
  b<-newmancommunities(z)        #Newman and Girvan compartments, z is igraph object
  d<-sum(x)                      #total number of interactions
  answer<-c(a,b$mod2,b$comp2,b$medsize2,b$avsize2,d)
  return(answer)}

```

```
#####
```

```
## Models of invader interactions
```

```
#Simple additive model
```

```
#average degree and connectance
```

```
x=expdat      #this is the matrix of interactions from the experiment,
              #it comprises 1s and 0s, or presence/absence of interactions
              #between insect visitors (cols) and plant species (rows)
```

```
obj<-data.frame(DEG=0,CON=0,INT=0)
```

```
obj2<-data.frame(DEG=0,CON=0,INT=0)
```

```
  #creates an empty dataframe to dump results into
```

```
for(i in 1:(ncol(x)/5)){      #generality in increments of 5
  for(j in 1:1000){          #1000 simulations
    a=rep(1,times=(i*5))      #repeat 1 for 1:# columns in x
    b=rep(0,times=(ncol(x)-length(a))) #have the rest of the vector be 0
    Inv=append(a,b)          #append these two for the Invader vector
    temp=rbind(x,Inv)        #bind the invader to the matrix
    obj[j,]<-t(firstorder(temp)) #fill empty dataframe with results
  }
  obj2[i,]<-colMeans(obj)
  write.table(obj2, 'add_1.csv', quote = FALSE, sep = ',', row.names = FALSE)
}
```

```
#comparmentalization and nestedness
```

```
obj<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)
```

```
obj2<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)
```

```
  #creates an empty matrix to dump results into
```

```
for(i in 1:(ncol(x)/5)){      #nested for loops
  a=rep(1,times=(i*5))        #repeat 1 for 1:# columns in x
  b=rep(0,times=(ncol(x)-length(a))) #have the rest of the vector be 0
  Inv=append(a,b)            #append these two for the Inv vector
  for(j in 1:1000){          #run this vector through the simulations
    Inv2=sample(Inv,replace=FALSE) #randomize interactions
    temp=rbind(x,Inv2)        #bind randomized invader to matrix
    temp.edge=web2edges(temp,out.files="edges",return=T) #make igraph object
    temp.graph=graph.edgelist(as.matrix(temp.edge[,1:2]))
    obj[j,]<-t(secondorder(temp,temp.graph))
  }
  obj2[i,]<-colMeans(obj)
  write.table(obj2, 'add_2.csv', quote = FALSE, sep = ',', row.names = FALSE)
}
```

```
#print these results to a csv file
```

```
#Competitive model
```

```
#average degree and connectance
```

```
obj<-data.frame(DEG=0,CON=0,INT=0)
```

```
obj2<-data.frame(DEG=0,CON=0,INT=0)
```

```
for(i in 1:(ncol(x)/5)){      #similar to additive model
```

```
  for(j in 1:1000){
```

```
    a=rep(1,times=(i*5))
```

```
    b=rep(0,times=(ncol(x)-length(a)))
```

```

Inv=append(a,b)
  temp=foo(x,Inv)      #run foo function
  obj[j,]<-firstorder(temp)
}
obj2[i,]<-colMeans(obj)
write.table(obj2, 'comp_1.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#comparmentalization and nestedness
obj<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)
obj2<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)

for(i in 1:(ncol(x)/5)){
  a=rep(1,times=(i*5))
  b=rep(0,times=(ncol(x)-length(a)))
  Inv=append(a,b)
  for (j in 1:1000){      #run this vector through the simulations
    Inv2=sample(Inv,replace=FALSE)    #randomize interactions
    temp=foo(x,Inv2)
    temp.edge=web2edges(temp,out.files="edges",return=T)
    temp.graph=graph.edgelist(as.matrix(temp.edge[,1:2]))
    obj[j,]<-t(secondorder(temp,temp.graph))
  }
  obj2[i,]<-colMeans(obj)
  write.table(obj2, 'comp_2.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

# Supergeneralist pollinator
#average degree and connectance
obj<-data.frame(DEG=0,CON=0,INT=0)
obj2<-data.frame(DEG=0,CON=0,INT=0)

for(i in 1:(ncol(x)/5)){
  for(j in 1:1000){
    a=rep(1,times=(i*5))
    b=rep(0,times=(ncol(x)-length(a)))
    Inv=append(a,b)
    Pol=rep(1,times=(nrow(x)+1)) #pollinator super generalist, vector of all 1s
    temp2=rbind(x,Inv)
    temp3=cbind(temp2,Pol) #bind supergeneralist pollinator
    obj[j,]<-firstorder(temp3)
  }
  obj2[i,]<-colMeans(obj)
  write.table(obj2, 'super_1.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#comparmentalization and nestedness
obj<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)
obj2<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)

for(i in 1:(ncol(x)/5)){
  a=rep(1,times=(i*5))
  b=rep(0,times=(ncol(x)-length(a)))
  Inv=append(a,b)
  Pol=rep(1,times=(nrow(x)+1)) #pollinator super generalist
  for (j in 1:1000){
    Inv2=sample(Inv,replace=FALSE)
    temp2=rbind(x,Inv2)
    temp3=cbind(temp2,Pol)
    temp.edge=web2edges(temp3,out.files="edges",return=T)

```

```

    temp.graph=graph.edgelist(as.matrix(temp.edge[,1:2]))
    obj[j,]<-t(secondorder(temp3,temp.graph))
  }
obj2[i,]<-colMeans(obj)
write.table(obj2, 'super_2.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

## Randomized pollinator matrix model
#average degree and connectance
x=expdat      #experimental interaction matrix
y=ctldat     #control interaction matrix
z=ncol(y)-ncol(x) #the difference in the number of columns
int=sum(y[,z:ncol(y)]) #the number of interactions of each added pollinator from the control
#so they are characterized by their degree, not by
#the identity of their links
obj<-data.frame(DEG=0,CON=0,INT=0)
obj2<-data.frame(DEG=0,CON=0,INT=0)

for(i in 1:(ncol(y)/5)){
  a=rep(1,times=(i*5))
  b=rep(0,times=(ncol(y)-length(a)))
  Inv=append(a,b)
  d=rep(1,times=int) #number of interactions of the pollinator, but no identity
  e=rep(0,times=((nrow(x)+1)*z)-length(d)) #the rest of the vector is 0s
  f=append(d,values=e)
  Pol=matrix(f,ncol=z,nrow=(nrow(x)+1))
  for (j in 1:1000){
    temp1=as.matrix(Pol) #so we're randomly allocating interactions
    rnm=rownames(Pol) #to new pollinators
    cnm=colnames(Pol)
    g=nrow(Pol)*ncol(Pol) #and then appending them to the matrix
    temp2=sample(temp1,g,replace=F) #and then adding an invader with a specific
    dim(temp2) <- c((nrow(x)+1),z) #number of interactions, randomized
    dimnames(temp2) <- list(rnm, cnm)
    Inv2=sample(Inv,replace=FALSE)
    temp3=rbind(x,Inv2)
    temp4=cbind(temp3,temp2)
    obj[j,]<-firstorder(temp4)
  }
obj2[i,]<-colMeans(obj)
write.table(obj2, 'random_1.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#comparmentalization and nestedness
obj<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)
obj2<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)

for(i in 1:(ncol(y)/5)){
  a=rep(1,times=(i*5))
  b=rep(0,times=(ncol(y)-length(a)))
  Inv=append(a,b)
  d=rep(1,times=int)
  e=rep(0,times=((nrow(x)+1)*z)-length(d))
  f=append(d,values=e)
  Pol=matrix(f,ncol=z,nrow=(nrow(x)+1))
  for (j in 1:1000){
    temp1=as.matrix(Pol) #so we're randomly allocating interactions
    rnm=rownames(Pol) #to new pollinators
    cnm=colnames(Pol)

```

```

g=nrow(Pol)*ncol(Pol)          #and then appending them to the matrix
temp2=sample(temp1.g,replace=F) #and then adding an invader with a specific
dim(temp2) <- c((nrow(x)+1),z) #number of interactions, randomized
dimnames(temp2) <- list(rnm, cnm)
Inv2=sample(Inv,replace=FALSE)
temp3=rbind(x,Inv2)
temp4=cbind(temp3,temp2)
temp.edge=web2edges(temp4,out.files="edges",return=T)
temp.graph=graph.edgelist(as.matrix(temp.edge[,1:2]))
obj[,j]<-t(secondorder(temp4,temp.graph))
}
obj2[i,]<-colMeans(obj)
write.table(obj2, 'random_2.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#Substitution model
#average degree and connectance
x<-y[!rownames(y) %in% "Impatiens.glandulifera",] #the control except for the invader

obj<-data.frame(DEG=0,CON=0,INT=0)
obj2<-data.frame(DEG=0,CON=0,INT=0)

for(i in 1:(ncol(x)/5)){      #putting the simulated invader back in
  for(j in 1:1000){
    a=rep(1,times=(i*5))
    b=rep(0,times=(ncol(x)-length(a)))
    Inv=append(a,b)
    temp=rbind(x,Inv)
    obj[,j]<-firstorder(temp)
  }
  obj2[i,]<-colMeans(obj)
  write.table(obj2, 'sub_1.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#compartmentalization and nestedness
obj<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)
obj2<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)

for(i in 1:(ncol(x)/5)){
  a=rep(1,times=(i*5))
  b=rep(0,times=(ncol(x)-length(a)))
  Inv=append(a,b)
  for (j in 1:1000){
    Inv2=sample(Inv,replace=F)
    temp=rbind(x,Inv2)
    temp.edge=web2edges(temp,out.files="edges",return=T)
    temp.graph=graph.edgelist(as.matrix(temp.edge[,1:2]))
    obj[,j]<-t(secondorder(temp,temp.graph))
  }
  obj2[i,]<-colMeans(obj)
  write.table(obj2, 'sub_2.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#####
#SUPPLEMENTAL MODELS
#preferential attachment
#average degree and connectance
x=expdat #the interaction matrix from the experimental plot

```

```

f=expfreq #the weighted interaction matrix
a=colSums(f) #the weighted degree of the pollinators
b=sum(a) #the total number of interactions
d=a/b #the normalized vector of interaction frequencies

obj<-data.frame(DEG=0,CON=0,INT=0)
obj2<-data.frame(DEG=0,CON=0,INT=0)

poll.prob=d #probabilities according to the proportion of total visits

for(i in 1:(ncol(x)/5)){
  for(j in 1:1000){
    Inv<-integer(ncol(x))
    Inv[sample.int(ncol(x),(i*5),prob=poll.prob)]<-1 #sampling according to the weighted interactions
    Inv2<-Inv #more generalist species have a higher probability
    Inv<-integer(ncol(x)) #of being selected
    temp2=rbind(x,Inv2)
    obj[j,]<-firstorder(temp2)
  }
  obj2[i,]<-colMeans(obj)
  write.table(obj2, 'pref_1.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#comparmentalization and nestedness
obj<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)
obj2<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)

for(i in 1:(ncol(x)/5)){
  for(j in 1:1000){
    Inv<-integer(ncol(x))
    Inv[sample.int(ncol(x),(i*5),prob=poll.prob)]<-1
    Inv2<-Inv
    Inv<-integer(ncol(x))
    temp=rbind(x,Inv2)
    temp.edge=web2edges(temp,out.files="edges",return=T)
    temp.graph=graph.edgelist(as.matrix(temp.edge[,1:2]))
    obj[j,]<-t(secondorder(temp,temp.graph))
  }
  obj2[i,]<-colMeans(obj)
  write.table(obj2, 'pref_2.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#Preserve the interaction structure of the matrix; static pollinator matrix
#average degree and connectance
ctlmdat->y #the interaction matrix from the control (invaded)
expdat->x #the interaction matrix from the experiment
z=ncol(y)-ncol(x) #the difference in the number of columns
obj<-data.frame(DEG=0,CON=0,INT=0)
obj2<-data.frame(DEG=0,CON=0,INT=0)

Pol=y[1:nrow(x),1:z] #the added pollinators from the control matrix

for(i in 1:(ncol(y)/5)){ #the total number of columns in the control
  for(j in 1:1000){
    a=rep(1,times=(i*5))
    b=rep(0,times=(ncol(y)-length(a)))
    Inv=append(a,b)
    temp=cbind(x,Pol) #first bind the pollinator matrix, not randomized
    temp2=rbind(temp,Inv)
    obj[j,]<-firstorder(temp2)
  }
}

```

```

}
obj2[i,]<-colMeans(obj)
write.table(obj2, 'static_1.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#compartmentalization and nestedness
obj<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)
obj2<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)

for(i in 1:(ncol(y)/5)){ #ncol(y) is equivalent to (ncol(Pol)+ncol(x))
a=rep(1,times=(i*5))
b=rep(0,times=(ncol(y)-length(a)))
Inv=append(a,b)
for(j in 1:1000){
temp=cbind(x,Pol)
Inv2=sample(Inv,replace=FALSE)
temp2=rbind(temp,Inv2)
temp.edge=web2edges(temp2,out.files="edges",return=T)
temp.graph=graph.edgelist(as.matrix(temp.edge[,1:2]))
obj[j,]<-t(secondorder(temp2,temp.graph))
}
obj2[i,]<-colMeans(obj)
write.table(obj2, 'static_2.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

## Matrix of pollinators that only interact with the invader; invader specific pollinators
#average degree and connectance
obj<-data.frame(DEG=0,CON=0,INT=0)
obj2<-data.frame(DEG=0,CON=0,INT=0)

for(i in 1:(ncol(y)/5)){
a=rep(1,times=(i*5))
b=rep(0,times=(ncol(y)-length(a)))
Inv=append(a,b)
e=rep(0,times=(nrow(x)*z)) #the number of entries in the matrix
Pol=matrix(e,ncol=z,nrow=(nrow(x))) #an all zero matrix
for(j in 1:1000){
temp1=as.matrix(Pol)
temp2=cbind(x,Pol) #attaching the zero interaction pollinator matrix
Inv2=sample(Inv,replace=FALSE) #the invader can interact with the new pollinators
temp3=rbind(temp2,Inv2)
obj[j,]<-firstorder(temp3)
}
obj2[i,]<-colMeans(obj)
write.table(obj2, 'invaderspecific_1.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#compartmentalization and nestedness
obj<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)
obj2<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)

for(i in 1:(ncol(y)/5)){
a=rep(1,times=(i*5))
b=rep(0,times=(ncol(y)-length(a)))
Inv=append(a,b)
e=rep(0,times=(nrow(x)*z))
Pol=matrix(e,ncol=z,nrow=(nrow(x)))
for(j in 1:1000){

```



```

temp1=as.matrix(Pol)
temp2=cbind(x,Pol)
Inv2=sample(Inv,replace=FALSE)
temp3=rbind(temp2,Inv2)
temp.edge=web2edges(temp3,out.files="edges",return=T)
temp.graph=graph.edgelist(as.matrix(temp.edge[,1:2]))
obj[j,]<-t(secondorder(temp3,temp.graph))
}
obj2[i,]<-colMeans(obj)
write.table(obj2, 'invaderspecific_2.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#Plants and pollinators added
#average degree and connectance
z=ncol(y)-ncol(x) #the difference in the number of columns
w=nrow(y)-nrow(x) #the difference in the number of rows
u<-y[!rownames(y) %in% "Impatiens.glandulifera",] #be sure to not include the invader
#its interactions are very different
obj<-data.frame(DEG=0,CON=0,INT=0)
obj2<-data.frame(DEG=0,CON=0,INT=0)

Pol=y[1:nrow(x),1:z]
Pln=u[1:w,1:ncol(y)]

for(i in 1:(ncol(y)/5)){
  for(j in 1:1000){
    a=rep(1,times=(i*5))
    b=rep(0,times=(ncol(y)-length(a)))
    Inv=append(a,b)
    temp=cbind(x,Pol) #bind the added pollinators
    colnames(Pln)<-colnames(temp) #include the names
    temp2=rbind(temp,Pln) #bind the added plants
    Inv2=sample(Inv,replace=FALSE) #resample the invader vector
    temp3=rbind(temp2,Inv2) #bind the invader vector
    obj[j,]<-firstorder(temp3)
  }
  obj2[i,]<-colMeans(obj)
  write.table(obj2, 'plantspollinators_1.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#compartmentalization and nestedness
obj<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)
obj2<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)

for(i in 1:(ncol(y)/5)){
  a=rep(1,times=(i*5))
  b=rep(0,times=(ncol(y)-length(a)))
  Inv=append(a,b)
  for (j in 1:1000){
    temp=cbind(x,Pol)
    colnames(Pln)<-colnames(temp)
    temp2=rbind(temp,Pln)
    Inv2=sample(Inv,replace=FALSE)
    temp3=rbind(temp2,Inv2)
    temp.edge=web2edges(temp3,out.files="edges",return=T)
    temp.graph=graph.edgelist(as.matrix(temp.edge[,1:2]))
    obj[j,]<-t(secondorder(temp3,temp.graph))
  }
  obj2[i,]<-colMeans(obj)
}

```

```

write.table(obj2, 'plantspollinators_2.csv', quote = FALSE, sep = ',', row.names = FALSE)
}

#Randomized control matrix
#average degree and connectance
y=ctldat

obj<-data.frame(DEG=0,CON=0,INT=0)
obj2<-data.frame(DEG=0,CON=0,INT=0)

for(i in 1:1000){
  temp1=as.matrix(y) #coerce it into a matrix
  rnm=rownames(y) #get the row and column names
  cnm=colnames(y)
  g=nrow(y)*ncol(y) #the number of potential interactions
  temp2=sample(temp1,g,replace=F) #resample the original matrix
  dim(temp2) <- c(nrow(y),ncol(y)) #build it in the same size
  dimnames(temp2) <- list(rnm, cnm) #reattach names
  obj[i,]<-firstorder(temp2) #calculate properties
}
colMeans(obj) #print the properties

#comparmentalization and nestedness
obj<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)
obj2<-data.frame(NODF=0,MOD=0,COMP=0,MED=0,AV=0,SIZE=0)

for(i in 1:1000){
  temp1=as.matrix(y)
  rnm=rownames(y)
  cnm=colnames(y)
  g=nrow(y)*ncol(y)
  temp2=sample(temp1,g,replace=F)
  dim(temp2) <- c(nrow(y),ncol(y))
  dimnames(temp2) <- list(rnm, cnm)
  temp.edge=web2edges(temp2,out.files="edges",return=T)
  temp.graph=graph.edgelist(as.matrix(temp.edge[,1:2]))
  obj[i,]<-secondorder(temp2,temp.graph)
}

colMeans(obj)

```

Appendix E

Chapter 6 Appendix: Positive effects of an invader

Generalized Linear Models

GLMs were used to compare the abundance of specimens in different insect orders, the abundance of non-Apoidean families, the abundance and species richness of bee species in thistle and non-thistle plots for the vacuum and composite insect traps, and the number of observations of different categories for observational data. For these models, we used the function `lmer` in the package `lme4` (R Development Core Team 2011).

We used the random effects of Week, Year, and Plot, with the fixed effect of thistle presence or absence. We checked for interactions between the random effects and fixed effects, and then performed model validation to remove factors that did not significantly contribute to the variation observed. The data were Poisson distributed.

Zero-inflated Models

Individual bee species, genera, and observation level groups were zero-inflated and we therefore compared thistle and non-thistle plots for those groups with the `zeroinfl` function in the package `pscl` (R Development Core Team 2011).

Similarly to the generalized linear models, we checked for significant interactions and performed model validation to find the most parsimonious model.

Table E1. Total biomass and flower counts of each of the background plant species in the plots with and without thistles. Values are averaged across plots and between years for the two treatments, with standard errors reported in parentheses. There was no significant effect of the presence of the thistle on the biomass or number of flowers produced by any of the species ($P > 0.05$).

		<i>C. cyanus</i>	<i>H. annuus</i>	<i>R. hirta</i>	<i>C. crista</i>	<i>L. trimestris</i>	<i>C. bipinnatus</i>	<i>G. pulchella</i>	<i>Melilotus</i>	<i>C. tripteris</i>
Biomass	Thistle	2.28 (\pm 0.61)	0.29 (\pm 1.82)	3.65 (\pm 0.75)	2.16 (\pm 0.37)	1.78 (\pm 0.71)	5.11 (\pm 0.98)	4.50 (\pm 0.54)	3.28 (\pm 0.40)	3.50 (\pm 0.68)
	Non-Thistle	2.89 (\pm 0.58)	2.58 (\pm 1.79)	3.76 (\pm 0.76)	2.07 (\pm 0.37)	3.45 (\pm 0.71)	4.92 (\pm 0.97)	5.06 (\pm 0.55)	3.39 (\pm 0.40)	3.63 (\pm 0.69)
Flower Counts	Thistle	39.67 (\pm 12.41)	2.78 (\pm 1.14)	26.78 (\pm 10.37)	13.89 (\pm 3.27)	39.44 (\pm 21.64)	25.72 (\pm 8.15)	1.06 (\pm 0.45)	--	84.89 (\pm 29.81)
	Non-Thistle	53.94 (\pm 17.51)	13.5 (\pm 6.38)	40.78 (\pm 22.61)	16.72 (\pm 4.44)	93.06 (\pm 41.5)	13.5 (\pm 5.37)	0.67 (\pm 0.4)	--	72.89 (\pm 30.6)

Table E2. List of all bee species captured by the vacuum trap over two years of sampling and where they were collected (i.e. in thistle or non-thistle plots).

Species	Non-thistle	Thistle
<i>Agapostemon virescens</i>	5	12
<i>Andrena aliciae</i>	1	3
<i>Apis mellifera</i>	3	2
<i>Augochlora pura</i>	4	39
<i>Augochlorella aurata</i>	1	2
<i>Bombus bimaculatus</i>	1	22
<i>Bombus impatiens</i>	18	80
<i>Bombus insularis</i>	0	5
<i>Bombus vagans</i>	0	3
<i>Ceratina calcarata</i>	5	55
<i>Ceratina dupla</i>	2	28
<i>Halictus confusus</i>	0	1
<i>Halictus ligatus</i>	21	46
<i>Lasioglossum admirandum</i>	3	3
<i>Lasioglossum fascipenne</i>	0	1
<i>Lasioglossum imitatum</i>	0	4
<i>Lasioglossum males</i>	0	8
<i>Lasioglossum pectorale</i>	1	1
<i>Lasioglossum versatum</i>	0	1
<i>Lasioglossum viridatum</i>	1	4
<i>Megachile mendica</i>	0	3
<i>Megachile pugnata</i>	1	0
<i>Melissodes agilis</i>	7	0
<i>Melissodes bimaculata</i>	2	4
<i>Melissodes desponsa</i>	0	5
<i>Melissodes subiliata</i>	0	11
<i>Melissodes trinodis</i>	7	1
<i>Triepeolus lunatus</i>	1	1
<i>Lasioglossum</i> sp. (damaged)	1	1
<i>Xylocopa virginica</i>	1	0

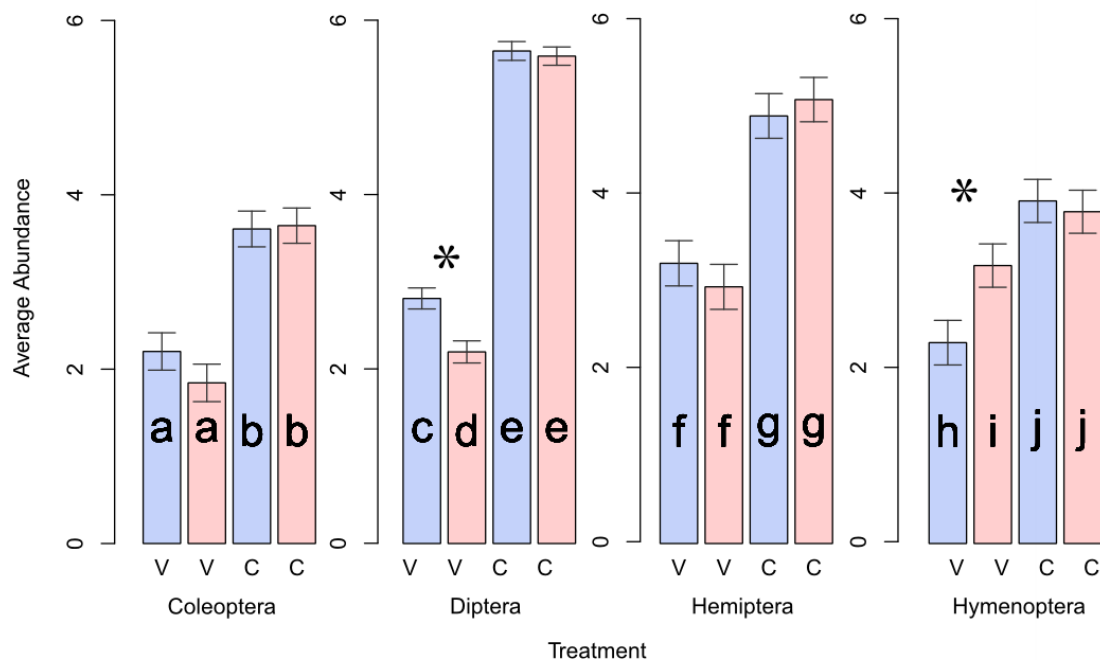
Table E3. Counts of specimens from non-Apoidean families of Hymenoptera. There were no significant differences ($P > 0.05$) detected between thistle or non-thistle plots.

Family	Non-thistle	Thistle	Total
Argidae	1	0	1
Bethylidae	31	25	56
Braconidae	46	35	81
Cephalidae	2	2	4
Ceraphronidae	13	7	20
Chalcididae	3	0	3
Charipidae	1	0	1
Chrysididae	4	6	10
Crabronidae	0	1	1
Cynipidae	11	7	18
Diapriidae	25	12	37
Dryinidae	1	1	2
Encyrtidae	3	6	9
Eulophidae	7	2	9
Eupelmidae	1	1	2
Eurytomidae	4	4	8
Figitidae	12	12	24
Formicidae	105	115	220
Gasteruptiidae	1	0	1
Heloridae	0	2	2
Ichneumonidae	51	50	101
Megaspilidae	4	3	7
Mymaridae	6	3	9
Pamphiliidae	0	1	1
Pelecinidae	1	0	1
Pergidae	0	1	1
Perilampidae	3	5	8
Platygastridae	7	8	15
Pompilidae	16	13	29
Proctotrupidae	3	2	5
Pteromalidae	13	5	18
Roproniidae	0	1	1
Scelionidae	3	2	5
Sclerogibbidae	1	0	1
Scoliidae	2	1	3
Sierolomorphidae	2	1	3
Siricidae	1	0	1
Sphecidae	11	12	23
Tenthredinidae	16	16	32
Tiphiidae	16	7	23
Torymidae	1	2	3
Unknown	21	20	41
Vespidae	7	15	22
Xiphydriidae	0	2	2
Vanhorniidae	0	1	1
Total	457	409	866

Table E4. Counts of specimens from 18 families of Hymenoptera that contain natural enemies of agricultural pests. There were no significant differences ($P > 0.05$) detected between thistle or non-thistle plots in either the vacuum trap (VT) or the composite insect trap (CIT).

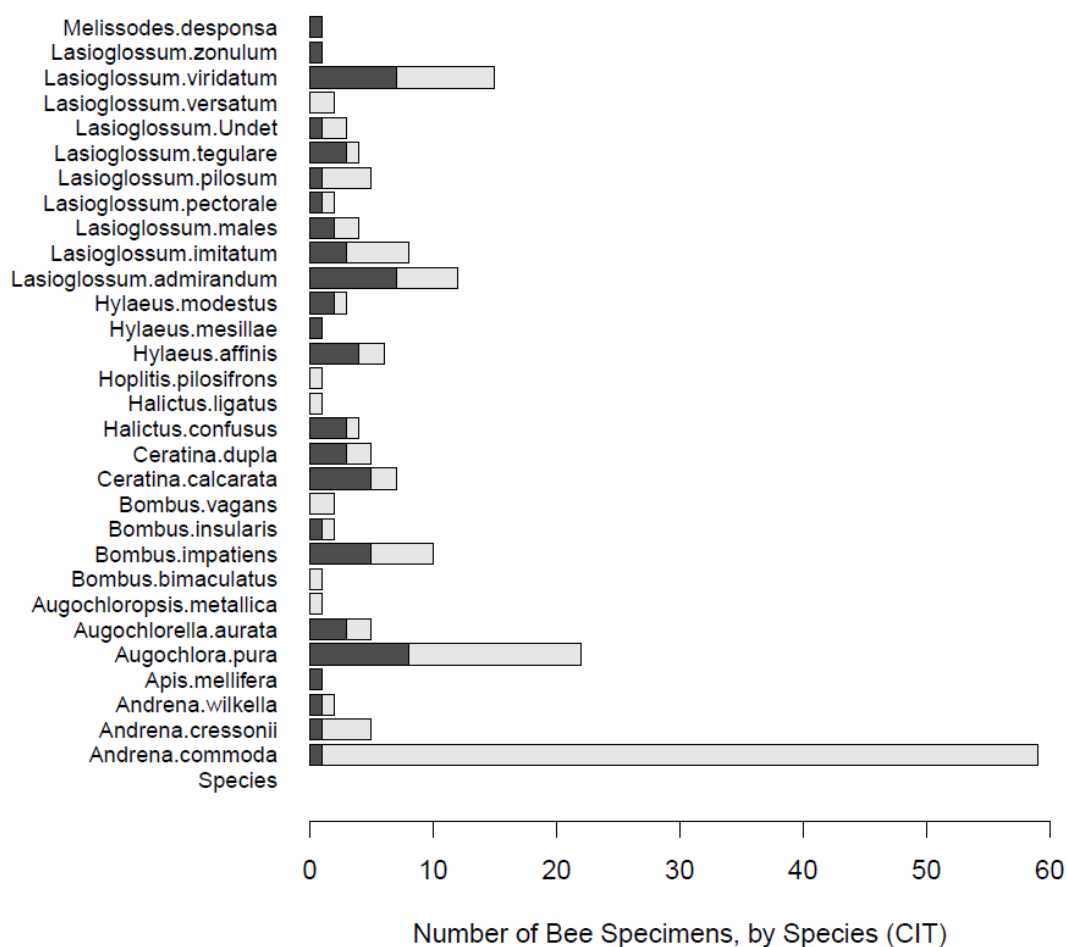
Family	Non-thistle VT	Thistle VT	VT Total	Thistle CIT	Non-thistle CIT	CIT Total	Grand Total
Bethylidae	1	0	1	25	30	55	56
Braconidae	9	10	19	25	37	62	81
Ceraphronidae	1	0	1	7	12	19	20
Chalcididae	0	0	0	0	3	3	3
Chrysididae	0	0	0	6	4	10	10
Diapriidae	0	1	1	11	25	36	37
Encyrtidae	0	1	1	5	3	8	9
Eulophidae	0	0	0	2	7	9	9
Figitidae	1	0	1	12	11	23	24
Megaspilidae	0	0	0	3	4	7	7
Proctotrupidae	0	0	0	2	3	5	5
Pteromalidae	0	0	0	5	13	18	18
Scelionidae	0	0	0	2	3	5	5
Tiphiidae	0	0	0	7	16	23	23
Vespidae	0	0	0	15	7	22	22
Platygastridae	1	0	1	8	6	14	15
Mymaridae	0	0	0	3	6	9	9
Ichneumonidae	10	10	20	40	41	81	101

Figure E1



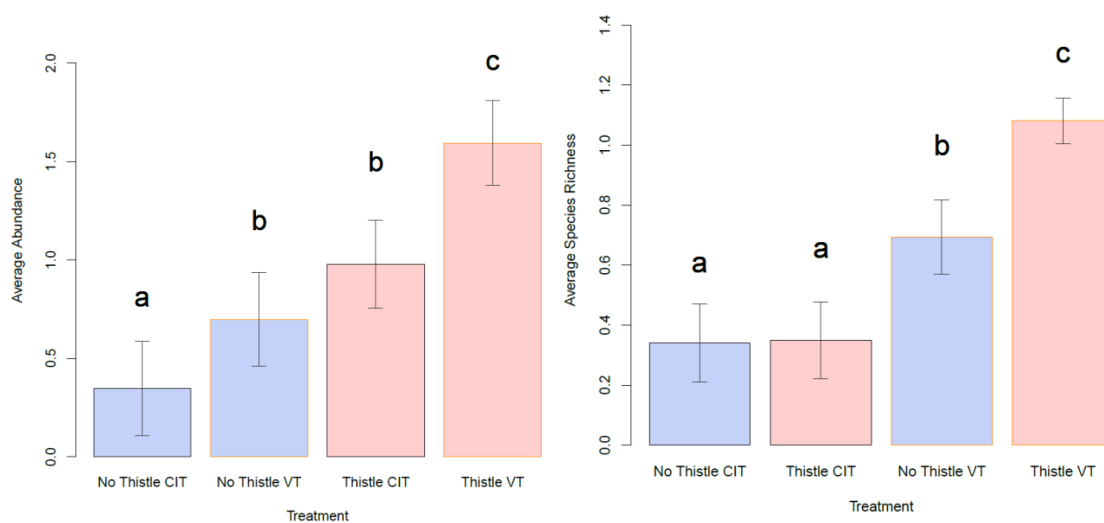
The average abundance of the four most abundant insect orders collected by vacuum (V) and composite (C) insect traps in thistle (red) and non-thistle (blue) plots, with standard errors represented by error bars. There were always significant differences between vacuum and composite traps ($P < 0.05$). The composite trap never detected significant differences between thistle and non-thistle plots ($P > 0.05$), but the vacuum trap detected a significant decrease in the average abundance of Diptera ($P < 0.05$) and a significant increase in the average abundance of Hymenoptera ($P < 0.05$). There were no significant differences between thistle and non-thistle plots in Coleoptera or Hemiptera for either trap type ($P > 0.05$).

Figure E2

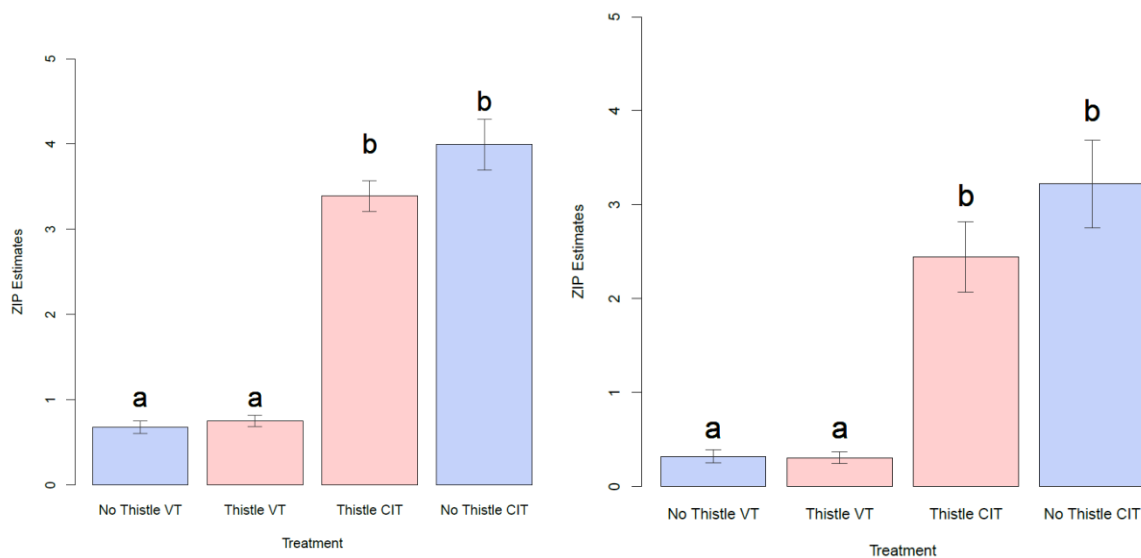


The absolute abundance of bee species in thistle (light grey) plots stacked on top of non-thistle (dark grey) plots across 2 years as collected by the composite insect trap. There was a significant increase in *Andrena commoda* in thistle plots ($P < 0.05$); however, this difference is likely due to an unusually high number of specimens caught at one plot-day (46 individuals), likely representing a nesting aggregation. No other species responded significantly ($P > 0.05$) and there was no difference in species richness in thistle versus non-thistle plots ($P > 0.05$).

Figure E3



The average abundance (left) and species richness (right) of bees collected by vacuum (VT) and composite insect traps (CIT) in thistle (red) and non-thistle (blue) plots over two years. There were significant differences between composite insect traps and vacuum traps ($P < 0.05$) and vacuum traps always had a significantly higher species richness ($P < 0.05$). When the outlier *Andrena commoda* specimens were accounted for, there was no significant difference in composite insect traps in either abundance or species richness ($P > 0.05$).

Figure E4

The estimates for abundance of non-Apoidea Hymenoptera (left) and Hymenopteran families of natural enemies (right) in thistle (red) and non-thistle (blue) plots as collected by the vacuum (VT) and composite (CIT) insect traps. The composite insect trap collected a higher abundance of both non-Apoidea Hymenoptera and families of natural enemies than the vacuum trap ($P < 0.05$), but there were no differences between thistle and non-thistle plots within either trap type ($P > 0.05$).

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PUBLICATIONS:

- **Russo, L,** DeBarros, N, Yang, S, Shea, K & Mortensen, D. (2013) Supporting crop pollinators with floral resources: network-based phenological matching. *Ecology and Evolution*, doi: 10.1002/ece3.703.
- **Russo, L,** *Stehouwer, R, *Heberling, JM & Shea, K. (2011) The Composite Insect Trap: a novel method for biologically diverse sampling. *PLoS ONE*, **6**(6).
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- **Russo, L,** Miller, A, Bjornstad, O, Tooker, J, and Shea, K. "The taxonomic structuring and conservatism of plants and their insect visitors." *in review*
- **Russo, L,** Memmott, J, Montoya, D, Shea, K, & Buckley, Y. "Characteristics of invader interactions determine network level impacts in plant-pollinator communities." *submitted*
- **Russo, L.,** *Nichol, C., & Shea, K. "Pollinator floral provisioning by a plant invader: quantifying beneficial effects of detrimental species." *submitted*
- **Russo, L.** & Shea, K. "Experimentally increasing the network connectance of a simple bee-plant community." *submitted*
- Campbell, C, **Russo, L,** Marins, A, DeSouza, O, Schonrogge, K, Mortensen, D, Tooker, J, Albert, R, and Shea, K. "Inferring characteristics of termite-guest communities through comparative network analysis of plant-pollinator communities." *In preparation*
- Marins, A, Costa, D, **Russo, L,** Campbell, C, Bjornstad, O, and Shea, K. "The role of mound size and builder identity in a termite-cohabitation network." *In preparation*

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- Biology 220 Populations and Communities. Taught two laboratory sections of 22 students. Pennsylvania State University, Spring 2010.
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OUTREACH:

- Provided recommendations for the development of a long term native plant pollinator garden at the Penn State Arboretum.
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