Ecological networks of grassland plants and arthropods

by

Ellen A. R. Welti

B.A., Kansas State University, 2012

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
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Manhattan, Kansas

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Abstract

Ecological communities are comprised both of species and their interactions. The importance of species interactions is embraced by ecological network analysis, a framework used to identify non-random patterns in species interactions, and the consequences of these patterns for maintaining species diversity. Here, I investigated environmental drivers of the structure of plant-pollinator and plant-herbivore networks. Specifically, I asked: (1) Do global-scale climate gradients shape mutualistic and antagonistic networks? (2) At a landscape scale (within a 3,487 ha research site), how do contrasting regimes of major grassland disturbances - fire frequency and grazing by bison (*Bison bison*) - shape plant-pollinator network structure? (3) How do fire and grazing affect plant-grasshopper network structure? And, (4) What is the role of plant species diversity in determining plant-herbivore network structure? At the global scale, variability in temperature was the key climatic factor regulating both antagonistic and mutualistic network structural properties. At the landscape scale, fire and grazing had major consequences for plant-pollinator and plant-herbivore communities. In particular, bison grazing increased network complexity and resistance to species loss for both plant-pollinator and plant-herbivore systems. Results from an experimental grassland restoration that manipulated plant diversity suggest that plant diversity directly affects plant-herbivore structure and increases network stability. Collectively, these results suggest that environmental gradients and plant species diversity regulate the network structure of ecological communities. Determining how the structure of ecological interactions change with environmental conditions and species diversity improves our ability to identify vulnerable communities, and to predict responses of biodiversity to global change.

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Co-Major Professor
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Ecological communities are comprised both of species and their interactions. The importance of species interactions is embraced by ecological network analysis, a framework used to identify non-random patterns in species interactions, and the consequences of these patterns for maintaining species diversity. Here, I investigated environmental drivers of the structure of plant-pollinator and plant-herbivore networks. Specifically, I asked: (1) Do global-scale climate gradients shape mutualistic and antagonistic networks? (2) At a landscape scale (within a 3,487 ha research site), how do contrasting regimes of major grassland disturbances - fire frequency and grazing by bison (*Bison bison*) - shape plant-pollinator network structure? (3) How do fire and grazing affect plant-grasshopper network structure? And, (4) What is the role of plant species diversity in determining plant-herbivore network structure? At the global scale, variability in temperature was the key climatic factor regulating both antagonistic and mutualistic network structural properties. At the landscape scale, fire and grazing had major consequences for plant-pollinator and plant-herbivore communities. In particular, bison grazing increased network complexity and resistance to species loss for both plant-pollinator and plant-herbivore systems. Results from an experimental grassland restoration that manipulated plant diversity suggest that plant diversity directly affects plant-herbivore structure and increases network stability. Collectively, these results suggest that environmental gradients and plant species diversity regulate the network structure of ecological communities. Determining how the structure of ecological interactions change with environmental conditions and species diversity improves our ability to identify vulnerable communities, and to predict responses of biodiversity to global change.

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Dedication

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Chapter 1 - Introduction

While interactions between two or three species have been a major emphasis of community ecology historically, consequences of interaction patterns at the community level remain poorly described and understood. Previous studies using an ecological network framework suggest that patterns of interactions among species at the community level are non-random, and that these patterns have implications for maintaining ecosystem stability (Bascompte 2010). Moreover, theoretical work to date concludes that networks of antagonistic and mutualistic interactions are stabilized by different structural properties (Thébault and Fontaine 2010). However, these network types are rarely studied together (Takemoto et al. 2014). In this dissertation, I constructed and analyzed plant-pollinator (mutualistic) and plant-insect herbivore (antagonistic) interaction networks in the same grassland habitats to characterize and determine what factors drive changes in interaction structure.

At the global-scale, I used a meta-analysis approach to examine constraints of climate and climate variability on antagonistic and mutualistic ecological networks. At the landscape level, I investigated impacts of fire and grazing disturbance regimes, two major practices for managing grassland habitats, on plant-floral visitor and plant-insect herbivore network structure across watersheds at Konza Prairie Biological Station, a Long-Term Ecological Research (LTER) site in the Flint Hills ecoregion of northeast Kansas. Additionally, because plant diversity acts as a major driver of plant-insect interaction network structure, and managing plant diversity is a potential conservation tool, I directly evaluated the relationship between plant diversity and plant-insect herbivore network structure in a plant diversity manipulation experiment at the local scale at Platte River Prairies, a site of The Nature Conservancy located in central Nebraska. By examining changes in network structural properties across climate gradients, grassland disturbance regimes, and experimentally manipulated plant diversity levels, my research provides insight into contributions and constraints of environmental factors affecting network structure acting at varying spatial and temporal scales.

My choice of grassland systems was motivated for two reasons: (a) Many critical ecological questions of general importance can be effectively studied using grassland systems, as is the case for looking at community network structure and its significance. Grasslands provide excellent opportunities to address change in network structural properties across environmental gradients: they occur across striking, large-scale orthogonal gradients of temperature and precipitation that result in large differences in ANPP and vegetation physical structure, grassland insects and plants are easily sampled, and they support moderate species richness sufficient to test ideas while remaining tractable (Knapp et al. 1998). (b) Second, grasslands are highly exploited by humans because of their high suitability for agricultural use, especially because of the suitability of both soils and climate for crop production. As such, many North American grasslands and especially the tallgrass prairie are rapidly disappearing as intact systems. Thus, understanding how grassland communities maintain species in the age of anthropogenic change is increasingly critical (Samson and Knopf 1994, Hoekstra et al. 2005).

Insects play many critical ecological roles and are exemplary for testing hypotheses at the community level. Insects are an exceptionally useful group for addressing questions regarding ecological communities because they are easy to sample in large quantities, are habitat- and resource-sensitive, and show high α and β diversity (McGeoch 1998, Pryke and Samways 2012). Insects provide many ecosystem services critical for other species, including acting as: a food source for higher trophic levels, natural enemies controlling pests, pollinators, and contributors to nutrient cycling (Rohr et al. 2007).

In the face of the current global environmental crises, it is increasingly crucial to understand the nature of relationships between environmental factors and the long-term maintenance of ecological communities. My work aimed to improve our ability to predict ecological community responses to future environmental change through improved understanding of the responses of plant-insect communities, interactions, and the implications of interaction patterns for changes in environmental variation. Here, I summarize and provide an overview of the topics in this dissertation to achieve these goals.

Chapter 2 Overview– Ecological networks across global-scale climate gradients

In Chapter 2, I analyzed data from published bipartite antagonistic and mutualistic networks to evaluate the potential consequences of broad-scale environmental gradients for the resulting underlying network structure of trophic and mutualistic networks. Results indicate that annual cumulative temperature (growing season length) and temperature variability are potential underlying drivers of network structural properties and, therefore, have major consequences for community stability by limiting the types and scope of species interaction patterns that persist. Importantly, trophic and mutualistic network structural properties always exhibited opposite responses to temperature gradients.

Critical analyses assessing modularity and nested structure in ecological networks have provided new and exciting insights into the importance of such internal network structure for community stability (Bascompte 2010, Thébault and Fontaine 2010, Pocock et al. 2012). To date, most network studies focused on the internal architecture of species interactions in individual communities, the relationship between network structural properties and species richness and abundance, and the possible inferences of such results for community stability (Jordano 1987, Bascompte et al. 2006, Montoya et al. 2006, Olesen et al. 2011). In this chapter, I extended these results in important and critical new directions – the search for repeatable responses of network structural properties along broad-scale environmental gradients of temperature and precipitation. This approach has much merit in that these factors covary with broad-scale latitudinal gradients that themselves often covary with species diversity. If species diversity covaries with environmental gradients, does this influence the internal structure of species networks, and indeed, do network structural properties vary in meaningful ways along such gradients if one removes the effect of species richness? Results indicate that all of these potential outcomes occur, they will be important for understanding networks in a comprehensive and comparative framework, and may be informative in predicting community-scale responses to global climate change.

Chapter 3 Overview – Fire and grazing drive plant-floral visitor network structural properties

In Chapter 3, I examined how grassland disturbance regimes shape flowering plant and insect floral visitor communities and, in turn, the consequences for network structure of plant-floral visitor interactions. Using a landscape-level experiment that manipulates fire frequency and bison (*Bison bison*) grazing in tallgrass prairie, I sampled plant-floral visitor interactions and independently sampled flowering plant and floral visiting insect communities. Both fire and grazing were important drivers of plant and insect communities and their accompanying interaction structure.

A body of literature using an ecological network framework hypothesizes that mutualistic networks exhibit nonrandom interaction structure, which decreases risk of local extinctions and prevents extinction cascades (Memmot et al. 2004, Olesen et al. 2007, Bastolla et al. 2009, Bascompte 2010). In Chapter 3, I examined plant-floral visitor networks at 12 sites subjected to landscape-level treatments of bison grazing and fire frequency. Additionally, and distinct from other studies of ecological network structure, this analysis includes independently sampled inflorescences and floral-visiting insects to provide a more mechanistic link between disturbance regime and network structural properties. This approach allowed me to test for fire and grazing effects on interaction structure, while controlling for differences in local plant and floral visitor communities. Fire and grazing altered both plant and floral visitor communities and affected network structural properties. The presence of grazers increased flowering plant species richness, network floral visitor species richness, and decreased network nestedness. Fire treatment strongly affected flowering plant and floral visitor community composition and increases in fire frequency lead to more specialized and nested networks. By sampling both plant and floral visiting insect communities and their interaction networks across a habitat matrix, this chapter illustrates the importance of the state of grassland disturbance for the robustness of ecological interaction networks with respect to species loss, and provides suggestions for managing critical plant- and insect floral visitor communities.

Chapter 4 Overview– Grassland drivers of plant-grasshopper networks

In Chapter 4, I investigated the effects of climate and grassland disturbance regime from fire frequency and bison grazing on plant-grasshopper trophic network structural properties. I constructed and characterized plant-grasshopper networks across 13-19 years and sites subjected to varying fire frequency and bison grazing treatments. Both disturbance and climate shaped plant-grasshopper network structural properties. Specifically, the presence of grazers increased plant-grasshopper network complexity and increased predicted robustness of the grasshopper community to endure plant species loss. Drought conditions decreased plant diversity and, in turn, decreased predicted grasshopper robustness to plant loss.

Unraveling the web of species interactions in ecological communities allows one to make predictions about how local species extinctions affect community stability (Montoya et al. 2006). Based on ecological network analysis of food webs, antagonistic interactions between plants and their insect herbivores are predicted to increase the stability of insect herbivore communities when interactions are arranged in a modular structure (Krause et al. 2003, Thébault and Fontaine 2010), and when herbivores are more generalist in diet choices (López-Carretero et al. 2014). Grasshoppers (Acrididae) are major herbivores affecting plant diversity and production in grassland ecosystems (Tscharntke and Greiler 1995, Belovsky and Slade 2000, Anderson et al. 2001, Branson et al. 2006). I sequenced gut contents of a grasshopper community to identify grasshopper diet composition. I then combined the determined potential feeding interactions with long-term surveys of plant cover and grasshopper abundances to assemble more than a decade of predicted annual plant-grasshopper networks across grassland disturbance regimes. Plant-grasshopper network structural properties and the stability of the grasshopper community were strongly influenced by climate and disturbance regime. Predicted grasshopper robustness to plant species loss decreased with increasing mean annual temperature. Grazing increased plant-grasshopper network modularity, generality of grasshopper diet, and predicted grasshopper community robustness to loss of plant species. My results suggest that future loss of grazed grasslands and drought conditions, predicted by climate models for North American grasslands (Seager et al. 2007, Dobrowski et al. 2013, Kunkel et al. 2013), will negatively impact grasshopper communities, a key group of grassland herbivores.

Chapter 5 Overview– Plant diversity and plant-herbivore networks

In Chapter 5, I characterized plant-herbivore interaction structure across an experimental grassland containing replicate plots of three levels of plant diversity. Higher plant diversity decreased connectance of plant-herbivore interactions, caused increased average diet generalism of insect herbivores, and increased herbivore robustness to plant species loss. I sampled plant and herbivore communities across three years and observed divergence in community structure across time, with the largest differences in plant-herbivore network structural properties occurring in the last year of sampling.

Previous manipulations of grassland plant diversity demonstrated a positive correlation between plant and insect species richness (Crutsinger et al. 2006, Johnson et al. 2006, Haddad et al. 2009, Laws and Joern 2013). While likely equally important for predicting ecosystem stability, characterizations of changes in plant-herbivore interaction structure across plant diversity have rarely been conducted as identifying species-specific trophic interactions remains a challenge (Haddad et al. 2009, López-Carretero et al. 2014). This is an important need as ecological network analysis emphasizes the importance of species interactions. Previous work within this community network framework identified nonrandom arrangements of species interactions in natural communities, and linked network structure to ecosystem stability (Thébault and Fontaine 2010). I sampled plants and arthropods across four replicate plots for each of three plant diversity treatments for three years. Through an extensive literature review of plants and herbivores in my study system, I identified 509 previously documented trophic interactions and used them to construct plant-herbivore interaction networks for each plot and year. Plant diversity increased arthropod diversity and altered plant-herbivore interaction structure, resulting in an herbivore community with broader diet breath that was more robust to potential plant species loss. Additionally, plant-herbivore structure was less temporally variable with higher plant diversity, indicating the importance of biodiversity for maintaining community stability. Understanding the causes of plant-herbivore structure aids in identification of vulnerable ecosystems and increases predictability of terrestrial communities to species loss.

Chapter 2 - The structure of trophic and mutualistic networks across broad environmental gradients

Abstract

This chapter aims to understand how inherent ecological network structural properties of nestedness and modularity vary over large geographic scales with implications for community stability. Bipartite networks characterized in previous research from 68 locations globally were analyzed. Using a meta-analysis approach, we examine relationships between the structure of 22 trophic and 46 mutualistic bipartite networks in response to extensive gradients of temperature and precipitation. Network structural properties varied significantly across temperature gradients. Trophic networks showed decreasing modularity with increasing variation in temperature within years. Nestedness of mutualistic networks decreased with increasing temperature variability between years. Mean annual precipitation and variability of precipitation was not found to have significant influence on the structure of either trophic or mutualistic networks. By examining changes in ecological networks across large-scale abiotic gradients, this study identified temperature variability as a potential environmental mediator of community stability. Understanding these relationships contributes to our ability to predict responses of biodiversity to climate change at the community level.

Introduction

Understanding changes in community dynamics along major environmental gradients is a major goal of community ecology. Substantial ecologically relevant gradients abound, including abiotic ones such as precipitation, temperature or salinity gradients (Crain et al. 2008, Kaspari et al. 2000), changes in biotic environments resulting from variable primary productivity, habitat structure, and gradients associated with competition or predation risk (Ripley and Simovich 2009, Ricklefs 2004). Changing species diversity along productivity gradients (Tilman et al. 2012), the relationship between food web complexity and stability (Krause et al. 2003), variable abiotic conditions and the likelihood of trophic cascades (Laws and Joern 2013), or changes with niche metrics such as diet breadth or overall community stability in response to species diversity (Haddad et al. 2011, Pianka 1973, Pianka 1966a,b) are all examples of long-standing interest in this context. Despite much success in identifying single species responses and ecosystem-level responses to underlying gradients, a great need remains to understand how networks of coexisting species respond (Bascompte 2010), or how the observed network structure reflects species diversity. For example, ecological gradients may affect community dynamics through limiting species richness (Dyer et al. 2007); alternatively, environmental conditions may directly influence species interactions and thus community stability and species richness. Here, I assess changes in communities over gradients of precipitation and temperature using an ecological network framework.

In studies of ecological networks, ecologists focus on the role played by species linkages to assess the overall functional stability or persistence of a network (Bascompte 2010), or they predict likely changes in community persistence when components are removed (Pocock et al. 2012). The ecological network approach emphasizes species interactions and internal architecture of linkages in communities as important factors affecting species persistence across changing environmental conditions (Pearson and Dawson 2003). The approach benefits from methodological contributions across many disciplines, including physics and sociology (Bascompte 2009).

In an ecological network framework, communities are represented as adjacency matrices with axes composed of plants (*i*) and consumer (*j*) species with the goal of assessing how network structural characteristics such as nestedness and modularity co-vary with community traits and stability (Montoya et al. 2006). Theoretical studies have linked nestedness to the stability of mutualistic networks (Thébault and Fontaine 2010). In nested networks, specialist species interact primarily with generalist species, which tend to be the more persistent and stable members of the community. A nested structure may allow rare specialist species to persist because the limited numbers of species with which they interact are maintained by generalist species (Jordano 1987). Recent work has found nestedness to be less important for individual species persistence than the simpler metric of number of mutualistic partners (James et al. 2012). However, nestedness may stabilize mutualistic networks at the community level by increasing the number of mutualistic animal partners shared by plants and the number of plants shared by animals, therefore decreasing competition between plants and between animals (Bastolla et al. 2009).

Modularity has been linked theoretically to stability in trophic networks (Thébault and Fontaine 2010), although empirical evidence is limited (Krause et al. 2003). Modularity is hypothesized to increase ecological network stability by limiting the spread of a perturbation to the confines of the compartment of the perturbation's origin (Montoya et al. 2006). Larger mutualistic networks have also been shown to be significantly modular (Olesen et al. 2007), although the significance of modularity in mutualistic networks is understudied.

By comparing network types, we can identify differences in community dynamics due to mutualistic versus antagonistic interactions (Thébault and Fontaine 2010). Here, I evaluate and compare trophic (insect herbivore-plant) and mutualistic (pollinator-plant and seed-disperser plant) networks, most of which are insect-plant interaction networks. Because insects and plants comprise disproportionately large groups of global biodiversity, studies of their interactions are well-represented in the literature and make insect-plant interaction networks a suitable choice for comparative analysis.

Network structure is often highly correlated with species richness (Olesen et al. 2007, Fonseca et al. 2005, Jordano 1987). Prior studies predict that modularity increases with species richness (Olesen et al. 2007) whereas nestedness decreases with species richness (James et al. 2012, Fonseca et al. 2005). We include species richness in our models predicting network structure in order to account for its contribution while we consider the effects of other factors, and consider the model with species richness as the only predictor variable to be our null model.

Annual cumulative temperature is an indicator of growing season length, a limiting factor for many plant and animal communities. A known source of nestedness in plant-pollinator networks is the preferential association of incoming pollinators with the most highly linked plants in a network (Olesen et al. 2008). We hypothesize that there will be a positive relationship between annual cumulative temperature and nestedness of mutualistic networks as the result of a longer growing season, allowing for further development of such linkages and therefore increasing nestedness. Variability in growing season length should disrupt this assembly process, potentially resulting in more fragmentation and network modularity. In trophic networks, we hypothesize that herbivores entering the community do not preferentially eat plants with the most links but instead are limited by nutritional niche space (Behmer and Joern 2008, Guimerá et al. 2010), phylogeny affecting host plant use (Rezende et al. 2009), plant defensive compounds and micronutrients (Rosenthal and Berenbaum 1992, Becerra 2007, Joern et al 2012). As growing season length increases, so does the number of interacting and coevolving insects and plants.

Variability in growing season length should reduce the size and number of these modules and force species to become more generalist in their resource use to survive, increasing network nestedness. If modularity is associated with stability in trophic networks and nestedness with the stability of mutualistic networks as predicted theoretically (Thébault and Fontaine 2010), ecological communities existing in areas with longer and less variable growing seasons are predicted to be more stable than those in areas with shorter and more variable growing seasons.

Likewise, precipitation should have a positive relationship with total resource availability for insects including increases in plant biomass for herbivorous insects and potentially flowering plant diversity for pollinating insects. We predict nestedness of mutualistic networks and modularity of trophic networks will increase with mean annual precipitation and decrease with precipitation variability.

Because changes in network structural properties are putatively associated with community stability (Thébault and Fontaine 2010), understanding the influence of environmental conditions on network structure should provide insight into causes of stability and fragility in ecological communities as conditions change in either time or space. As such, knowing the relationships between ecological gradients and ecological network structural properties could help to predict persistence of species facing global climate change.

Methods

Datasets

Bipartite mutualistic and trophic networks were collected from published studies (Dyer et al. 2007, Rezende et al. 2007, Joern 1983, for full list of network sources please see Appendix A). Mutualistic networks included 25 plant-pollinator and 21 seed-disperser networks. Trophic networks included 22 plant-insect herbivore networks.

Environmental variables

The geographic location of each network was plotted using Google Earth, and all points were converted into a kmz document. This document was overlaid on NASA Earth Observatory (NEO) (EOS Project Science Office 2013) cumulative monthly data maps of precipitation and temperature for all months from 2001-2012. These years were selected for analyses because they are years for which NEO data were available for all months. Point values were extracted in ArcGIS 10.1 (ESRI 2012) for each map at each network location. We used these data to calculate 12-year averages, coefficient of variation (CV) among years, and CV within years for precipitation and temperature for each network site. Precipitation maps provided data only for locations between 35^{o} N and S latitude. Because of this constraint, networks from the source studies located outside of this range were not included in the analysis.

Network structural properties

We analyzed nestedness and modularity of all networks using standard metrics. Modularity is the tendency for organisms to interact in subgroups (called modules), and not interact with organisms outside of their module. Modularity calculations were made using the Newman and Girvin (2004) algorithm in the software BIPMOD (Thébault 2013). Nestedness is a measure of the degree to which specialist species' interactions are a subset of generalist species' interactions (Bascompte 2010). The NODF (nested metric based on overlap and decreasing fill) metric for nestedness was used in this study. NODF is preferred to alternate metrics based on deviations from a maximum nestedness value, which have been shown to inflate Type I Error (Almeida-

Neto et al. 2008). NODF was calculated using the software ANINHADO ver. 3.0.3 3 (Guimarães and Guimarães 2006).

Statistics

Relationships between environmental variables and their variability and network structural properities were analyzed following Akaike's Information Criterion (ΔAIC_c) (Burnham and Anderson 2002). Global models for the response variables of nestedness and modularity were analyzed using combinations of predictor variables including species richness, mean annual cumulative temperature, the coefficient of variation (CV) of temperature between years, the CV of temperature within years, mean annual cumulative precipitation, the coefficient of variation (CV) of precipitation between years, and the CV of precipitation within years. The global model and all reduced additive models from the global model were fitted using the *dredge* function in the MuMIn package (Barton 2012) in R ver. 3.0.2 (R Development Core Team 2014). The model with only species richness as a predictor variable was considered the null model; if this null model had a ΔAIC_c <2, other models were considered irrelevant. Otherwise, models with ΔAIC_c <2 were considered equally parsimonious. The relative importance values (RIV) for each predictor variable, computed as the sum of Aikaike weights (w_i) were also calculated. Because nestedness and species richness were lognormally distributed in previous studies (Dalsgaard et al. 2013, Fonseca et al. 2005, Bengtsson 1994), nestedness and species richness were $\log_{10}$ transformed for all analyses. Variability of environmental variables was measured as the coefficient of variation (CV) between years (CV of annual cumulative sums) and within years (CV of monthly sums).

Results

Nestedness of mutualistic networks

The likelihood of 127 competing models comprising the global models and all reduced forms of the global model were assessed using AIC_c analysis. The predictor variables in from the global model and their abbreviations are listed in Table 2-1. Only one model explaining variation in the nestedness of mutualistic networks out of the 127 competing models had a $\Delta AIC_c < 2$, indicating it is the best fitting model. This model included species richness and the CV of temperature between years as the only variables explaining variation in the nestedness of mutualistic networks (Table 2, Part A). The CV of temperature between years also had a high RIV indicating it is an important predictor of the nestedness of mutualistic networks (Table 3, Part A). Nestedness of mutualistic networks decreased with both species richness and the CV of temperature between years (Fig. 2-1).

Modularity of mutualistic networks

Four top models with $\Delta AIC_c < 2$ resulted from the AIC_c analysis of the modularity of mutualistic networks. These models included a model with mean annual cumulative temperature, a model including the coefficient of variation (CV) of temperature between years and a model including the CV of temperature within years. However, the null model (model including only species richness as a predictor of modularity) was also included in the top models (Table 2-2, Part B); therefore, there is no strong evidence for a correlative relationship between environmental variables and the modularity of mutualistic networks. Likewise, the RIVs of environmental variables as predictors of the modularity of mutualistic networks are uniformly low (Table 2-3, Part B).

Nestedness of trophic networks

There were seven top models with ΔAIC_c <2 predicting nestedness of trophic networks. Mean annual cumulative temperature, the coefficient of variation (CV) of temperature between years, the CV of temperature within years, the coefficient of variation (CV) of precipitation between years, and the CV of precipitation within years were all included in the top models. However, the null model including only species richness as a predictor of the nestedness of trophic networks was also included in the top models (Table 2-2, Part C), so the additional models are considered ecologically irrelevant. The RIVs of environmental variables were also low, indicating a lack of evidence for the influence of environmental variables on the nestedness of trophic networks (Table 2-3, Part C).

Modularity of trophic networks

Three top models with ΔAIC_c <2 resulted from the AIC_c analysis of the modularity of trophic networks. Besides species richness, the CV of temperature within years explained a significant portion of the variation in the modularity of trophic networks and was included in all three top models (Table 2-2, Part D). The CV of precipitation within years and cumulative mean annual temperature were also included as predictor variables in plausible models but the RIV of the CV of temperature within years is more than twice as high all other environmental variables (Table 2-3, Part D). Modularity of trophic networks increased with species richness and decreased with the CV of temperature within years (Fig. 2-2).

Discussion

Ecological networks have inherent structure (Bascompte 2010). Although many possible drivers of ecological network structure have been proposed, sources of variation in network structure remain elusive (Thébault and Fontaine 2010). Network nestedness has been hypothesized to arise from network size (Bastolla et al. 2009, James et al. 2012), interaction strength (Okuyama and Holland 2008, Suweis et al. 2013), interaction switches (Zhang et al. 2011), extinction events (Thébault and Fontaine 2010), and phylogenic relatedness (Rezende et al. 2007, Rezende et al. 2009). Modularity has been hypothesized to be linked to network size (Olesen et al. 2007), habitat structure (Pimm and Lawton 1980), niche space (Guimerá et al. 2010), trait matching (Joppa and Williams 2013), phylogeny (Rezende et al. 2007), and rate of temperature change (Dalsgaard et al. 2013). In sum, much theoretical modeling of ecological networks predicts that network structure arises from combined contributions from multiple sources. Here, we assess how environmental conditions may influence network structure directly, or they may work in tandem with other sources of network structure. Moreover, predicted relationships between network structure and species richness often do not match available empirical data (James et al. 2012), suggesting that further comparative synthesis of current models and empirical testing of hypothesized drivers of network structure is needed. This study investigates the hypothesis that broad-scale environmental conditions are drivers of network structure and explain much variation in community network structure observed at a geographic scale.

Network structure and environmental variables

We document an inherent difference of network structure between trophic and mutualistic networks in response to temperature variation over broad geographic gradients. In mutualistic networks, increases in temperature variation between years corresponded to decreases in nestedness. In trophic networks, as temperature variation within years increased, modularity decreased. The effect of temperature variation on network structure was strong even when the effect of species richness was included in the models. These results are consistent with our hypothesis that temperature variability should decrease nestedness in mutualistic networks and modularity in trophic networks. Neither mutualistic nor trophic network structural properties were significantly correlated with precipitation variables. This suggests that precipitation and variability of precipitation is not a primary driver of network structure, although it may influence network structure through other effects on species richness such as net primary productivity.

To our knowledge, this is the first study to test the potential of broad-scale temperature and precipitation gradients to predict both trophic and mutualistic network structure on a global scale. This extends greatly projections of previous case studies that found correlations between structure and environmental properties. Modularity decreased with latitude and contrary to our results, precipitation was strongly correlated with nestedness and modularity in a comprehensive analysis of 54 mutualistic networks (Trøjelsgaard and Olesen 2013). In a comparative study of stream food webs, Thompson and Townsend (2005) linked network connectance to fine particulate matter. Soil fertility across a forest-brush gradient in southern Brazil accompanied network connectance through controlling species richness (Fonseca et al. 2005). Phenophase length was correlated with number of links per species in a study of temporal changes in a Greenland plant-pollinator network (Olesen et al. 2008). Such examples suggest that large-scale environmental factors can influence network assembly.

Our results also extend inferences of a small number of large-scale studies that examined potential effects of environmental drivers on network structure. Net primary productivity explained 17% of the variance in 14 multi-trophic food webs (Vermaat et al. 2009). In plant-pollinator networks, the number of interactions per plant species decreased on islands compared to mainland, and connectance of residuals increased from highland to lowland (Olesen and Jordano 2002). An analysis of the effects of global climate change on plant-pollinator networks showed reduced modularity in pollination networks when associated with high rate of climate change in the Quaternary (last 2.6 million year) (Dalsgaard et al. 2013). In light of these studies, there is definitely reason to expect changes in network structure along macro-scale environmental gradients.

Network structure and species richness

While species richness can be significantly correlated with network modularity (Jordano 1987, Olesen and Jordano 2002) and nestedness (James et al. 2012, Dalsgaard et al. 2013), the causal significance of species richness to network structure remains a longstanding, unresolved question. Some studies suggest mutualistic networks increase in nestedness as they increase in size (Okuyama and Holland 2008, Suweis et al. 2013). In agreement with our results, recent meta-analyses of mutualistic networks found nestedness decreased significantly with increased species richness (James et al. 2012, Dalsgaard et al. 2013). Modularity has been shown to increase with species richness (Olesen et al. 2007, Dalsgaard et al. 2013), a result duplicated in our analysis.

One hypothesis for explaining the relationship between network structure and species richness is that the size of the network constrains network structure (Fontaine 2013). For example, the nature of a smaller network requires it to have high connectivity for all network members to be included (Fonseca et al. 2005). Likewise, modularity is not expected in small networks because the limited number of interactions is not sufficient to allow partitioning into modules. Another hypothesis suggests that increases in network size are driven by network structure (Okuyama and Holland 2008, Bascompte 2009, Suweis et al. 2013). Because the relationship between species richness and network structure did not differ regardless of network type, our results do not refute either of these hypotheses. Therefore, the effect of species richness must be accounted for when testing for the influence of other variables on network structure (Bengtsson 1994, Fonseca et al. 2005).

Potential sources of error

As for most community-scale studies, results of this study may be biased by incomplete data from missing species. Network structure reflects species presence and the organization of species interactions. If species or interactions are not included in the network, the calculated network structure is incomplete, potentially altering conclusions. Because most trophic networks used in this study are based on studies with multiple years of sampling, we feel they are reliable. The mutualistic networks are more variable in degree of sampling completeness, but represent the best available at the geographic scale of this study.

While ecological networks go beyond species richness in describing community structure by including species interactions, bipartite networks such as those used here clearly do not fully capture the full complexity of ecological communities. Incorporating weighted matrices, where interaction strength is measured, is a logical next step in ecological network studies. However, the sampling effort necessary to accurately identify all species in a community, the species with whom they interact, and the weight of interactions remains a great challenge.

Importance and future directions

Our study seeks to understand the longstanding question of why and how environmental gradients influence species richness and community dynamics. Documenting only changes in species richness is not sufficient for determining changes in ecosystem functioning and services. For example, in one well-sampled and taxonomically well-resolved study of bees, important insect pollinators, the community decreased in diversity by 50% in the last 120 years. However, the number of interactions between the bee species and the angiosperm species in the system decreased at a greater rate of 76% over the same time frame (Burkle et al. 2013). Interaction number in plant-pollinator communities is more important than number of pollinator species for the desired ecosystem service of pollination.

May (1972) noted that stability is not an inherent property of complexity in random communities, although modularity was proposed as the missing structure that stabilizes such food webs (Lawlor 1978). Ecological modelling has since provided much additional support for the hypothesis that non-random network structural properties such as modularity and nestedness increase the stability of ecological networks (Okuyama and Holland 2008, Thébault and Fontaine 2010). Ecological models have been less succinct in predicting the causes of network structure, as different models demonstrated that multiple factors may be drivers of the same network structure. We show that network type and temperature variables can influence network structure over broad environmental gradients, to then be refined by local conditions and interactions.

Future research must assess whether environmental conditions such as those we evaluated actually drive network structure or are correlated for other reasons (i.e., correlation does not always translate into causation). If environmental properties drive network structure, understanding the timescale over which change in environment effects change in network structure and the robustness of networks to changes becomes critical. Due to the difficulty of experimental tests of changes in network structure, especially under field conditions, empirical evidence for direct causes of observed structure along large environmental gradients remains elusive. However, understanding network structure is critical as it offers potential insight into community resilience and stability, leading to better predictions of the impacts of changing environmental conditions at the global level on ecological communities and ecosystem function in this period of unprecedented change.

Tables

Table 2-1. Variables included in global model for analyses of relationships between network structural properties (nestedness and modularity) and environmental variables. Species richness was included to account for network structure variation due to network size.

Abbreviation	Variable
spp_richness	Total number of species in the network (plants + animals)
precip	12 year average mean annual precipitation (mm)
CVprecipBTWyrs	Coefficient of variation of mean annual precipitation between years
CVprecipW/INyrs	Coefficient of variation of mean annual precipitation within years
temp	12 year average mean cumulative annual temperature (°C)
CVtempBTWyrs	Coefficient of variation of mean cumulative temperature between years
CVtempW/INyrs	Coefficient of variation of mean cumulative temperature within years

Table 2-2. AIC$_c$ statistics for models for network nestedness and modularity for mutualistic and trophic networks. AIC_c= AIC corrected for small sample size, *LL*= log likelihood, *df*= degrees of freedom, R^2= adjusted regression coefficient, *P*= model p-value, ΔAIC_c= difference between the top model and given model AIC$_c$, w_i= model weight. Only models with $\Delta AIC_c < 2$ are shown for each network structure/network type comparison. If the model with only species richness was included as a model with $\Delta AIC_c < 2$, the accompanying models were not considered statistically meaningful.

Model variables	AIC$_c$	LL	df	R^2	P	ΔAIC$_c$	w_i
A. Nestedness of Mutualistic Networks							
spp_richness, CVtempBTWyrs	8.02	0.5	4	0.44	2E-06	0	0.33
B. Modularity of Mutualistic Networks							
spp_richness, CVtempBTWyrs	-66.37	37.7	4	0.25	7E-04	0	0.14
spp_richness	-65.76	36.2	3	0.22	6E-04	0.62	0.10
spp_richness, temp	-65.52	37.2	4	0.24	0.001	0.85	0.09
spp_richness, CVtempW/INyrs	-65.32	37.1	4	0.24	0.001	1.06	0.08
C. Nestedness of Trophic Networks							
spp_richness, CVtempBTWyrs	15.07	-2.4	4	0.73	1E-06	0	0.13
spp_richness, CVtempW/INyrs	15.32	-2.5	4	0.73	2E-06	0.25	0.11
spp_richness	16.04	-4.4	3	0.70	9E-07	0.97	0.08
spp_richness, temp	16.08	-2.9	4	0.72	2E-06	1.01	0.08
spp_richness, CVprecipW/INyrs	16.29	-3.0	4	0.72	2E-06	1.22	0.07
spp_richness, CVprecipBTWyrs, CVtempBTWyrs	16.69	-1.5	5	0.74	4E-06	1.62	0.06
spp_richness, CVprecipBTWyrs, CVprecipW/INyrs	16.88	-1.6	5	0.74	5E-06	1.81	0.05
D. Modularity of Trophic Networks							
spp_richness, CVtempW/INyrs	-52.53	31.4	4	0.89	3E-10	0	0.30
spp_richness, CVprecipW/INyrs, CVtempW/INyrs	-50.83	32.3	5	0.89	2E-09	1.70	0.13
spp_richness, CVprecipW/INyrs, temp, CVtempW/INyrs	-50.55	34.1	6	0.90	4E-09	1.98	0.11

Table 2-3. Relative Importance Values of predictor variables for all models.

spp_richness	precip	CVprecip BTWyrs	CVprecip W/INyrs	temp	CVtemp BTWyrs	CVtemp W/INyrs
A. Nestedness of Mutualistic Networks						
1	0.23	0.22	0.24	0.28	0.74	0.31
B. Modularity of Mutualistic Networks						
0.97	0.31	0.29	0.23	0.33	0.43	0.3
C. Nestedness of Trophic Networks						
0.99	0.2	0.34	0.37	0.25	0.34	0.32
D. Modularity of Trophic Networks						
1	0.16	0.17	0.36	0.34	0.29	0.76

Figures

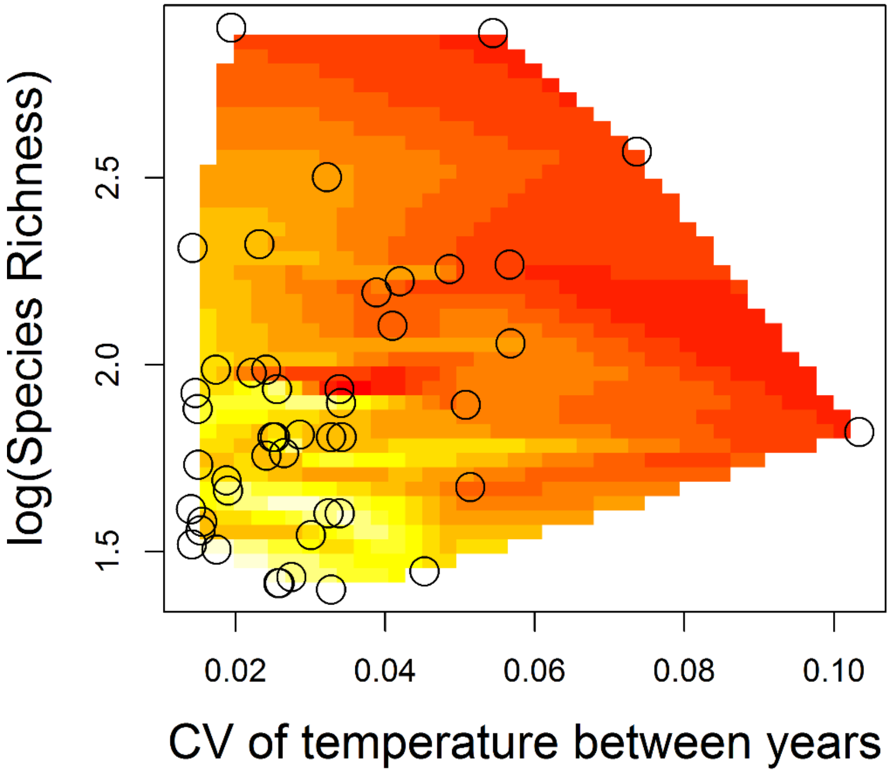

Figure 2-1. Contour plot for the relationships between the coefficient of variation of temperature between years, species richness, and nestedness of mutualistic networks. Color is used to represent nestedness. Lighter colors (yellow) indicate high nestedness values while darker colors (red) indicate low nestedness values.

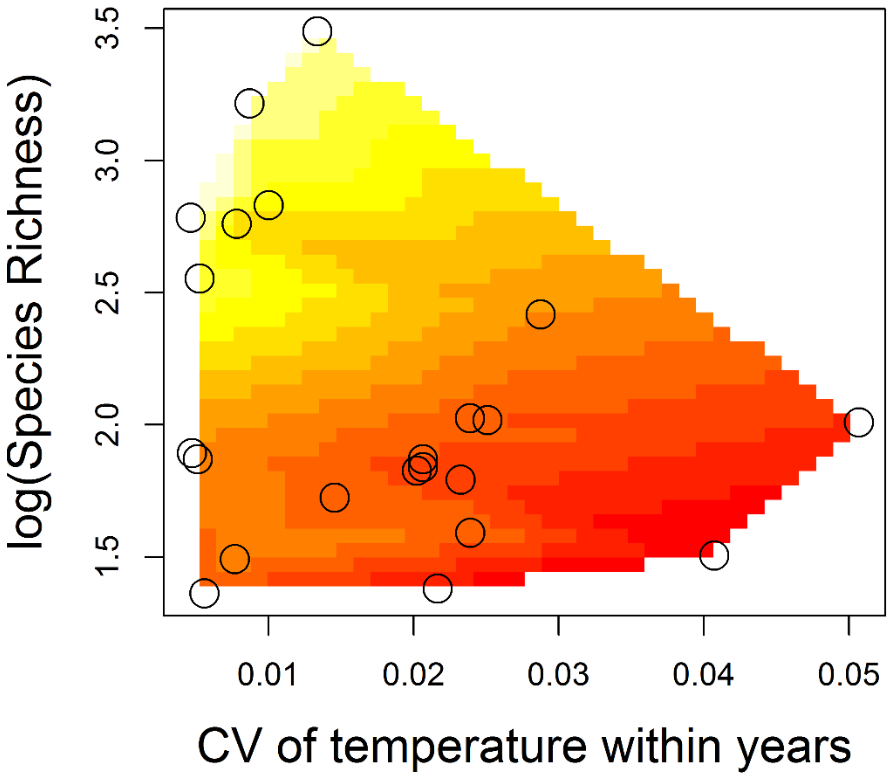

Figure 2-2. Contour plot for the relationships between the coefficient of variation of temperature within years, species richness and modularity of trophic networks. Color is used to depict modularity. Lighter colors (yellow) indicate high modularity and darker colors (red) indicate low modularity values.

Chapter 3 - Fire and grazing modulate the structure and resistance of networks of grassland plants and floral visitors

Abstract

Significant loss of pollinator taxa and their interactions with flowering plants has resulted in growing reductions to pollination services globally. Ecological network analysis is a useful tool for evaluating factors that alter the interaction structure and resistance of systems to species loss, but is rarely applied across multiple empirical networks sampled within the same study. The non-random arrangement of species interactions within a community, or “network structure” such as nested or modular organization, is predicted to prevent extinction cascades in ecological networks. How ecological gradients such as disturbance regimes shape network structural properties remains poorly understood despite significant efforts to quantify interaction structure in natural systems. Here, we examine changes in the structure of plant-floral visitor networks in tallgrass prairie using a decadal and landscape-scale experiment that manipulates interacting fire frequency and ungulate grazing, resulting in different grassland states. Plant-floral visitor network structural properties shifted with grassland fire and grazing regimes, demonstrating that grassland state has important implications for the resistance of flowering plant and floral visitor communities to species loss. The presence of grazers increased flowering plant species richness and network floral visitor species richness, and decreased network nestedness. Fire treatment strongly affected flowering plant and floral visitor community composition and increases in fire frequency led to more specialized and nested networks.

Introduction

Pollinators are currently facing global declines (Potts el at. 2010, Swengel et al. 2011, Burkle et al. 2013), seriously affecting delivery of a critical service for the approximately 87.5% of all flowering plants that rely on animal pollinators (Ollerton et al. 2011). The response of ecological communities to species loss is dependent on their taxonomic composition and the type, strength and topological structure of species interactions (Bascompte 2010). In plant-insect communities, loss of interaction structure and function may decline more steeply than would be predicted by insect species loss alone (Burkle et al. 2013). Although the insects included in our study are limited to the four major pollinator orders, Coleoptera, Diptera, Hymenoptera and Lepidoptera, we use the term "floral visitor" instead of "pollinator" as we did not robustly discriminate between true pollinators and insects visiting flowers for other purposes, such as nectar thieves.

An ecological network approach provides an ideal framework for examining shifts in the interactions between plants and their associates as it emphasizes the importance of species interactions, describes inherent community-level structural properties among interacting species groups, and examines how nonrandom network structural properties can increase community resistance to extinctions (Bascompte 2010, Thébault and Fontaine 2010, Pocock et al. 2012). Environmental conditions shape and constrain network structure, but to date have been considered only in a small set of studies (Rezende et al. 2009, Dalsgaard et al. 2013, Welti and Joern 2015).

Understanding grassland community dynamics, including the dynamics of plant-insect interactions, is important from a conservation perspective as grasslands are exceedingly impacted by humans, primarily through loss due to conversion to agriculture (Hoekstra et al. 2005). In particular, tallgrass prairie in North America only covers ~4% of its original extent (Samson and

Knopf 1994). Plant and floral visitor relationships are of particular importance in grassland systems and the majority of grassland plant species depend on insect pollination (Jakobsson and Ågren 2014, Fantinato et al. 2016). Naturally-occurring ecological disturbances (e.g., fire-grazing interactions) shape grasslands and alter habitat conditions, thus playing a central role in structuring communities (Connell 1978, Sousa 1984, Picket and White 1985, Huston 1994, Archibald et al. 2005). Tallgrass prairie is ideal for studying disturbance effects as it is characterized and maintained by frequent disturbance from the interaction between ungulate grazing and fire (Vinton et al. 1993, Fuhlendorf and Engle 2004, Collins and Calabrese 2012). Community response to habitat state resulting from naturally-occurring and varying disturbance regimes has only occasionally been examined in the context of network structure, an approach which offers significant insight into community resistance to species loss (Yoshihara et al. 2008, Vanbergen et al. 2014, Lázaro et al. 2016).

Bison are primarily grass feeders and their grazing activity increases the abundance and diversity of forbs (Steuter et al. 1995, Collins and Calabrese 2012). Unlike grasses, forbs are primarily insect-pollinated and comprise the majority of plants with floral visitors in tallgrass prairie. Like bison grazing, fire is a major regulator of grassland biomass (Bond and Keeley 2005), but unlike grazing, frequent fire by itself decreases plant diversity and leads to grass-dominated systems (Collins and Smith 2006, Spasojevic et al. 2010, Collins and Calabrese 2012). As a central theme of this study, fire and grazing drive plant community composition, which in turn constrain the range of floral visitor species dependent on taxon-specific flower resources (Ghazoul 2006, Ebeling et al. 2008, Moranz et al. 2012).

Many definitions and measures have been proposed to characterize the stability and persistence of ecological communities (Connell and Sousa 1983, Ives and Carpenter 2007). In the face of current global pollinator declines, we aimed to evaluate the resistance of plant-pollinator communities to species loss. Resistance is the ability of a system to reduce the likelihood of perturbation or to be minimally effected by perturbation. Of the numerous network metrics described in the literature (Bersier et al. 2002, Blüthgen et al. 2008), we selected the following to characterize our plant-floral visitor networks as they are commonly reported in studies of resistance in mutualistic ecological networks: nestedness (Bascompte et al. 2003, Fortuna and Bascompte 2006, Burgos et al. 2007), modularity (Olesen et al. 2007, Thébault and Fontaine 2010), network specialization (H_2') (Blüthgen et al. 2008, Schleuning et al. 2012), and robustness (Memmott et al. 2004).

Previous studies demonstrated that size of an interaction matrix (flowering plant and pollinator species richness) can affect network structural properties (Dalsgaard et al. 2013, Bascompte et al. 2003). However, it is not known to what extent this simply reflects mathematical constraints on matrix size *per se*, or represents an inherent difference in the topology of interactions as the availability of the resource of flowering plants for floral visitors varies. For example, if a smaller network has greater connectivity, is this due to limited sampling of rare specialists or does it reflect the necessity of generalization by pollinators when resources are scarce? Here, we surveyed blooming inflorescences and collected insects in addition to our plant-floral visitor interaction dataset. By obtaining independent estimates of the abundance and diversity of interaction partners available for plants and floral visitors, we were able to test for effects of locally available flowering plant and floral visitor richness, abundance and community composition on network structural properties.

Here, we investigated effects of fire frequency and grazing by bison (*Bison bison*) on the network structural properties of interacting floral visitor and flowering plant communities in tallgrass prairie. We hypothesized that because grazed grasslands have higher forb species diversity, resistance of plant-floral visitor networks will increase with the presence of grazers as measured by network structural properties (H_2', modularity, nestedness, and robustness). We also predicted that the fire-grazing interaction, specifically increased fire frequency in the absence of grazers, would decrease network resistance through reductions in flowering plant diversity. Nestedness decreases with the species richness of the network (Dalsgaard et al. 2013, Welti and Joern 2015, James et al. 2012). Therefore, we predicted nestedness would be lower in watersheds with no bison grazing and increased fire frequency due to decreases in flowering plant diversity. To determine how grassland state alters network structure, we documented flowering plant and insect floral visitor communities and plant-floral visitor network structural properties across a landscape-level experiment subjected to a crossed design of two grazing treatments (grazed by bison and not grazed) and three prescribed fire frequency treatments (1, 4 and 20 year frequencies).

Methods

Site description

Floral visiting insects and flowering plant communities were sampled in summer 2014 at Konza Prairie Biological Station (KPBS), a tallgrass prairie research station in northeastern Kansas, USA (39°05’ N, 96°33’ W). KPBS consists of 3,487 ha of primarily unplowed native tallgrass prairie. Beginning in 1972, KBPS was divided into replicated watershed-level fire return interval treatments (1, 2, 4, and 20 year fire frequencies). Bison were reintroduced to KPBS in 1987 and the current population of ~300 adult individuals is allowed free access to a 1,012 ha area containing replicate watersheds subjected to 1, 2, 4, and 20 year fire interval treatments (Collins and Calabrese 2012). Sampling for this study was conducted on 12 KPBS watersheds consisting of 6 watersheds grazed by bison and 6 non-grazed watersheds crossed in a balanced design with 3 fire frequency treatments (1, 4, and 20 year fire frequencies) (Fig. 3-1).

Network sampling

Sampling was conducted twice during the flowering season of 2014, in early June and early July, following a phenological shift in the availability of both flower and pollinator communities. Within each watershed, one upland and one lowland location were selected haphazardly as topography affects soil types that may affect plant community composition. At each location, pollinating insects were collected directly on flowering plants using sweep nets, by hand, and by observation for large and easily identified insects for 1 person-hour within a 1ha area by random walk. Networks were assembled from a combination of the two sampling locations (upland and lowland) within each watershed. These watershed-level networks are snapshots of networks at the larger, landscape level. A preliminary analysis found no evidence for an effect of topography on network structural properties.

Insects were collected only if they belonged to one of four orders: Coleoptera, Diptera, Hymenoptera and Lepidoptera. Insects were separated by the species of flowering plant on which they were collected, later sorted to morphospecies, and subsequently identified to the lowest possible taxonomic level by taxonomists. Analyses of insect communities using morphospecies provide reliable estimates of species richness (Oliver and Beattie 1996). Plant species by insect morphospecies matrices were assembled from which network metrics could be calculated.

Flower sampling

A count of blooming inflorescences was conducted at each location. Counts of individual inflorescences for each flowering plant species were recorded for each of ten, 5m x 5m subplots within a 5m x 50m area plot. Flower census data were used to calculate the watershed-level species richness of blooming flowering plants and inflorescence abundance. Both flowering plant species richness and inflorescence abundance exhibited a skewed distribution and were log transformed before all subsequent analyses. A two dimensional Non-Metric Multidimensional Scaling (NMDS) ordination of the flower community was conducted to summarize variation in flower species composition among communities. Scores of the two NMDS dimensions were used as indices of flower community composition.

Floral visitor sampling

For separate estimates of insect floral visitor availability, insects were collected using transects consisting of 12, 70cm tall, elevated 9 oz. pan traps (two each of six inflorescent colors: purple, blue, yellow, orange, pink, and white) each spaced 5 meters apart, and left out for three days at each of the 24 sites during each sampling period (Roulston et al. 2007, Grundel et al. 2011). Consistent with sampling for interaction networks, only insects from the four major pollinator orders of Coleoptera, Diptera, Hymenoptera, and Lepidoptera were included in sorting and analyses. Collected insects were washed, stored in 70% alcohol, sorted to morphospecies, and identified to lowest possible taxonomic level. Pan trapped insect data were used to calculate watershed-level floral visiting insect species richness and abundance. Insect species richness and abundance were log transformed for all subsequent analyses. Indices of insect community composition were calculated as the site scores of a two dimensional NMDS ordination of the log transformed insect community matrix.

Network metric description

Nestedness measures the degree to which specialist species' interactions are a subset of, or are "nested within", generalist species' interactions. Nestedness is predicted to increase system resistance by decreasing the probability of local extinction of specialists, which are considered the most vulnerable network members (Fortuna and Bascompte 2006, Joppa et al. 2010). In a highly nested network, specialist plants and insects interact primarily with generalists, thus providing specialists with more stable resources while increasing local persistence of specialists (Joppa et al. 2010). Modularity refers to the clustering of interactions between sub-groups of plants and insects. Modularity limits species loss by preventing the spread of perturbations outside of modules (Thébault and Fontaine 2010, Dupont and Olesen 2012). H_2' measures the degree of specialization between plants and floral visitors at the network level (Blüthgen et al. 2006; 2007; 2008).

An additional measure of network resistance, termed "robustness", comes from a model developed by Memmott et al. (2004). Memmott et al. (2004)'s model randomly removes species from the community and then predicts secondary extinctions based on the assumption that every community member needs one interaction partner to remain in the community. Robustness is calculated as the average area under the curve describing the relationship between the independent variable "primary extinctions" (species randomly removed by the model) and the dependent variable "secondary extinctions" (species predicted to be lost from the community because they have no remaining interaction partners).

Network metric calculation

H_2', modularity, nestedness and robustness were calculated from quantitative plant-floral visitor interaction matrices for each watershed and sampling period using the networklevel, computeModules and second.extinct functions in the Bipartite package (Dormann et al. 2008, Dormann et al. 2009) in R ver. 3.2.4 (R Development Core Team 2016). Robustness analyses were conducted for system level robustness (robustness of plants and floral visitors simultaneously) and were run 1000 times and averaged to mitigate bias caused by the order of species removal.

RDA of disturbance regimes and plant and insect communities

While not part of our primary objectives, understanding how grassland disturbance regimes affect plant and insect communities, aids in our interpretation of how both grassland disturbance regime and community factors affect network structural properties. Redundancy analysis (RDA) is a standard ordination technique in community ecology for relating community species composition and environmental data (Legendre and Anderson 1999). We performed two partial RDAs conditional on date (early or late sampling period) to examine effects of grassland disturbance regime management treatments on (1) flowering plant and (2) floral visitor communities (arrow 1 in Fig. 3-2). Forward stepwise selection was used to determine which grassland disturbances significantly affected plant and insect communities. RDA analyses was conducted using the vegan package (Oksanen et al. 2015) in R ver. 3.2.4 (R Development Core Team 2016).

AIC_c analysis of network structural property drivers

To determine which community (arrow 2 in Fig. 3-2) and grassland disturbance regime (arrow 3 in Fig. 3-2) properties were related to plant-floral visitor network structural properties, we relied on Akaike's Information Criterion (AIC_c) (Burnham and Anderson 2002) for variable selection. Community and disturbance regime variables are listed in Fig. 3-2. Multiple measures of fire treatment (fire return interval, days since fire, and number of fires) are used as these may have varying importance for plant and insect communities. A global community model included all variables listed in the community box in Fig. 3-2 as predictors for network structure metrics. A global grassland disturbance model included all variables listed in the disturbance box in Fig. 3-2 as predictor variables and an interaction between bison grazing and fire return interval. AIC_c calculations and model comparisons were conducted using the MuMIn package (Barton 2012) in R ver. 3.2.4 (R Development Core Team 2016). Models with a $\Delta AIC_c<2$ were considered competitive and equally parsimonious (Burnham and Anderson 2002). If the null model (model with no predictor variables) was included in the models with a $\Delta AIC_c<2$, the models were not considered a good fit (Burnham and Anderson 2002) and no models were reported.

Global models and all reduced additive models were compared to determine Relative Importance Values (RIVs), a summed and standardized indicator of predictor variable rank across all possible models. RIVs are calculated as the sum of Akaike weights (w_i), of community and disturbance regime predictor variables of each of the four calculated network metrics (Burnham and Anderson 2002). For models where predictor variables had RIV >0.45, we preformed simple linear regressions and Welch's t-test (not assuming equal variance) and provide graphs and results of these tests in Appendix B.

Results

Collection of insects on flowering plants included 7,070 individuals belonging to 369 morphospecies. Insects were collected on 44 flowering plant species. 29,324 individual inflorescences of flowering plant species were counted in flower resource availability surveys. Pan trapping collected 5,081 individual insects belonging to 416 morphospecies. NMDS ordinations of plant and insect communities had stress values of <0.2 (0.14 for the plant community and 0.18 for the insect community) indicating the two dimensions were able to capture community composition variation.

Disturbance regimes and plant communities

Following stepwise selection, the top Redundancy Analysis (RDA) model relating flowering plant communities to grassland disturbance regimes included the grassland disturbance regime variables of: days since last fire, fire return interval, grazer treatment, fire-grazing interaction, and the conditional variable of sampling date. The RDA revealed a significant relationship between disturbance regime and the resulting flowering plant community ($F_{4,18}$=4.91, *P*<0.001, F distribution based on 999 permutations; Fig. 3-3a). In particular, flowering plant communities were tightly linked to bison grazing and days since the last fire (Table 3-1). Grassland disturbance regime variables demonstrated no variance inflation (all variance inflation <4). Variation in community composition was largely captured in the first RDA axis ($F_{1,18}$=14.66, *P*>0.001); the second axis was nonsignificant ($F_{1,18}$=3.48, *P*=0.085).

Disturbance regimes and insect communities

The top model examining effects of grassland disturbance on insect communities included the grassland disturbance regime variables of fire return interval, number of fires (since 1981), grazing treatment, fire-grazing interaction, and was conditional on date of sampling. These measures of grassland disturbance regimes significantly affected insect communities ($F_{4,18}$=5.62, *P*<0.001, F distribution based on 999 permutations; Fig. 3-3b). Insect communities were tightly linked to the interaction between bison grazing and fire return interval (Table 3-2). Grassland disturbance regime variables demonstrated no variance inflation (all variance inflation <4). Variation in community composition was captured in the first RDA axis ($F_{1,18}$=14.0, *P*=0.002) and the second axis ($F_{1,18}$=6.96, *P*=0.008); the third axis was nonsignificant ($F_{1,18}$=1.44, *P*=0.47).

Plant and insect communities and network structural properties

Characteristics of plant and insect communities were good predictors of H_2' (specialization) and modularity, but not of nestedness and robustness of plant-floral visitor networks (Table 3-3). RIVs for community predictors (listed under "Community" in Fig. 3-2) and network structural properties (listed under "Network Structure" in Fig. 3-2) and relationships which had RIVs>0.45 are provided in Appendix B. AIC_c analysis revealed important relationships between plant and insect community composition both for H_2' and modularity of plant-floral visitor networks (Table 3-3; Appendix B, Fig. B1). Top models for H_2' also included negative effects of flowering plant species richness, inflorescence abundance and a positive effect of insect abundance (Table 3-3). Top models for modularity included a negative effect of inflorescence abundance (Table 3-3; Appendix B, Fig. B3). The null model (model with no predictor variables) was included in the models with a $\Delta AIC_c<2$ for nestedness and robustness. Therefore, models of community predictors of nestedness and robustness were not considered a good fit.

Disturbance regimes and network structural properties

Grassland disturbance characteristics and sampling date were good predictors of H_2', modularity and nestedness, but not robustness of plant-floral visitor networks (Table 3-3). RIVs for these grassland disturbance regime predictors (listed under "Disturbance" in Fig. 3-2) and network structural properties (listed under "Network Structure" in Fig. 3-2) and relationships which had RIVs>0.45 are provided in Appendix B. H_2' was strongly affected by fire regime with higher H2' in more frequently and recently burned watersheds (Table 3-4; Appendix B, Fig. B2 & Fig. B4e). Sampling date was also included in top models predicting H_2', with higher H_2' in the June (than July) sampling date (Table 3-4). Modularity was affected only by sampling date, with higher network modularity in July sampling (Table 3-4; Appendix B, Fig. B2 & Fig. B4f). Top models for nestedness included an increase in nestedness at one and four year fire intervals compared to 20 year intervals (Table 3-4; Appendix B, Fig. B4b), a decrease in nestedness with days since last fire (Table 3-4), higher nestedness in watersheds not grazed by bison (Table 3-4; Appendix B, Fig. B4a), and higher nestedness in June sampling compared to July (Table 3-4; Appendix B, Fig. B4c). The null model (model with no predictor variables) was included in the models with a $\Delta AIC_c<2$ for robustness (Table 3-4D) and therefore models of grassland disturbance regime predictors of robustness were not considered a good fit.

Discussion

Understanding how disturbance regimes shape networks of flowering plants and their insect visitors and predicting network resistance to species loss is increasingly important as pollination systems are currently facing significant global declines in both taxa (Ollerton et al. 2011) and number of interactions with plants (Burkle et al. 2013). While understanding changes in network structural properties in response to varying ecological drivers provides opportunities to predict the effects of alternate environmental states on system resistance to species loss, these changes are rarely evaluated due to the difficulty of obtaining sufficient data for network construction at multiple sites (Devoto et al. 2012).

Bison grazing and fire treatments altered plant and floral visitor communities in this system, and subsequently shaped plant-floral visitor network structural properties. Additionally, although the interaction between fire and grazing significantly affected the floral visiting insect community, this interaction had a low RIV for all network structural properties, and instead, effects of disturbances on network structural properties were additive. Bison grazing strongly affected the plant community. Most likely this effect is through preferential grazing on non-insect-pollinated grass species (Vinton et al. 1993). Watersheds grazed by bison showed increased species richness of flowering plant species, and grazing decreased nestedness of plant-floral visitor networks. Floral visiting insect species richness and abundance from pan trapping were unexpectantly higher in watersheds not grazed by bison. This could be due to a known bias of pan trapping, in which trapping effectiveness decreases with increases in flower resource availability (Baum and Wallen 2011). Within networks from interaction sampling, watersheds with bison grazing had higher species richness of floral visitors (averaging 48.75 species +/-3.53SE) than not grazed watersheds (averaging 33.92 species +/-2.78SE). The higher flower species richness and higher network floral visitor species richness in grazed watersheds are probable causes of the decrease of nestedness in grazed watersheds, mirroring previous reports of decreasing nestedness with network species richness (Dalsgaard et al. 2013, Welti and Joern

2015). The results are likely caused by sensitivity of nestedness to sampling intensity (Blüthgen et al. 2008, James et al. 2012, Suweis et al. 2013).

Similar to grazing, fire is a natural driver of grassland ecosystems and increased fire frequency reduced the species richness of forbs, the primary insect-pollinated flowering plants in grassland systems (Collins and Calabrese 2012). Fire treatments did not have major effects on flowering plant or floral visiting insect species richness or abundance, but did strongly affect plant and insect community composition. The fire variable most affecting the plant community, days since last fire (Table 3-1), was correlated with the second NMDS axis of flowering plant community composition (Fig. 3-3). The interaction between fire and grazing significantly affected the insect community (Table 3-2), and this interaction was correlated with the second NMDS axis of insect community composition (Fig. 3-3). Fire treatment had a strong effect on network H_2', with some measure of fire frequency occurring in all top models predicting H_2', and H_2' increasing with fire frequency and decreasing with days since last fire (Table 3-4; Appendix B, Fig. B4e). We hypothesize this is due to the dominance of weedy flowering plants visited by generalists (e.g. *Melilotus officinalis* and *Securigera varia*) in unburned areas. Nestedness was also higher in watersheds with more frequent fire return intervals (Table 3-4), but this relationship was not significant (Appendix B, Fig. B4b).

Our expectations based on past research predicted tight correlations between network structural properties and network size (Bascompte et al. 2003, Olesen et al. 2007, Blüthgen and Klein 2011, Dalsgaard et al. 2013). However, the relationships described in these studies are constrained by non-independence as they all used the number of plant and insect species included in the networks themselves rather than obtaining independent estimates of the local availability of insects and flowering plants. Relationships between network size and network structural properties are themselves interesting, but it is unclear whether they reflect sampling bias or inherent biologically meaningful differences (Blüthgen et al. 2008). H_2' was the only network structural property observed in this study that had species richness of either group included in top models, showing a negative relationship with flower species richness. Abundance of inflorescences also decreased H_2' and modularity, whereas insect abundance increased H_2' (Table 3-3), indicating the abundance of interaction partners can alter network structure. As H_2' is known to not be affected by sampling bias (Blüthgen et al. 2006), increases in H_2' may reflect real increases in network-level specialization with decreases in inflorescence abundance, and with increases in insect abundance. The negative effect of inflorescence abundance on modularity is likely linked to the effect of sampling date on modularity and reflects shifts in the flower community with time. June sampling included more plant species with large number of inflorescences (e.g., *Cornus drummondii*) which were pollinated by more generalist pollinators and likely reduced modularity.

As with ecological questions in general, the appropriate scale for sampling ecological networks is not clear. Ecological network structure can change with sampling scale (e.g., Dalsgaard et al. 2013, Olesen et al 2007, Blüthgen et al. 2008). We choose a snapshot approach to increase the number of networks across grassland disturbance regimes while maintaining a feasible sampling effort. A caveat of our approach is the small time scale for sampling individual networks (2hrs of sampling interactions for each network). More work is needed on the biological relevance of the scale of ecological networks. With this caveat in mind, our approach allows us to examine networks across a landscape experiment of interacting fire and grazing disturbance regimes.

Different network structural properties offer different means to resist species loss. For example, grazed grasslands support more flowering plant and floral visiting insect species and may therefore increase redundancy and decrease network susceptibility to extinction cascades. Non-grazed grasslands with more nested plant-floral visitor networks may decrease the likelihood of the extinction of specialist species (Thébault and Fontaine 2010). Fire treatment had major consequences for plant and insect community composition, with composition in turn shaping network level specialization and modularity. Understanding relationships between environmental drivers and interaction structure improves our ability to predict changes in ecological communities under changing habitat conditions. Moreover, understanding how fire and grazing disturbance regimes affect network structure provides useful management options for maintaining resistant plant-floral visitor networks in grassland ecosystems.

Tables

Table 3-1. ANOVA table for the RDA of the effects of grassland disturbance characteristics on flowering plant community structure (Fig 3a). Grassland disturbance variables are days since last fire (DSF), fire return interval (fire), bison grazing (graze), and the interaction between fire return interval and grazing. Sampling date was included as a conditional variable. Plant and community variables are listed in the community box in Fig. 3-2. F distributions and p-values were generated using 999 permutations.

	df	**Variance**	**F**	***P***
DSF	1	0.56	7.12	0.003
fire	1	0.15	1.86	0.139
graze	1	0.64	8.11	<0.001
fire*graze	1	0.20	2.56	0.064
Residual	18	1.43		

Table 3-2. ANOVA table for the RDA of the effects of grassland disturbance characteristics on insect community structure (Fig. 3b). Grassland disturbance variables are number of fires since 1981 (fire #), fire return interval (fire), bison grazing (graze), and the interaction between fire return interval and grazing. Sampling date was included as a conditional variable. Insect community variables are listed in the community box in Fig. 3-2. F distributions and p-values were generated using 999 permutations.

	df	**Variance**	**F**	***P***
fire #	1	0.37	4.89	0.010
fire	1	0.34	4.40	0.11
graze	1	0.57	7.41	<0.001
fire*graze	1	0.44	5.81	0.002
Residual	18	1.37		

Table 3-3. Final models for relationships between network structural properties and plant and insect communities (arrow 2 in Fig. 3-2). (-) indicates negative relationships and (+) indicates positive relationships between network structural properties and plant and insect species richness and abundances. Full variable names for abbreviated community variables are listed in Fig. 3-2.

Model variables	AIC_c	LL	df	R^2	P	ΔAIC_c	w_i
A. H_2'							
FC2	-22.2	14.7	3	0.14	0.038	0	0.06
FC2, IC1	-21.8	16.0	4	0.19	0.040	0.33	0.05
FSR* (-), *FC2	-21.6	15.9	4	0.19	0.044	0.54	0.04
IC2	-21.4	14.3	3	0.012	0.058	0.78	0.04
IC1, IC2	-21.2	15.7	4	0.17	0.052	0.93	004
FA* (-), *FC2	-21.2	15.6	4	0.17	0.054	1.01	0.03
FC1,FC2	-20.9	15.5	4	0.16	0.060	1.026	0.03
FSR* (-), *FC1, IC2	-20.4	16.9	5	0.21	0.050	1.76	0.03
***FC2, IA* (+)**	-20.2	15.2	4	0.14	0.082	1.97	0.02
B. Modularity							
FA* (-), *IC1	-10.6	10.4	4	0.34	0.005	0	0.14
IC1	-9.7	8.4	3	0.26	0.006	0.96	0.09
FA* (-), *FC1	-8.8	9.4	4	0.29	0.011	1.88	0.06
C. Nestedness							
D. Robustness							

Table 3-4. Final models for relationships between network structural properties and grassland disturbance characteristics (arrow 3 in Fig. 3-2). (-) indicates negative and (+) indicates positive relationships between network structural properties and habitat variables. (-) following date indicates higher network metric values in June (than July) sampling, whereas (+) following date indicates higher network metric values in July (than June) sampling. (-) following graze indicates higher network metric values in not grazed versus grazed watersheds. Full variable names for abbreviated habitat variables are listed in Fig. 3-2.

Model variables	AIC_c	LL	df	R^2	P	ΔAIC_c	w_i
A. H_2'							
***firenum* (+)**	-23.5	15.3	3	0.19	0.012	0	0.14
***DSF* (-)**	-23.2	15.2	3	0.18	0.023	0.26	0.13
***firenum* (+), *date* (-)**	-22.4	16.2	4	0.21	0.032	1.08	0.08
***firenum* (+), *DSF* (-)**	-22.2	16.1	4	0.21	0.034	1.23	0.08
***DSF* (-), *date* (-)**	-22.1	16.1	4	0.21	0.035	1.32	0.07
***fire* (-)**	-21.8	14.5	3	0.13	0.046	1.64	0.06
B. Modularity							
***date* (+)**	-7.7	7.4	3	0.25	0.018	0	0.35
C. Nestedness							
***fire* (-), *graze* (-), *date* (+)**	158.8	-72.8	5	0.40	0.004	0	0.16
***fire* (-), *graze* (-)**	159.4	-74.6	4	0.34	0.005	0.52	0.12
***DSF* (-), *graze* (-), *date* (+)**	159.8	-73.2	5	0.38	0.006	0.95	0.10
***DSF* (-), *graze* (-)**	160.1	-75.0	4	0.31	0.007	1.29	0.09
D. Robustness							

Figures

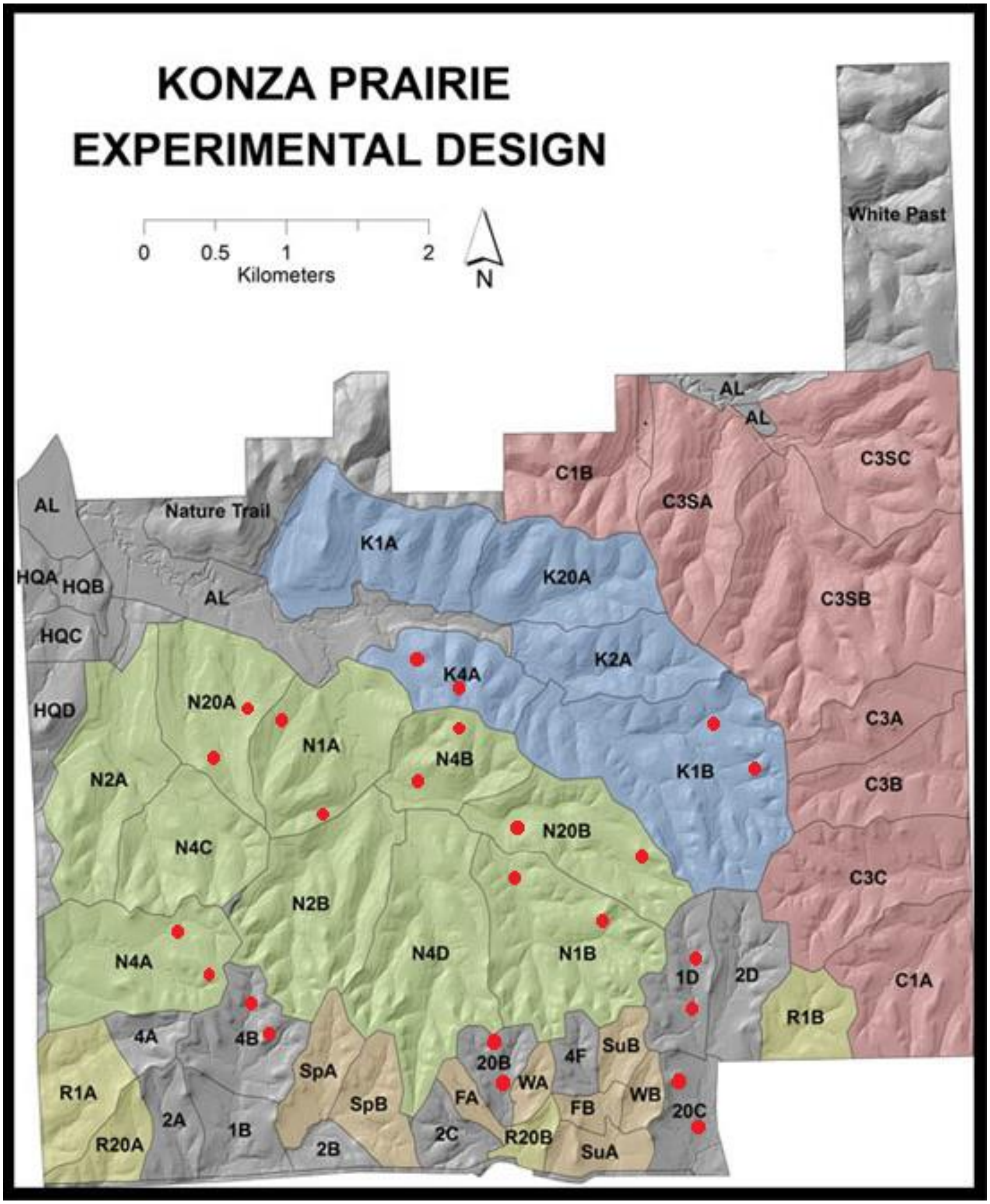

Figure 3-1. Experimental design for KPBS with sampling locations indicated (red dots). Figure modified from the experimental design illustration available on the Konza website: http://lter.konza.ksu.edu/. Gray outlines denote watershed divisions. Watersheds grazed by bison are labeled "N", cattle grazed watersheds (not included in this study) are labeled "C" and all other watersheds are not grazed. Numbers indicate fire frequencies.

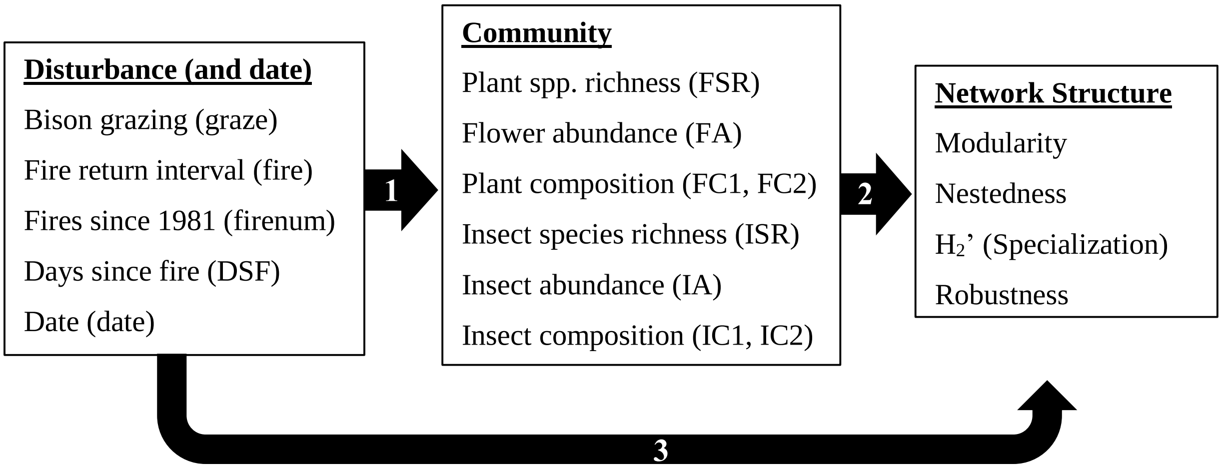

Figure 3-2. Conceptual model for disturbance, community and network structural property relationships and variable abbreviations.

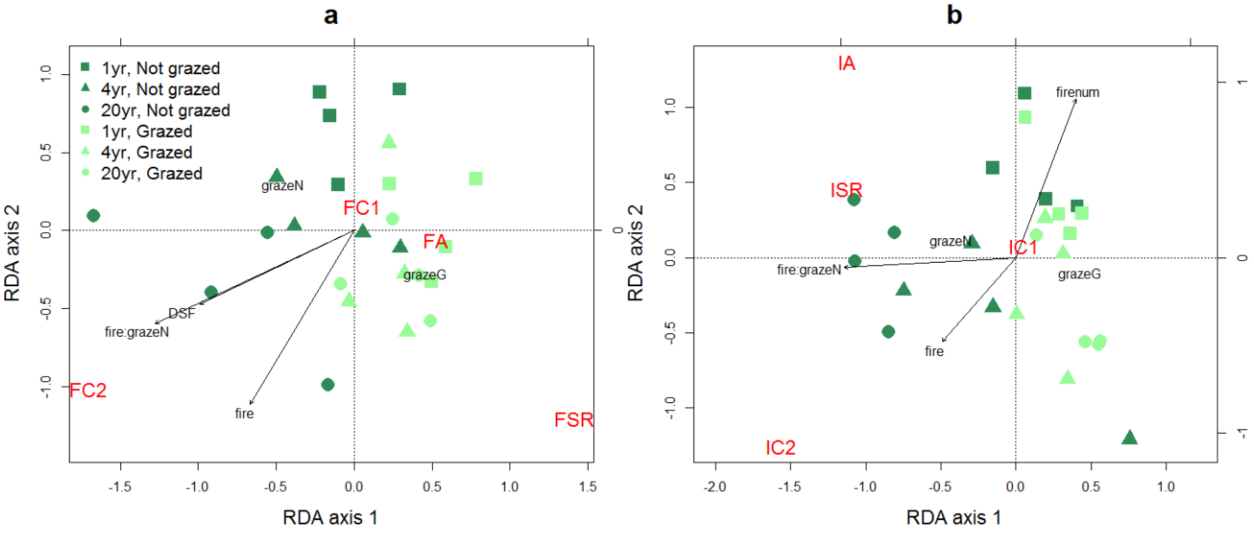

Figure 3-3. Partial RDA biplots relating plant community variables (plot a) and insect community variables (plot b) with grassland disturbance variables. Disturbance variable (in black) and plant and insect community variable (in red) abbreviations are listed in Fig. 3-2. Species richness and abundance variables were log transformed before conducting ordination. Points represent sampling sites. Date of sampling was included as a conditional factor to remove effects of season. ANOVA tables for RDAs are given in Table 3-1 (plant community) and Table 3-2 (insect community).

Chapter 4 - Fire, grazing and climate interactions shape the network structure of plant-grasshopper trophic interactions in a tallgrass prairie

Abstract

Species interactions are integral to the function of ecological communities. Understanding patterns of these interactions allows predictions of how species extinctions affect other species directly and indirectly. We examined variation in plant-grasshopper network structure in response to three major factors that shape grassland systems: periodic fire, ungulate grazing and climate. We sequenced a plant barcoding gene from extracted grasshopper gut contents to characterize diet variability of 26 grasshopper species. Resulting grasshopper species' diets were combined with long term plant and grasshopper surveys to assemble plant-grasshopper networks across 13-19 years for 6 watersheds subjected to contrasting fire and grazing treatments. Network modularity, generality, and predicted grasshopper robustness to plant species loss all increased in grazed watersheds. Predicted grasshopper robustness to plant species loss decreased under drought conditions. Dynamic grassland disturbance regimes and a variable climate modulate the interaction structure between plants and grasshoppers and thus grasshopper community stability.

Introduction

The local persistence of a species depends on its interactions with other species in a community, and the suite of species interactions at the community level modulates local species loss (Thébault and Fontaine 2010). Understanding plant-herbivore interactions is particularly important, as they are critical building blocks of terrestrial food webs. While we know of no precise estimates of the global proportions of different interaction types (Fontaine et al. 2011), the diversity of multicellular terrestrial species is dominated by plants and their insect consumers. Plant species comprise ~20% and herbivorous insects ~25% of known non-microbial terrestrial biodiversity, suggesting that ~45% of known non-microbial terrestrial organisms likely participate in plant-insect herbivore interactions (Price 2002). Identifying plant-herbivore interactions within a community remains challenging (Novotny and Basset 2005), and more community-level studies are needed to elucidate drivers of plant-herbivore interaction structure.

To date, studies of ecological networks have focused predominately on identifying non-random patterns in interaction networks and assessing how these network structural properties contribute to community stability. Stability of ecological communities has been defined in multiple ways with definition-dependent implications for stability responses (Ives and Carpenter 2007); here we define stability as robustness (ability to lose species without resulting secondary species loss). More recent studies have examined how habitat and climatic conditions shape network structural properties (Rezende et al. 2009, Daalgard et al. 2013, López-Carretero et al. 2014, Welti and Joern 2015).

Modularity and generalism of herbivore diet are metrics of species interaction networks which provide insight into the structure of these networks. Modularity is hypothesized to be an important structural property stabilizing trophic networks (Montoya et al. 2006, Rezende et al. 2009, Thébault and Fontaine 2010). A modular species interaction structure results when species interact primarily within groups and do not interact with species outside of their group. Modularity increases robustness to extinctions in a network either by limiting the spread of perturbation due to species loss from radiating throughout the network, or by reducing competition between community members in different modules (Rezende et al. 2009). Species within a highly modular network maintain tight interactions primarily with other species within their sub-module, and are therefore less sensitive to fluctuations in populations of species outside their sub-module (Montoya et al. 2006). The degree of herbivore diet generalism is alternatively predicted to buffer herbivores against plant species loss. More generalist herbivore species should be less susceptible to changes in resource availability as generalists can turn to alternate plant hosts if some plant hosts become locally extinct (López-Carretero et al. 2014).

In addition to network structural properties, community robustness to extinction can be predicted through extinction simulation models when the interactions between species in a community are known. The "robustness" of an herbivore community in response to plant species loss can be modeled by simulating plant species extinction, followed by predicting secondary extinctions of herbivores based on the assumption that every herbivore species needs at least one plant host to remain in the community. This allows one to quantify the tolerance of the herbivore community to plant species loss (Memmott et al. 2004).

Insect communities in particular can be influenced by a suite of environmental factors (Andrewartha and Birch 1954, Farkas and Singer 2013), leading to abrupt fluctuations in local abundances and species richness. Identifying which factors are most important for determining insect abundance and community composition remains a major challenge, and more long-term studies are needed to tease apart the contributions from different environmental drivers (Jonas and Joern 2007). Insects contribute substantially to global biodiversity, but are understudied both taxonomically and as contributors to ecosystem function. Grasshoppers (Acrididae) in particular are functionally important above-ground herbivores in grassland systems (Branson et al 2006) that contribute substantially to turn-over of plant biomass (Tscharntke and Greiler 1995, Anderson et al. 2001) and nutrient cycling (Belovsky and Slade 2000), and are an important food for higher trophic levels (Joern 2002).

Many grassland ecosystems are structured by exposure to periodic fire, grazing and climate variability. These disturbances affect plant community composition through a combination of varying tolerance to disturbance and altered competitive dominance (Collins et al. 2002), shaping vegetation quality and structure (Hartnett et al. 1996). Fire and grazing regimes indirectly modify grasshopper communities through their effects on plant communities and structure (Jonas and Joern 2007). Large ungulate vertebrates preferentially graze on particular plant species, removing them or otherwise reducing their impact in the community, thus reducing the competitive pressures on other plant taxa in the process (Towne et al. 2005). Previous studies report both positive and negative effects of grazing by large vertebrates on grasshopper communities (Joern and Laws 2013). Increases in fire frequency indirectly affect grasshopper communities by removing accumulated plant litter, altering the soil and near surface environment, facilitating fire tolerant plant species and enhancing dominance of several species of warm-season grasses, thus modifying the potential availability of host plants. Despite the strong influence of fire on plant responses, effects of fire frequency on grasshopper communities are minimal, although some grasshopper species are highly responsive to variation in historic fire frequency (Joern 2004, Joern 2005, Branson 2005). However, the interaction between fire and grazing shapes mesic grassland plant communities, which in turn can be important to grasshopper communities. In the absence of grazing, frequent fires decrease plant species richness and increases spatial homogeneity, whereas these plant responses to fire are ameliorated in the presence of grazing (Collins and Smith 2006), supporting more host plant species and in turn, greater grasshopper diversity. Annual variation in precipitation and temperature also affect grasshopper populations both directly and indirectly through effects on host plants, predators and pathogens (Jonas and Joern 2007).

Here, we couple DNA barcoding of gut contents of 26 grasshopper (Acrididae) species with long-term grasshopper and plant datasets from Konza Prairie Biological Station (KPBS) to assemble putative plant-grasshopper networks across 13-19 years. We calculated plant-grasshopper interaction weights for each interaction for each watershed and year using (1) proportion of plant species in grasshopper species diets, (2) grasshopper species frequency, and (3) mean plant species cover. We then compared plant-grasshopper networks across KPBS watersheds subjected to different fire and grazing treatments to characterize effects of these grassland disturbances on plant-grasshopper network structural properties.

We predicted that through enhanced habitat heterogeneity, nutrient availability and plant species diversity, bison grazing would lead to shifts in plant-grasshopper network structural properties. Predicted shifts in network structural properties include (1) increased modularity, through increases in plant species richness allowing the addition of plant-grasshopper network modules, especially between forbs and forb-feeding grasshoppers, (2) increased grasshopper feeding generality as a result of increased plant species richness improving the success of a generalist feeding strategy as it improves the chance of maintaining a balanced diet (Bernays et al. 1994, Unsicker et al. 2010), and (3) increased grasshopper robustness to local plant species loss due to increased plant species redundancy in grasshopper diets. Because of its negative effect on plant species diversity, we expected increased fire frequency to decrease the modularity, generality and robustness of plant-herbivore networks, especially in the absence of grazers. Increases in annual precipitation could either increase modularity, generality and robustness by increasing host plant availability or decrease these network structural properties by decreasing food quality or decreasing grasshopper abundance due to negative effects of grasshopper fungal pathogens. Increase in mean annual temperature was predicted to decrease modularity, generality and robustness as higher temperatures stress both plant and grasshopper communities.

Methods

Site description

Grasshopper and plant data were collected at Konza Prairie Biological Station (KPBS), a 3,487 ha native tallgrass prairie located in NE Kansas, in the Flint Hills ecoregion. KPBS had a mean annual precipitation of 835mm and a mean annual temperature of 12.9^{o} C over the period of this study (1996-2014). Beginning in 1977, a landscape-level fire-frequency experiment has been conducted at KPBS, with individual watersheds subjected to fire-frequency treatments of 1, 2, 4, or 20 years. In 1987, bison were reintroduced to a portion of KPBS. The current herd of ~280 animals have free access to 10 watersheds (~1,000 ha), encompassing an area containing replicated watersheds subjected to combinations of all fire-frequency treatments.

Grasshopper collection

Grasshoppers (Acrididae) used for diet analyses were collected as encountered with sweep nets across KPBS in August 2014. We attempted to collect 20 individuals (10 adult males and 10 adult females) of each grasshopper species present; however, some samples sizes were smaller for uncommon species (total grasshopper individuals =452; see Appendix C, Table C-1 for number of individuals sampled per species). Twenty-nine species of grasshopper were collected, and we were able to extract, amplify and sequence gut content DNA from 26 species. With the exception of one relatively common grass-feeding grasshopper (*Mermiria bivittata*) from which we were unable to extract DNA, these 26 species comprise the majority of the grasshopper species at KPBS that overwinter as eggs.

DNA extraction, PCR and Illumina MiSeq sequencing

To characterize grasshopper diets, plant DNA was extracted from the dissected crop of each individual grasshopper using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) according to manufacturer's protocol, quantified with a Nanodrop ND2000 spectrophotometer (Thermo Scientific, Wilmington, Delaware, USA) and templates standardized to 2 ng/µL. DNA amplicons were performed using primers targeting the ~600bp chloroplast trnL intron (forward primer: CGAAATCGGTAGACGCTACG, reverse primer: GGGGATAGAGGGACTTGAAC$^{\text{12bp\_tag}}$) (Taberlet et al. 1991, Jurado-Rivera et al. 2009, Avanesyan 2014). Reverse primers were synthesized with 26 unique 12 bp 3' tags (Appendix C, Table C1) to facilitate multiplex sequencing and identification of samples originating from the 26 grasshopper species. Each sample was amplified in a 25 μL reaction consisting of 1 μM of forward and reverse primers, 10 ng of template DNA, 200 μM of dNTPs, 1 μM of $MgCl_2$, 0.5 units of proofreading Phusion Green Hot Start II High-Fidelity DNA polymerase (Thermo Scientific, Wilmington, Delaware, USA) and 5 μL of 5X Green HF PCR buffer (Thermo Scientific, Wilmington, Delaware, USA). PCR thermal cycler parameters followed Taberlet et al. (1991). Briefly, PCR conditions were 98°C for 5 min, followed by 35 cycles with denaturation at 94°C for 1 min, annealing for at 50°C for 30 s, extension at 72°C for 1 min, with final extension at 72°C for 10 min. Negative controls were performed where template DNA was withheld and none produced amplicons.

To clean up PCR amplicons and remove residual PCR primers, we first used ExoSAP-IT (Thermo Scientific, Wilmington, Delaware, USA) followed by the Agencourt AmPure SPRI system (1:1 ratio of bead solution to PCR volume). Purified PCR products were visualized on a 1.5% agarose (w/v) gel to ensure successful amplification. Amplicon DNA concentration from each grasshopper species was measured and pooled across species at equal amounts (300 ng each). The amplicon libraries were paired-end sequenced using the MiSeq Personal sequencing system 600 cycles kit (Illumina, San Diego) at the Kansas State University Integrated Genomics facility.

Bioinformatics

Sequencing data were processed and assembled into contigs using mothur (version 1.33.3, Schloss et al. 2009) for each grasshopper species. Sequences were removed using mothur (version 1.33.3) based on the following criteria: (1) containment of any ambiguous bases, (2) more than two mismatches to the primers, (3) any mismatches to unique 12 bp 3’ tags (4) homopolymers longer than 8 bp, (5) were <250 bp in length, (6) any containing chimeric properties as determined using the UCHIME algorithm (Edgar 2010) (7) any without a minimum overlap of 50 bp. This yielded 1,067,005 total contigs. Sequences were then clustered as Operational Taxonomic Units (OTUs) based on a 97% similarity using nearest neighbor joining that conservatively assigns sequences to OTUs.

To identify the assembled contigs from each grasshopper species to plant taxa, the chloroplast trnL gene for all plant genera found at KPBS (for list of KPBS plants, we used Towne 2002) were downloaded from GenBank (31,765 sequences, defined as the trnL database). Assembled contigs were compared to the trnL database with the BLASTN tool of BLAST v2.2.31 (Altschul et al. 1990). The best BLAST hit of each contig was used for plant identification (e-value <10^{-20}). Sequences from gut contents matched 47 plants at the species level and 19 at the genus level. Only plants with >5 contig matches per grasshopper species were used to assemble plant-grasshopper networks.

Konza LTER datasets and plant-grasshopper network assembly

Long-term grasshopper and plant community composition datasets from selected KPBS watersheds were collected as part of the National Science Foundation Long-Term Ecological Research (NSF LTER) program. Datasets and detailed information on sampling protocols for these datasets are available on the KPBS LTER website: http://lter.konza.ksu.edu/data-explorer-dashboard (grasshopper dataset code: CGR02, plant dataset code: PVC02). Six watersheds included in both datasets and consisting of a crossed experimental design of bison grazing (three watersheds grazed by bison and three non-grazed watersheds) and fire-frequency (two watersheds of each with fire return intervals of 1, 4, and 20 years) were selected for analysis in this study (watershed codes: 1D, 4B, 20B, N1B, N4D and N20B).

Data from 1996-2014 were analyzed for non-grazed watersheds. Grasshopper sampling on grazed watersheds did not begin until 2002; data from 2002-2014 were analyzed for grazed watersheds. Grasshoppers for the KPBS long-term grasshopper dataset were sampled using sweep nets twice per year in mid-summer at two locations on each watershed. At each location and date, collections of ten samples were taken, each consisting of 20 sweeps along a transect for a total of 40 samples and 400 sweeps per watershed per year. The frequency of each grasshopper species was calculated as the proportion of the 40 samples in which the specific grasshopper species was collected.

Plant composition for the KPBS long-term plant dataset was sampled across eight 50 m transects per watershed, each consisting of five, evenly spaced 10-m^2 plots. For each plant species present in each plot, a percent cover category of 0-1%, 2-5%, 5-25%, 25-50%, 50-75%, 75-95% or 95-100% was recorded. We used the median of each selected range for each species in each plot (Ratajczak et al. 2014) and averaged the median of all plots per watershed per year to estimate mean percent cover of each plant species within each watershed. For plant species identified only to the genus level from DNA extracted from grasshopper gut content, all plant species within the genus were averaged when calculating estimated mean percent plant cover per watershed per year.

Precipitation and temperature data from KPBS were collected from the KPBS meteorological station and archived by the Climate and Hydrology Database Project with support from the NSF LTER and USDA forest service (available at http://climhy.lternet.edu/).

Interaction weights for each plant species or genus ($\boldsymbol{i}$) and grasshopper species ($\boldsymbol{j}$) interaction within each watershed ($\boldsymbol{w}$) and year ($\boldsymbol{y}$) were calculated as:

$$\mathbf{Interaction\ weight}_{\boldsymbol{ijwy}} = \mathbf{C}_{\boldsymbol{ij}}/\mathbf{T}_{\boldsymbol{i}} * \mathbf{P}_{\boldsymbol{iwy}} * \mathbf{G}_{\boldsymbol{jwy}}$$

where **C** is the number of contigs of the plant taxa ($\boldsymbol{i}$) sequenced from the grasshopper species ($\boldsymbol{j}$) gut contents, **T** is the total number of contigs sequenced from that grasshopper species ($\boldsymbol{j}$)'s gut contents (**C/T** is the estimated proportion of a grasshopper species ($\boldsymbol{j}$)'s diet comprised by a given plant ($\boldsymbol{i}$)), **P** is the mean percent plant cover of plant taxa ($\boldsymbol{i}$) from plant sampling within each watershed ($\boldsymbol{w}$) and year ($\boldsymbol{y}$), and **G** is the frequency of sampled grasshopper species ($\boldsymbol{j}$) within each watershed ($\boldsymbol{w}$) and year ($\boldsymbol{y}$).

Diets from DNA barcoding were only determined once, from grasshoppers collected in 2014, leading to two underlying and important assumptions for our study: (1) DNA barcoding results accurately captured the proportions of plant species included in grasshopper species diets; and (2) annual changes in the proportion of a grasshopper species diet, comprised by a given plant species, are due primarily to changes in the availability of host plant species (as measured by mean plant cover), and that inclusion of particular plant species in the diet is otherwise largely conserved for individual grasshopper species.

Network analyses

Network indices of generality, modularity and robustness were calculated for each plant-grasshopper network from each watershed and year using the Bipartite package (Dormann et al. 2009) in program R (version 3.3.0, R Development Core Team 2016). Generality (generality.HL, calculated using the *networklevel* function in the Bipartite package) measures the weighted mean number of plant species per grasshopper species in the network (Bersier et al. 2002, Tylianakis et al. 2007). Modularity (modularity, calculated using the *computeModules* function in the Bipartite package) measures the weighted degree of compartmentalization using Newman (2004)'s algorithm (Dormann and Strauß 2013). Grasshopper robustness to plant species loss (robustness.HL, calculated using the *robustness* function in the Bipartite package) measures the average area under the curve of 1000 simulations of the relationship between random removal of plant species (primary extinctions) and predicted loss of grasshopper species (secondary extinctions) due to loss of all host plants (Memmott et al. 2004, Burgos et al. 2007).

Statistical analyses

Models predicting plant-grasshopper network structural properties using fire and grazing treatments and climate variables were compared using an Akaike Information Criterion (AIC) framework (Burnham and Anderson 2002). A global linear mixed model, fit by REML, for each of the three network indices (generality, modularity and robustness) included the specific fixed predictor variables of grazing (presence or absence of bison), fire-frequency treatment, years since last fire, annual cumulative precipitation, and mean annual temperature. Individual watersheds were included as a random variable in models to account for repeated observations (Zuur et al. 2009). Linear mixed models were conducted using the lme4 package (Bates et al., 2015) in program R (version 3.3.0, R Development Core Team 2016). Global and all reduced additive models were compared using the *dredge* function in the MuMIn package (Barton 2012) in program R (version 3.3.0, R Development Core Team 2016). Models were considered competitive and equally parsimonious when they had a $\Delta AIC_c<2$ and substantially improved log likelihood for models which contained one additional parameter to the top model (Burnham and Anderson 2002, Arnold 2010). Relative importance values were calculated as the sum of all AIC weights containing the specific predictor variable (Burnham and Anderson 2002)

In addition to examining effects of fire, grazing and climate on network structural properties directly, and as we expected these drivers to have indirect effects through altering plant and grasshopper communities, we used a piecewise structural equation model (SEM) to identify these indirect pathways. Unlike traditional SEM, piecewise SEM does not assume complete independence of observations, is less constrained by sample size, and is evaluated using a Fisher's C statistic and associated p-value (nonsignificant p-values indicate good fitting models) (Shipley 2013, Lefcheck 2015). To quantify differences in plant and grasshopper community composition across watersheds and years, we conducted two dimensional Non-Metric Multidimensional Scaling (NMDS) ordinations for both the plant and the grasshopper communities separately. The site scores from both axes of the plant and the grasshopper ordinations were used as community composition metrics.

Results

Grasshopper gut content analyses revealed 572 individual plant-grasshopper feeding interactions between 26 grasshopper species and 66 plant taxa at either the species or genus levels. The full plant-grasshopper interaction web assembled from gut content sequencing is available in Appendix C, Fig. C1. Grazing in particular affected plant-grasshopper network structural properties, appearing in the top model for modularity and grasshopper diet generality (Table 4-1). Plant-grasshopper communities in grazed watersheds had more species and more complex interaction networks than those in non-grazed watersheds (see Fig. 4-1 for a visual representation example of grazing and fire treatment effects on plant-grasshopper networks) and the presence of bison grazers increased modularity, generality and grasshopper robustness (Fig. 4-2). Temperature strongly affected grasshopper robustness to plant species loss; mean annual temperature was the only fixed effect in the robustness top model (Table 4-1), and was negatively correlated with robustness (Fig. 4-2F). Relative importance values showed grazer treatment and mean annual temperature to be the top drivers of plant-grasshopper network structural properties (Appendix C, Fig. C2). The piecewise SEM examining effects of grassland drivers on plant and grasshopper communities, in turn shaping network structural properties, was a good fit to the data (n=96, Fisher's C=105.99, df=92, *P*=0.15; Fig. 4-3).

Modularity

The model predicting modularity of plant-grasshopper networks contained the fixed predictor variable of grazer treatment (Table 4-1). However, the null model containing the random variable of watershed was also included in the top modularity models. In watersheds not grazed by bison, modularity of plant-grasshopper networks tended to be higher in less frequently burned watersheds, whereas there was no relationship between modularity and fire frequency in grazed watersheds (Fig. 4-2A; Fig. 4-2D). In our piecewise SEM, modularity was most affected by changes in plant community composition (plant NMDS1) which in turn was positively affected by year since fire and negatively related to mean annual temperature (Fig. 4-3). Modularity was also positively related to changes in generality of grasshopper diets which in turn was largely shaped by plant and grasshopper community composition (Fig. 4-3). While mean annual temperature had the second largest relative importance value, after grazing, of all predictor variables for modularity (Appendix C, Fig. C2), and temperature had a negative effect on modularity mediated through changes in the plant community (Fig. 4-3), the temperature-modularity relationship was highly variable (Fig. 4-2D) and not significant (Appendix C, Fig. C3D).

Generality

Generality responded to grassland drivers in a manner similar to modularity, with the top model predicting grasshopper diet generality (the weighted number of plant species consumed by each grasshopper species) including bison presence as the only fixed effect (Table 4-1). While the null model (model including only watershed to account for repeated observations) was also including in top models, grazers clearly increased grasshopper diet generality between watersheds (Fig. 4-

2B; Fig. 4-2E). Within grazed watersheds, generality was higher and more variable in 4 year burns (Fig. 4-2B; Fig. 4-2E). In our piecewise SEM model, grasshopper diet generality was affected by plant and grasshopper community composition which in turn were affected by all of our examined grassland drivers (Fig. 4-3). The positive effect of grazing was mediated by both plant (plant NMDS2) and grasshopper (Acrididae NMDS1 and Acrididae NMDS2) community composition (Fig. 4-3). The second most important predictor, mean annual temperature (Appendix C, Fig. C2), had a positive effect on grasshopper diet generality mediated through plant community composition (plant NMDS2), and a negative effect on grasshopper generality mediated through grasshopper community composition (Acrididae NMDS1) (Fig. 4-3) resulting in no overall strong effect of mean annual temperature on grasshopper diet generality (Fig. 4-2E; Appendix C, Fig. C3E).

Grasshopper robustness

One competitive model predicted grasshopper robustness to plant species loss included the fixed predictor of mean annual temperature, as well as the random effect of watershed to account for repeated observations (Table 4-1). The negative effect of mean annual temperature is evident in the changes in grasshopper robustness across years (Fig. 4-2F). The hottest (mean 14.9^{o}C) and driest (569mm cumulative precipitation) year (2012) had the lowest values of grasshopper robustness (Fig. 4-2C; Fig. 4-2F). While cumulative annual temperature was positively correlated with grasshopper robustness (Fig. 4-2C; Appendix C, Fig. C3C), precipitation was not an important predictor of grasshopper robustness in our AIC modeling (Table 4-1; Appendix C, Fig. C2). Grasshopper robustness tended to be higher in bison grazed watersheds but was not strongly affected by fire treatment (Fig. 4-2C; Fig. 4-2F). In our piecewise SEM analysis grasshopper robustness was most affected by plant community composition (plant NMDS2), positively related to plant richness, and negatively related to grasshopper richness (Fig. 4-3). Mean annual temperature had a negative effect on plant richness and a positive effect on grasshopper richness, resulting in two indirect paths contributing to the negative effect of temperature on grasshopper robustness (Fig. 4-3). The positive effect of grazing on grasshopper robustness was primarily mediated through increased plant species richness in grazed watersheds (Fig. 4-3).

Discussion

Few previous studies have examined long-term changes in community interaction patterns (Olesen et al. 2008; Lenhart et al. 2015). Understanding what drivers shape interaction patterns is critical for conserving ecological communities, as species interactions are key mediators of community stability (Thébault and Fontaine 2010). Long-term data on patterns of interaction allows one to include the effects of climatic variability, and provides a more robust understanding of the effects of disturbances, such as fire and grazing. It is clear from our analyses that bison grazing and variation in annual temperature had strong effects on grasshopper-host plant network structural properties. Modularity and generality were primarily impacted by bison grazing whereas grasshopper robustness was primarily driven by changes in mean annual temperature.

Previous work documented both positive (Jonas and Joern 2007, Karban and Myers 2012, Zhong et al. 2014) and negative (Belovsky 1984, Branson and Haferkamp 2014) effects of grazing by large ungulates on grasshopper species richness and abundance. Positive effects are more prevalent in grasslands with higher primary productivity, potentially due to reduced interspecific competition (Branson and Haferkamp 2014). Large ungulates directly modify plant community composition and vegetation structure through preferential grazing on particular plant species, and in turn indirectly affect grasshopper communities (Hartnett at al. 1996, Jonas and Joern 2007). Previous studies of grasshopper communities at our study site, Konza Prairie Biological Station (KPBS), revealed increased grasshopper species richness in grazed watersheds (Joern 2005, Jonas and Joern 2007), and 2.5 times higher grasshopper densities in areas grazed by bison than in non-grazed watersheds (Joern 2004). In less productive U.S. shortgrass steppe, livestock grazing can have no effect (Colorado: Newbold et al. 2014) or even decrease grasshopper abundance, potentially through interspecific competition (Montana: Branson and Haferkamp 2014). Bison grazing on KPBS increased plant-grasshopper network modularity, generality and grasshopper robustness. Grazing by large ungulates may therefore be a potentially useful management tool to preserve grasshopper effects on ecosystem function and decrease grasshopper outbreaks caused by dominance of one grasshopper species.

Fire-frequency interacted with bison grazing in its effect on plant-grasshopper network structural properties. In non-grazed watersheds, less frequent prescribed burning increased plant-grasshopper modularity and grasshopper diet generality. Watersheds subjected to less frequent fires have increased fuel loads when they burn, leading to hotter fires that potentially cause direct mortality to grasshoppers and their eggs (Branson 2005). Additionally, fire events produce concentrated but transient pulses of some nutrients (i.e., P and cations) and long-term reductions in others (i.e., N) (Blair 1997), and more frequently burned grasslands show increased primary production in the absence of grazing (Collins and Steinaur 1998). In grazed watersheds, the highest levels of modularity and generality occurred in watersheds burned every four years. This intermediate fire-frequency combined with grazing may therefore optimize plant-grasshopper network structural properties.

While climate factors were not altered directly by management treatments at the landscape level in this study, it is important to understand how these critical grassland drivers interact with climate to impact ecological communities in order to predict the effects of climate variability and directional climate change. Increases in precipitation generally increase host plant quantity and species richness, but can have negative effects on plant quality (Mopper and Whitham 1992, Richie 2000, Warne et al. 2010, Cleland et al. 2013). While the effects of drought stress on host plants can increase grasshopper growth and reproductive success for individual species (Franzke and Reinhold 2011), drought stress at the community level can reduce grasshopper species richness (Lenhart et al. 2015). Fungal pathogens of grasshoppers are known to have major impacts on populations (Pickford and Riegert 1964, Kistner and Belovsky 2016), and tend to increase in wetter years (Brust et al. 2007). Temperature can likewise alter host plant quality and quantity and directly affect grasshopper developmental rates (Mukerji and Randell 1975, Carter et al. 1998). Climate change predictions for central North American grasslands generally include warmer temperatures and increased precipitation variability leading to more frequent and severe periodic droughts (Seager et al. 2007, Dobrowski et al. 2013, Kunkel et al. 2013). Long-term monitoring of grassland plant communities indicates drought decreases grassland plant species richness (Harrison et al. 2015). Drought conditions can also decrease grasshopper diversity (Lenhart et al. 2015).

Here, grasshopper robustness to plant species loss strongly decreased with mean annual temperature and was negatively correlated with cumulative annual precipitation. Based on previously reported plant community responses, prolonged drought and increasing temperatures are predicted to decrease plant species richness in this system and in turn make grasshopper communities more susceptible to additional host plant loss (Schleuning et al. 2016). Effects of mean annual temperature on grasshopper robustness in our results were indirect and mediated by shifts in plant community composition, plant and grasshopper species richness. As our piecewise SEM model was strongly improved by the addition of a direct relationship between mean annual temperature and grasshopper robustness (n=96, Fisher's C=80.31, df=90, *P*=0.76), there may be an unknown additional factor mediating this relationship not accounted for in our model. While the mechanism of the effect of temperature was not fully accounted for, the strength of the negative relationship with grasshopper robustness is striking.

Caveats

Our analysis of plant-grasshopper interaction networks was based on quantification of grasshopper diets coupled with long-term plant and grasshopper community composition datasets, which provided a unique opportunity to evaluate changes in plant-grasshopper interactions across years. However, we wish to acknowledge several key assumptions of these analyses. As mentioned above, we quantified grasshopper diet composition using grasshoppers collected in 2014, and then extrapolated results to examine plant-grasshopper network over multiple years. We assume that diet preferences of grasshopper species do not change substantially among years, and that our constructed annual plant-grasshopper diet matrices, based on annual changes in plant species availability and grasshopper species population levels, are a reasonable representation of grasshopper diets across years. In our view, this assumption is valid and allows us to assess how plant-grasshopper networks change over time. Moreover, using the number of DNA contigs to estimate proportion of diet can lead to potential error in bias for or against particular plant species during grasshopper digestion, DNA extraction, amplification and sequencing. However, bias exists in all methods of diet sampling, the relative number of contigs represents our best estimates of proportion of plant species in a diet from this study, and its influence on interaction weight is tempered by inclusion of grasshopper species frequency and plant species cover in interaction weight calculation. While we may have missed some plant-grasshopper interactions for rare species, we do not expect major annual shifts in grasshopper diet in terms of plant species identity (Joern 1979). Many traits such as insect herbivore diets are conserved (e.g. Rowell-Rahier 1984, Pérez-Harguindeguy et al. 2003), as are many other ecological traits (Wiens et al. 2010), so we do not expect species-specific diets to shift much among years. While herbivores are known to adjust diet selection in response to plant species abundances (Bernays and Chapman 1970, Singer and Stieman 2001), we account for variation resulting from shifts in plant species abundances, as well as grasshopper species abundances, as mean plant cover and grasshopper frequency are included in our calculation of interaction weights. Combining these datasets allowed us to create a novel dataset of long term plant-grasshopper interactions, and thus examine possible major habitat and climatic effects on plant-grasshopper interaction structures.

Conclusions

Identifying drivers affecting changes in invertebrate communities is critical as invertebrates are at high risk for biodiversity loss (Thomas et al. 2004). Invertebrates are highly responsive to changes in habitat quality, making them good indicator species (Harms et al. 2014). Grasshoppers in particular are important drivers of grassland ecosystem function (Branson et al. 2006, Whiles and Charlton 2006) as they are dominant grassland herbivores (Anderson et al. 2001) and a major food source for many species of conservation concern, such as grassland songbirds (Joern 2002). Grasslands are highly endangered ecosystems. Tallgrass prairie in North

America is particularly endangered, with only ~4% of its original extent remaining (Samson and Knopf 1994). Maintaining plant-grasshopper network structure and grasshopper diversity in remaining grasslands is crucial, considering the functional importance of grasshoppers in grassland systems.

Based on our results, ungulate grazing and potentially intermediate fire-frequency are management practices that maximize habitat heterogeneity and resulting grasshopper robustness. Grazing by cattle, the functional replacement for native bison, in the majority of the remaining intact grasslands of North America may increase grasshopper community resilience as long as grasslands are not overstocked. However, homogenization of remaining rangeland grassland systems through annual burning and high stocking densities (Gossner et al. 2016) will likely decrease grasshopper robustness. Only through long-term monitoring can we examine anticipated effects of climate trends on ecological communities. Although it is difficult to predict community robustness to directional climatic shifts, our results for plant-grasshopper communities studied here predict that anticipated warmer and drier conditions in North American grasslands will decrease grasshopper robustness to plant species loss, making them more vulnerable to extinction.

Table

Table 4-1. AIC_c statistics for competitive models (see Statistics in Methods for criterion). AIC statistics include: AIC_c = AIC statistic, adjusted for small sample size, LL = log likelihood, df=degrees of freedom, t = t-value, *P* = p-value, ΔAIC_c = AIC_c minus top model AIC_c, w_i = model weight. Predictor variables the fixed effects: grazing = grazing treatment, MAT = mean annual temperature and the random variable: watershed.

Model Variables	**AIC_c**	**LL**	**df**	**t**	***P***	**ΔAIC_c**	**w_i**
Modularity							
grazing, watershed	-172.0	90.2	4	3.8	<0.001	0	0.48
watershed	-170.9	88.6	3	3.4	<0.001	1.12	0.27
Generality							
grazing, watershed	-14.9	11.7	4	2.7	0.006	0	0.46
watershed	-13.7	10.0	3	10.9	<0.001	1.2	0.25
Grasshopper robustness							
MAT, watershed	-491.7	250.1	4	-5.49	<0.001	0	0.79

Figures

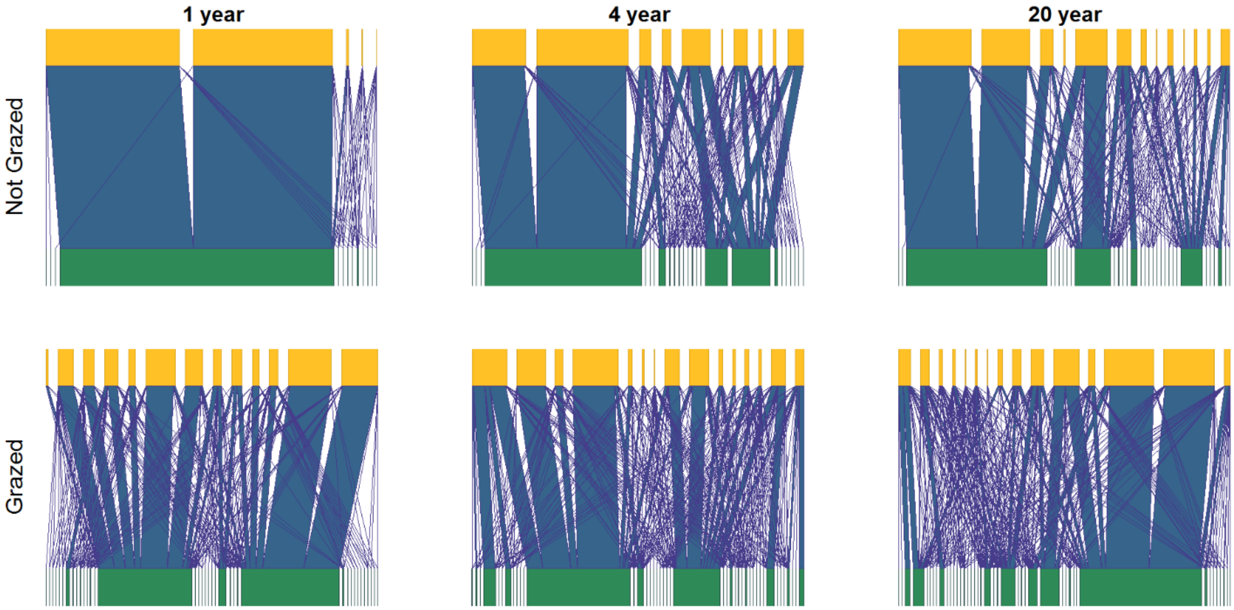

Figure 4-1. Comparison of representative example plant-grasshopper networks from the most recent year of data (2014). The top yellow bars represent grasshopper species; the bottom green bars represent plant species in the network. The blue lines connecting plant and grasshopper species represent feeding links and the thickness of the lines corresponds to the strength of the species' interaction. Networks are arranged by treatment with row indicating grazing treatment (watersheds not grazed by bison in the top row and grazed watersheds in the bottom row) and columns indicating fire return interval (first column=annually burned watersheds, second column=watersheds burned every four years, third column=watersheds burned every 20 years).

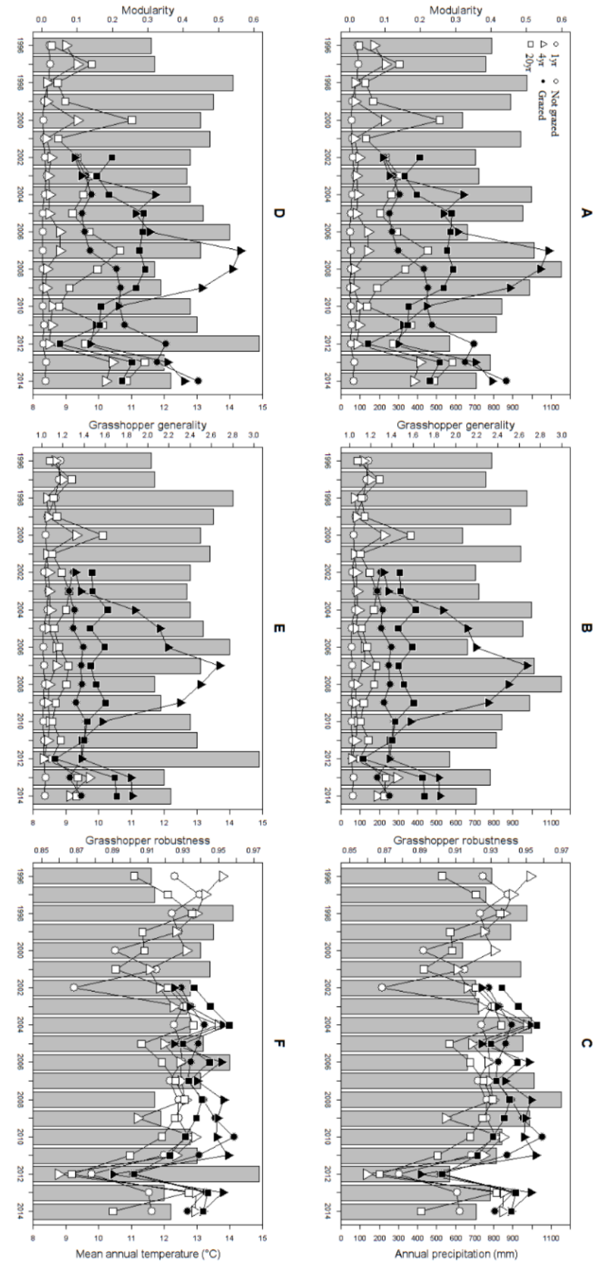

Figure 4-2. Change in network structural properties across years with underlying barplots depicting cumulative annual precipitation (Panels A-C) and mean annual temperature (Panels D-F). Point shape indicates fire return interval (1, 4, and 20 year frequencies) and point fill indicates grazing treatment (NG=not grazed, G=grazed). Although prevalent in top models, cumulative annual precipitation mean annual temperature did not significantly affect the modularity or generality of plant-grasshopper networks (*P*>0.05, Panels A & B). Cumulative annual precipitation correlated positively with grasshopper robustness to plant species loss (*P*<0.001, Panel C). Mean annual temperature did not significantly affect the modularity or generality of plant-grasshopper networks (*P*>0.05, Panels D & E) but correlated negatively with grasshopper robustness to plant species loss (*P*<0.001, Panel F). Regression relationships are shown in Appendix C, Fig. C3.

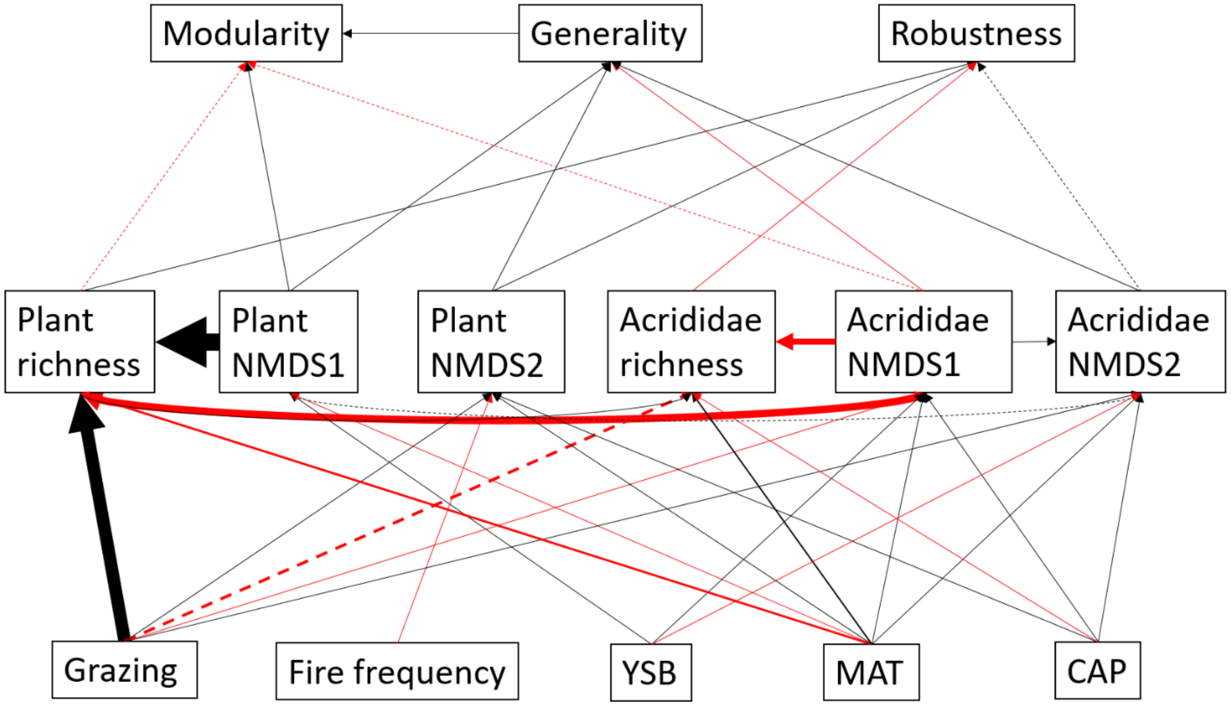

Figure 4-3. Piecewise structural equation model predicting effects of grassland drivers (grazing = presence/absence of bison grazers, fire frequency = historical burn interval, YSF = years since last fire, MAT = mean annual temperature, CAP = cumulative annual temperature) on the plant and grasshopper communities (plant richness = plant species richness, plant NMDS1 = the first axis of the NMDS of the plant community, plant NMDS2 = the second axis of the NMDS of the plant community, Acrididae richness = grasshopper species richness, Acrididae NMDS1 = the first axis of the NMDS of the grasshopper community, Acrididae NMDS2 = the second axis of the NMDS of the grasshopper community), in turn affecting plant-grasshopper network structural properties (modularity, grasshopper diet generality, and grasshopper robustness to plant species loss).

Chapter 5 - Impacts of plant diversity on arthropod communities and plant-herbivore network architecture

Abstract

At the local scale, herbivore diversity is limited by available plant diversity. Increasing plant diversity should result in more herbivore species and trophic links, but less is known about how the critical interaction structure between plants and herbivores changes across local plant diversity gradients. Ascertaining plant-herbivore interaction structure at the ecological community level is important for predicting responses of herbivores to plant species loss. We sampled arthropods and plants across three years, beginning in the fourth year of an experimental prairie restoration containing three treatment levels of initial plant seeding diversity. Plots initially planted with higher seed diversity had higher plant and arthropod species richness. Seeding diversity treatments strongly affected plant-herbivore interaction structure: the increased seeding diversity treatment decreased plant-herbivore connectance, increased average herbivore diet generalism, and increased herbivore robustness to plant species loss. Treatments diverged over time with the last year of sampling showing the greatest difference between treatments in plant and arthropod species richness, and plant-herbivore network structural properties. Network structural properties of the high seeding diversity treatment were the least variable temporally. However, divergence of plant and arthropod communities between diversity treatments was not found in traditional ordinations of community taxonomic composition. While previous comparisons of plant-herbivore interaction networks across ecological gradients treated network size (biodiversity) as a confounding factor, we demonstrate that plant diversity can directly shape

network structure in this grassland, and higher plant diversity maintains less temporally variable ecological networks of herbivores and their host plants.

Introduction

While plant diversity is often positively correlated with insect diversity and affects insect community composition (Crutsinger et al. 2006, Johnson et al. 2006, Haddad et al. 2009, Laws and Joern 2013), changes in the structure of interactions between insects and their host plants across a plant diversity gradient are expected but not well understood (Blüthgen et al. 2008, Haddad et al. 2009). Plant-herbivore interactions are the foundation of terrestrial food webs, and are among the most ubiquitous of ecological associations (Forister et al. 2014). An ecological network approach examines relationships between community-level species interactions and ecosystem stability (Pascual and Dunne 2006, Bastolla et al. 2009, Bascompte 2010, Pocock et al. 2012), providing a suitable framework for studying consequences of changing ecological interaction structure. The relationship between network structure and biodiversity is a point of longstanding debate in network studies (Dunne et al. 2002), but has rarely been evaluated empirically.

North American tallgrass prairie is an endangered ecosystem due to conversion to agricultural production (especially row crops), fire suppression, and spread of invasive species (Samson and Knopf 1994). Restored grasslands, including tallgrass prairies, often support reduced biodiversity over time in the absence of ongoing propagule inputs, but the causes of these decreases are unclear (Camill et al. 2004, Baer et al. 2016). Loss of plant diversity has implications for arthropod communities. For example, insect outbreaks are more likely in human-derived monocultures of both agricultural and forest systems (Haddad et al. 2011). Here, we evaluate responses of plant and arthropod communities and plant-herbivore interaction network structural properties to three treatment levels of plant seeding diversity (plots seeded as high diversity, low diversity, and monoculture) over three years in an experimental, restored grassland. Experimental comparison of plant-insect interaction networks across a local diversity gradient allows us to test predictions about changes in vulnerability to extinctions by herbivores caused by changes in plant diversity. Studies of changes in ecological network structure over time have rarely been conducted due to the difficulty of obtaining community-level interaction data (Bascompte and Jordano 2007, Olesen et al. 2008, López-Carretero et al. 2014). Sampling a recently restored prairie across three growing seasons following a 4-year establishment phase allows us to evaluate emerging interaction structures. By manipulating plant diversity in a community context, we can directly examine the effects of frequently confounding diversity effects, and improve our ability to predict ecological community responses to ongoing environmental change.

From among a wide variety of descriptive metrics characterizing network structure proposed in the ecological network literature (Blüthgen 2010), we selected four commonly reported network indices predicted to be important for system stability: connectance, modularity, generality of herbivore diet, and herbivore robustness to plant species loss. The connectance-stability relationship continues to be a subject of debate (May 1972, Dunne et al. 2002, Jacquet et al. 2016). However, as connectance generally decreases with increased biodiversity (Rejmánek and Starý 1979, Welti and Joern 2015), we predict decreasing connectance in a plant-herbivore interaction in response to increasing plant diversity treatments. Modularity, or nonrandom clustering of interactions, may stabilize bipartite antagonistic networks such as plant-herbivore networks by limiting the spread of perturbation across the network and/or by limiting competition across modules (Paine 1980, Pimm and Lawton 1980, Krause et al. 2003, Montoya et al. 2006, Rezende et al. 2009, Thébault and Fontaine 2010). Generality of herbivore diets is predicted to maintain herbivore diversity by increasing herbivore flexibility to plant host availability, and is reportedly less biased by network size than other measures of network structural properties (Blüthgen et al. 2008). Finally, robustness predicts herbivore community response to plant species loss using a plant extinction simulation algorithm (Memmott et al. 2004). We predicted increases in plant diversity would lead to more modular plant-herbivore networks with higher herbivore diet generalism and robustness to plant species loss. In the face of current global environmental challenges, it is increasingly crucial to understand the origin and long term maintenance of persistence in communities, and how stabilizing mechanisms can change with environmental gradients (Tilman et al. 2006, Bascompte and Stouffer 2009). Through collection and assembly of plant-herbivore interaction networks across three plant diversity levels in a restored prairie, we tested directly the effect of plant diversity on plant-insect herbivore network structural properties.

Methods

Site description

Plant-herbivore networks were constructed based on data from grassland restoration plots at Platte River Prairies, a site owned and managed by The Nature Conservancy in south-central Nebraska (40°44'37.8"N 98°35'23.9"W) in the Central Platte River ecoregion. The sampling design consists of twelve 60m x 60m plots with four replicates each of three levels of seeding diversity treatments. Plots were planted in 2010 (monoculture plots were seeded in February 2010, and low and high seeding diversity plots were seeded in March 2010) in a former agricultural field previously planted with corn. Plots have not been weeded since initial 2010 seeding. The three treatments consist of: **(1)** a monoculture seeding treatment originally seeded with only

Andropogon gerardii (big bluestem, a C_4 grass), **(2)** a low seeding diversity treatment originally seeded with a mixture harvested from a combine in the fall and primarily consisting of *Andropogon gerardii*, *Elymus canadensis* (Canadian wildrye, a C_3 grass), *Panicum virgatum* (switchgrass, a C_4 grass), *Sorghastrum nutans* (Indiangrass, a C_4 grass), and a few late season forbs, and **(3)** a high seeding diversity treatment originally seeded with 102 native prairie grass and forb species (hereafter the treatments shall be referred to as the M treatment [monoculture seeding treatment], the L treatment [low seeding diversity treatment], and the H treatment [high seeding diversity treatment]). All plots were burned in late March of 2013 and April of 2015.

Sampling

Plants and insects were sampled in all twelve plots for three years (2013, 2014 and 2015) following a 4-year establishment period. Plant height was indexed with a pasture disk meter with measurements taken every 5m along three 30m transects per plot. Pasture disk meters measure the height that a thick plastic disk of constant weight, dropped from a set height is supported by the vegetation canopy; this measure is highly correlated with plant biomass (Vartha and Matches 1977). Plant diversity was quantified as counts of each plant species within nine Daubenmire frames (50cm x 50cm) per plot arranged in a 3 x 3 grid spaced 15m apart. Forb and woody plants were individually counted whereas grass counts were estimated from percent cover of each grass species within the Daubenmire frame. Within each plot, arthropods were sampled along three vacuum (30m x 5m area) and four sweep net (25 sweeps) transects. Arthropod samples were placed in plastic bags, frozen, and taken back to the lab for later sorting. All arthropods were sorted to morphospecies and identified to the lowest possible taxonomic level. Identification of the major herbivore orders of Hemiptera and Orthoptera was prioritized and insects from these groups were mostly identified to species.

Plant-herbivore network construction

Feeding interactions between hemipteran and orthopteran herbivores, and plants sampled in this study were identified from previously published diet records. Scientific names of plant and herbivore species were separately searched in Web of Science (Thomson Reuters 2017) to find publications documenting host plant relationships. In total, 509 previously documented trophic interactions between the plant and herbivore species sampled in this study were identified from the literature. References for herbivore diets are given in Appendix D. All documented host plant relationships between plants and herbivores sampled in this study were recorded into a plant-herbivore matrix (Appendix D, Fig. D-1). Plot-level plant-herbivore networks for each year were then assembled from this interaction matrix combined with plot-level plant and herbivore composition. Interaction strength for each plant ($\boldsymbol{i}$) and herbivore ($\boldsymbol{j}$) interaction within each plot ($\boldsymbol{r}$) was calculated as:

$$\textbf{Interaction strength}_{ijr} = \mathbf{F}_{ij} * \mathbf{P}_{ir} * \mathbf{H}_{jr}$$

where **F** is the presence or absence of the feeding interaction (1 or 0) between plant species ($\boldsymbol{i}$) and herbivore species ($\boldsymbol{j}$), **P** is the sum of the counts of plant individuals of species ($\boldsymbol{i}$) from plant sampling within each plot ($\boldsymbol{r}$), and **H** is the sum of the herbivore individuals of species ($\boldsymbol{j}$) from sweep net and vacuum sampling within each plot ($\boldsymbol{r}$).

Statistical analysis

All plant-herbivore networks for each replicate plot and year were log transformed before performing network analysis using the disttransform function in the package BiodiversityR (Legendre and Gallagher 2001, Kindt and Coe) in program R (version 3.3.2, R Development Core Team 2016). Calculation of connectance, herbivore diet generalism (generality.HL) and herbivore robustness to loss of plant species (robustness.HL) were calculated using the networklevel, and network modularity was calculated using the computeModules function, both in the Bipartite package (Dormann et al. 2009) in program R (version 3.3.2, R Development Core Team 2016).

We performed Canonical Correspondence Analysis (CCA) to examine relationships between our two habitat variables (seeding diversity treatment and year) and community composition of the plant community, the entire arthropod community, and the insect herbivores. Herbivores are a subset of all arthropods but were included in a separate analysis to determine if they responded similarly to other arthropods to treatment and year effects. All site by species composition matrices were log+1 transformed before CCA was conducted. CCA is an ordination technique used in community ecology for relating community composition and habitat data (Ter Braak 1986, Legendre and Legendre 2012). CCA was conducted in the vegan package (Oksanen et al. 2015) in program R (version 3.3.2, R Development Core Team 2016). Treatment and year effects on community composition were tested using F distributions are based on 999 permutations. ANOVA was used to test for effects of the two dependent variables of plant diversity treatment and year on arthropod abundance, arthropod richness, plant biomass, plant richness, and the four selected network metrics. Tukey’s Honestly Significant Difference tests were used to infer differences between each group of plots within a plant diversity treatment and year of sampling.

Results

Plant responses

A total of 76 plant species across all plots and years were identified in plant sampling. As intended, the seeding diversity treatments resulted in significant changes in plant richness, including both seeded and volunteer species (n=36, $F_{2,31}$=98.3, $P<0.001$). Within year and treatment, the H treatment had significantly higher plant richness than the L and M treatments for all three years (Fig. 5-1A). Year of sampling did not significantly affect plant richness (n=36, $F_{2,31}$=2.0, $P=0.15$), although plant species richness tended to decrease somewhat across years in the M treatment (Fig. 5-1A). Increased differences between all treatments were found for plant Shannon's diversity (Appendix D, Fig. D-3B) and evenness (Appendix D, Fig. D-4B). The decrease in plant Shannon's diversity for the M treatment was significant from 2013 to 2015 (Appendix D, Fig. D-4A).

Plant height, as estimated by pasture meter, increased with year (n=36, $F_{2,31}$=80.11, $P<0.001$), and was affected by seeding diversity treatment (n=36, $F_{2,31}$=6.52, $P=0.004$), with a tendency for highest plant biomass in the L treatment (Fig. 5-1B).

Arthropod abundances

In total, over the three years of sampling, 10,075 individual arthropods (H treatment=4,336, L treatment=2,952, and M treatment=2,787 individuals) were collected. Across years, seeding diversity treatments significantly affected arthropod abundance (n=36, $F_{2,31}$=7.12, $P=0.003$), as did year of sampling (n=36, $F_{2,31}$=8.43, $P=0.001$). The H treatment plots had the highest mean plot-level abundances of total arthropods, and 2014 had higher abundances than 2013 and 2015. However, Tukey tests for differences between means of specific year and treatment groups did not detect significant differences between mean arthropod abundances (Fig. 5-1C). Within arthropod orders, the abundances of Coleoptera, Hemiptera, and Hymenoptera were most responsive to treatments and tended to increase with increasing levels of seeding diversity (Appendix D, Fig. D-2). Across years, 2014 tended to have higher abundances of arthropods (Fig. 5-1C), driven by increases in Coleoptera, Hemiptera, Lepidoptera, and Orthoptera (Appendix D, Fig. D-2). Spider (Araneae) abundance did not show strong responses to plant diversity treatments, but decreased with year of sampling in all treatments (Appendix D. Fig. D-2G).

Arthropod diversity

A total of 321 arthropod morphospecies (H treatment=250, L treatment=206, and M treatment=194 morphospecies) were collected from the study plots during the three years of sampling. The increased seeding diversity treatment led to increased arthropod richness (n=36, $F_{2,31}=23.55$, $P<0.001$). Differences between treatments increased over time, where Tukey tests found significant differences between the H treatment and M treatment were in 2015 (Fig. 5-1D). Arthropod richness decreased significantly with year of sampling (n=36, $F_{2,31}=9.45$, $P<0.001$), with the largest decrease occurring in the M diversity treatment (Fig. 5-1D). Within arthropod orders, species richness of Hemiptera and Hymenoptera were most responsive to treatments, with increasing levels of seeding diversity having a positive effect on species richness of those orders (Appendix D, Fig. D-3). Additionally, differences between mean species richness of Hemiptera and Hymenoptera between treatments increased over time (Appendix D,

Fig. D-3). Richness of Diptera and Orthoptera were notably non-responsive to changes in plant diversity treatments (Appendix D, Fig. D-3). Spider richness decreased over time with significant differences between 2013 and 2015 for all seeding diversity treatment levels (Appendix D, Fig. D-3G).

Plant, arthropod, and herbivore community composition

CCA revealed strong effects of seeding diversity and year of sampling on plant (Fig. 5-2A), arthropod (Fig. 5-2B), and herbivore (Fig. 5-2C) community composition. CCA of plant, arthropod, and herbivore communities had no variance inflation of the dependent variables of treatment and year (all variance inflation <2). Plant community composition was significantly affected by seeding diversity treatment (n=36, $F_{2,32}$=1.4, P=0.014) and year of sampling (n=36, $F_{1,32}$=1.9, P=0.005). Arthropod community composition was significantly affected by seeding diversity treatment (n=36, $F_{2,32}$=1.5, P=0.005) and year of sampling (n=36, $F_{1,32}$=4.2, P<0.001). Herbivore community composition responded similarly to community composition of all arthropods with significant responses to treatment (n=36, $F_{2,32}$=2.1, P=0.005) and year of sampling (n=36, $F_{1,32}$=7.3, P<0.001). Plant, arthropod and herbivore CCA all had two significant axes (plant: CCA1 P=0.002, CCA2 P=0.02, CCA3 P=0.92; arthropod: CCA1 P<0.001, CCA2 P<0.001, CCA3 P=0.17; herbivore: CCA1 P<0.001, CCA2 P<0.001, CCA3 P=0.42), allowing the majority of variation in community composition to be displayed in two dimensions. For all CCAs, variation in community composition caused by year of sampling was primarily represented by the first CCA axis, and variation caused by seeding diversity treatment was summarized in the second CCA axis.

Plant-herbivore network structure

A total of 5,629 individuals belonging to 72 species were hemipteran and orthopteran herbivores, and therefore were included in network analyses. Representative depictions of plant-herbivore networks from the first replicate plot of each of the three seeding diversity treatments across the three years of study are shown in Figure 5-3. Network connectance was strongly affected by seeding diversity treatment (n=36, $F_{2,31}$=22.1, *P*<0.001) and year of sampling (n=36, $F_{2,31}$=6.24, *P*=0.005), where connectance decreased significantly with year of sampling in the M treatment (Fig. 5-4A). Modularity of plant-herbivore networks was not significantly different between treatments (n=36, $F_{2,31}$=1.74, *P*=0.19), but was significantly different between years (n=36, $F_{2,31}$=7.82, *P*=0.002); 2015 showed the lowest modularity values (Fig. 5-4B). Seeding diversity treatment levels positively corresponded to an increase in the generality of herbivore diet (n=36, $F_{2,31}$=17.8, *P*<0.001), while year of sampling did not significantly affect herbivore generalism (n=36, $F_{2,31}$=0.24, *P*=0.79; Fig 5-4C). Similarly, herbivore robustness to plant species loss increased with seeding diversity treatment (n=36, $F_{2,31}$=13.0, *P*<0.001), but did not change across sampling year (n=36, $F_{2,31}$=0.31, *P*=0.74; Fig 5-4D).

Discussion

Increases in plant seeding diversity levels in prairie restoration plots resulted in increased plant and arthropod diversity. Arthropod abundance also tended to be highest in high plant diversity plots while plant biomass tended to be highest in plots with an intermediate level of plant diversity. Arthropod and specifically herbivore community composition were strongly controlled by seeding diversity treatment and year. While still significantly affected by year and seeding diversity treatments, plant community composition was more variable, with the community composition of the M treatment becoming more similar to the other treatments across years.

Seeding diversity affected plant-herbivore network structural properties, with higher seeding diversity increasing generalism of herbivore diet and herbivore robustness to plant species loss. Connectance decreased with increasing levels of seeding diversity, consistent with previous studies that found a negative connectance-diversity relationship (Rejmánek and Starý 1979, Pimm 1980, Jordano 1987, Blüthgen et al. 2008, Heleno et al. 2012). Contrary to our predictions, modularity was not affected by our treatments. Differences in community and network structural properties increased across the years of this study, with 2015 showing the greatest differences between plant diversity treatments.

Our results have implications for ongoing theoretical work on the consequences of network size for network structure; showing that network size directly affects network structural properties. These results have practical implications for managing prairie restorations, showing that higher initial diversity of seeding inputs decreases the temporal variability of plant and arthropod diversity and of plant-herbivore interaction structure. In comparative studies, network size (number of species in the network) correlates with ecological network structural properties, leading to criticisms that variation in observed network structural properties may actually reflect sampling bias (Blüthgen et al. 2008). Through our experimental approach, plant diversity is not merely a covarying factor, but a likely driver of plant-herbivore network structure. Resulting increases in generality of herbivore diets with increasing levels of seeding diversity were expected. As more plants were available, herbivore diet breadth increased. However, higher generality of diets in high plant diversity plots does not mean that herbivores in the monoculture and low diversity plots are specialists, but instead likely reflects limited host plant availability. A study of changes in leafhopper diversity across a gradient of land use intensity showed decreases in diversity with increased land use intensity were driven by loss of specialist species (Nickel and Achtziger 2005). We expected a similar pattern in our study across the plant diversity gradient. However, higher realized diet breadth in generalist species in higher plant diversity plots overshadowed this pattern and caused higher measured diet generality. Increases in herbivore robustness to plant species loss with increasing seeding diversity were expected and likely reflect higher herbivore diet generality; herbivores are less likely to become locally extinct due to local extinction of plant species in the high seeding diversity treatment because they have additional host plant options.

The lack of a modularity response to seeding diversity was unexpected as modularity should increase with network size for trophic networks across natural plant diversity gradients at macro-scales (Welti and Joern 2015). Although modularity is perhaps the most commonly cited network structural property hypothesized to increase stability of trophic networks, causal mechanisms that lead to modularity in ecological systems remain unclear (Rezende et al. 2009). Previously proposed mechanisms of modularity in trophic systems include: habitat structure (Pimm and Lawton 1980, Krause et al. 2003), variation in body size (Williams and Marinez 2000, Petchey et al. 2008), and variation in phylogenetic relatedness (Rezende et al. 2007, Rezende et al. 2009). We initially predicted modularity would decrease with decreasing plant diversity due to a smaller resource base upon which to build trophic compartments. Additionally, fewer plant species may limit possible causal mechanisms in their ability to increase modularity, potentially reducing habitat structure and the breadth of variability in body size and phylogenic divergence. Our result that seeding diversity treatments did not significantly affect plant-herbivore network modularity may reflect other mechanisms underlying the expression of modular structure, or this outcome may be explained by the early successional stage of the restoration examined here where the community network pattern is unresolved to this point.

While the L and M treatments included fewer forbs and forb-eating herbivores, the loss of these species appears to have reduced the size of or eliminated forb-related modules, but did not reduce overall network modularity.

Differences in plant and arthropod communities and plant-herbivore network structural properties increased across years of this study. Differences in network connectance between the M and H treatments within years were not significant in 2013, but became significantly different in 2014, and showed even greater divergence in connectance values in 2015 (Fig. 5-4A). Only in the last year of sampling (2015) did the M and H treatments exhibit significantly different mean plot generality of herbivore diet (Fig. 5-4C), herbivore robustness to plant species loss (Fig. 5-4D), and arthropod richness (Fig. 5-1D). Changes in arthropod richness were primarily driven by divergence of Hemiptera (Appendix D, Fig. D-3C) and Hymenoptera (Appendix D, Fig. D-3D). For all of these indicators of divergence between seeding diversity treatments, the M treatment changed the most across time, whereas the H treatment plots remained relatively static temporally. This effect is also evident in changes of plant diversity across time with the M treatment showing the greatest decrease in plant diversity across years (Appendix D, Fig. D-4A). Such evidence for divergence was not found in the CCA of plant and arthropod communities; in the plant analysis, community composition became more similar over time (Fig. 5-2). The exception to this pattern is changes in spider (Araneae) abundance and richness decreasing across time for all seeding diversity treatments (Appendix D, Fig. D-2G & Fig. D-3G). Loss of this critical arthropod predator order over time is a potentially dismaying result for prairie restoration efforts. In addition to spider community shifts, the increase in plant height over time (Fig. 5-1B) is an indicator of ongoing community assembly processes.

Tallgrass prairie is a highly endangered ecosystem (Hoekstra et al. 2005), and restoration efforts have the potential to reestablish lost grassland, improve degraded habitat and decrease fragmentation for grassland species. However, community assembly on prairie restorations can be unpredictable, and restorations often show decreased plant species richness across time (Camill et al. 2004, Baer et al. 2016). Results of this study indicate that increased initial plant seeding diversity can result in maintained plant and arthropod diversity, and of plant-herbivore trophic structure. The increased robustness of herbivores to plant species loss indicates that higher plant diversity planting will be less impacted by future changes to community composition.

Figures

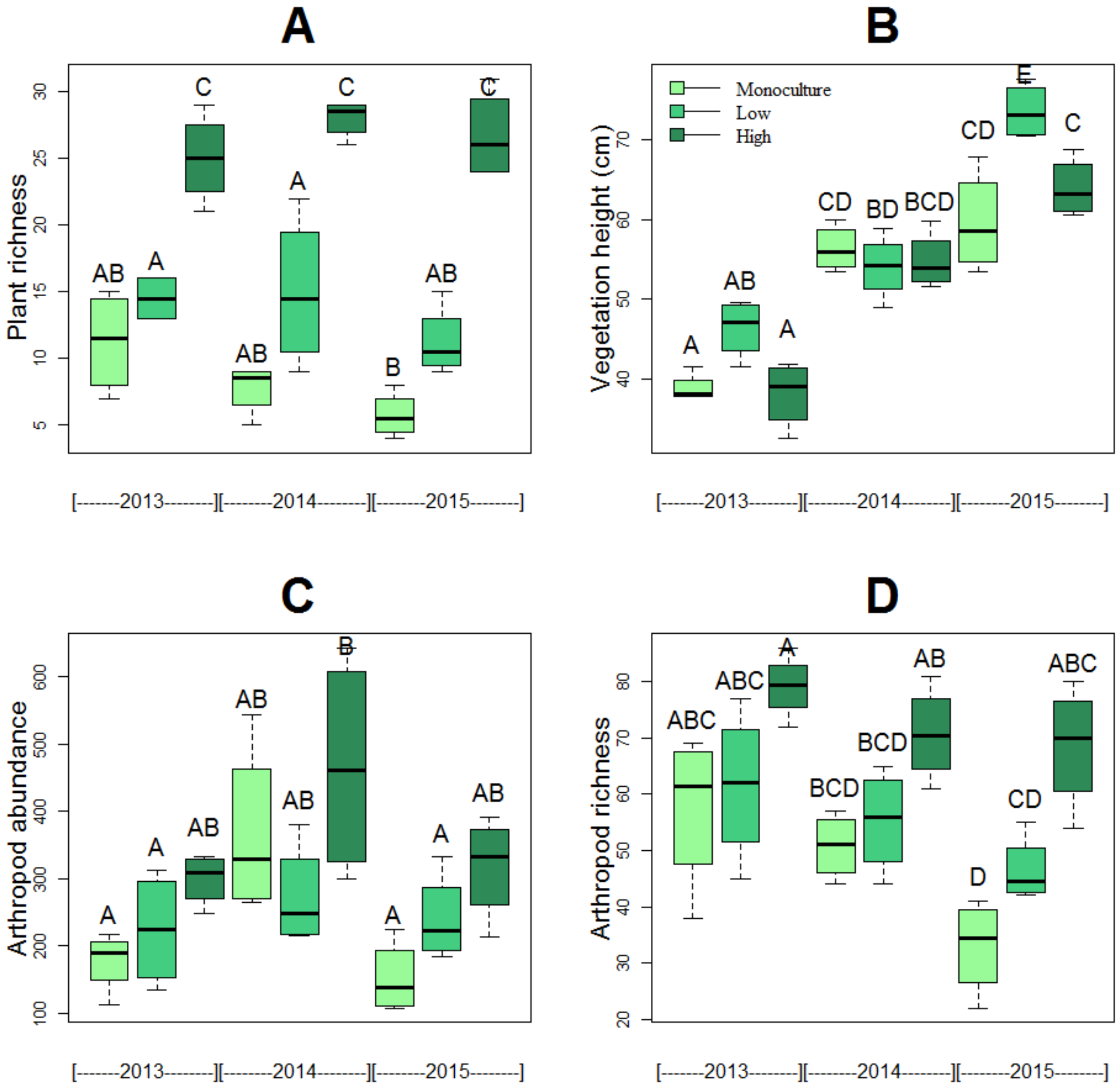

Figure 5-1. Changes in plot-level plant species richness (A), plant height (B), and arthropod abundance (C), richness (D), with the three plant diversity treatments and year of sampling. Letters represent the results of Tukey's Honestly Significant Differences test (α=0.05).

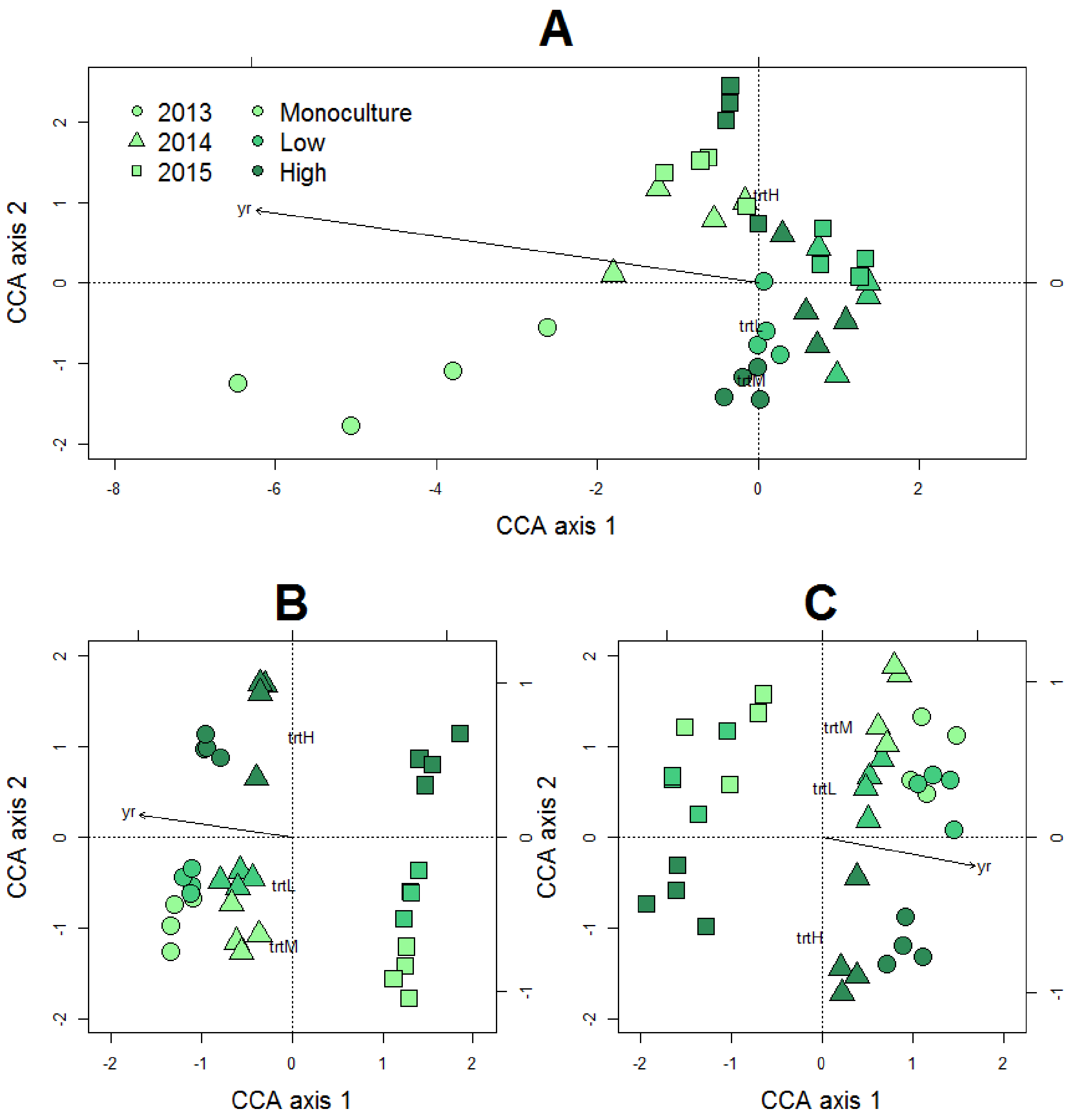

Figure 5-2. CCA ordinations examining the effects of plant seeding diversity treatment (trtM = M treatment, trtL = L treatment, and trtH = H treatment) and year (yr) on the plant community (A), arthropod community (B) and herbivores only (C). CCA were significant for plant (n=36, $F_{3,32}$=1.6, P<0.001), arthropod (n=36, $F_{3,32}$=2.5, P<0.001) and herbivore communities (n=36, $F_{3,32}$=3.8, P<0.001). F distributions are based on 999 permutations.

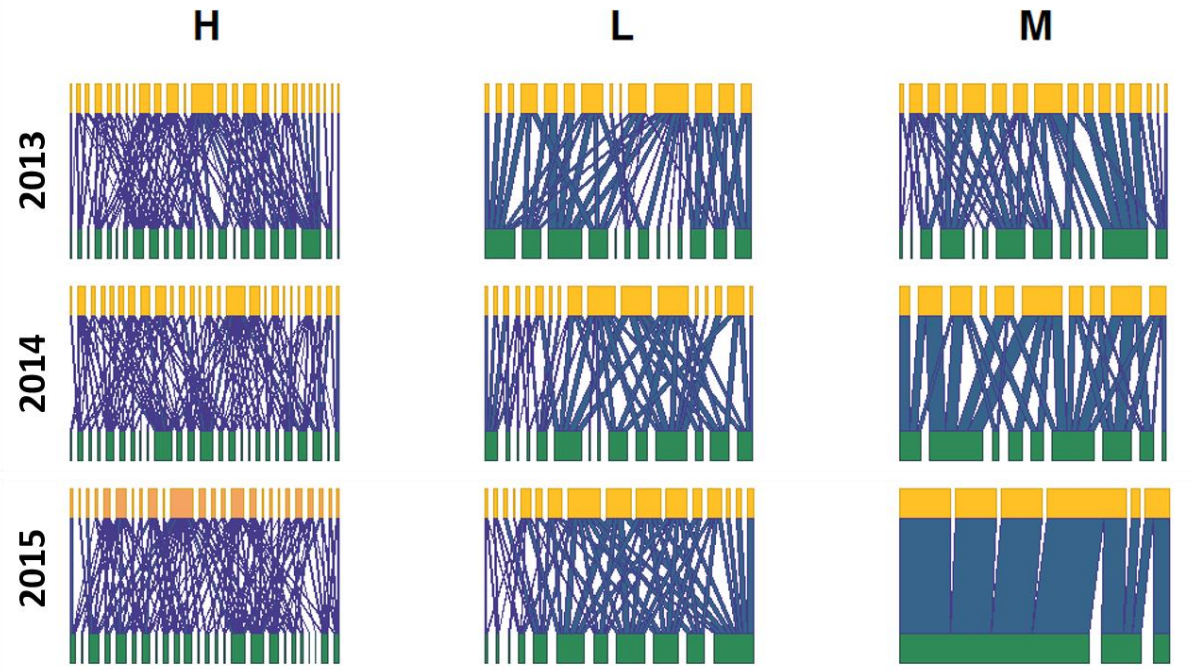

Figure 5-3. Example visualization of plant-herbivore networks for the first replicate plot (1 of 4) of each of the three diversity treatments. Top bars (yellow) represent herbivore species and bottom bars (green) represent plant species sampled within the plot and year. Blue lines connecting herbivore and plant species represent herbivore feeding interactions. Network depictions are arranged by diversity treatment in columns (H=high diversity, L=low diversity, M=monoculture treatment) and by year in rows.

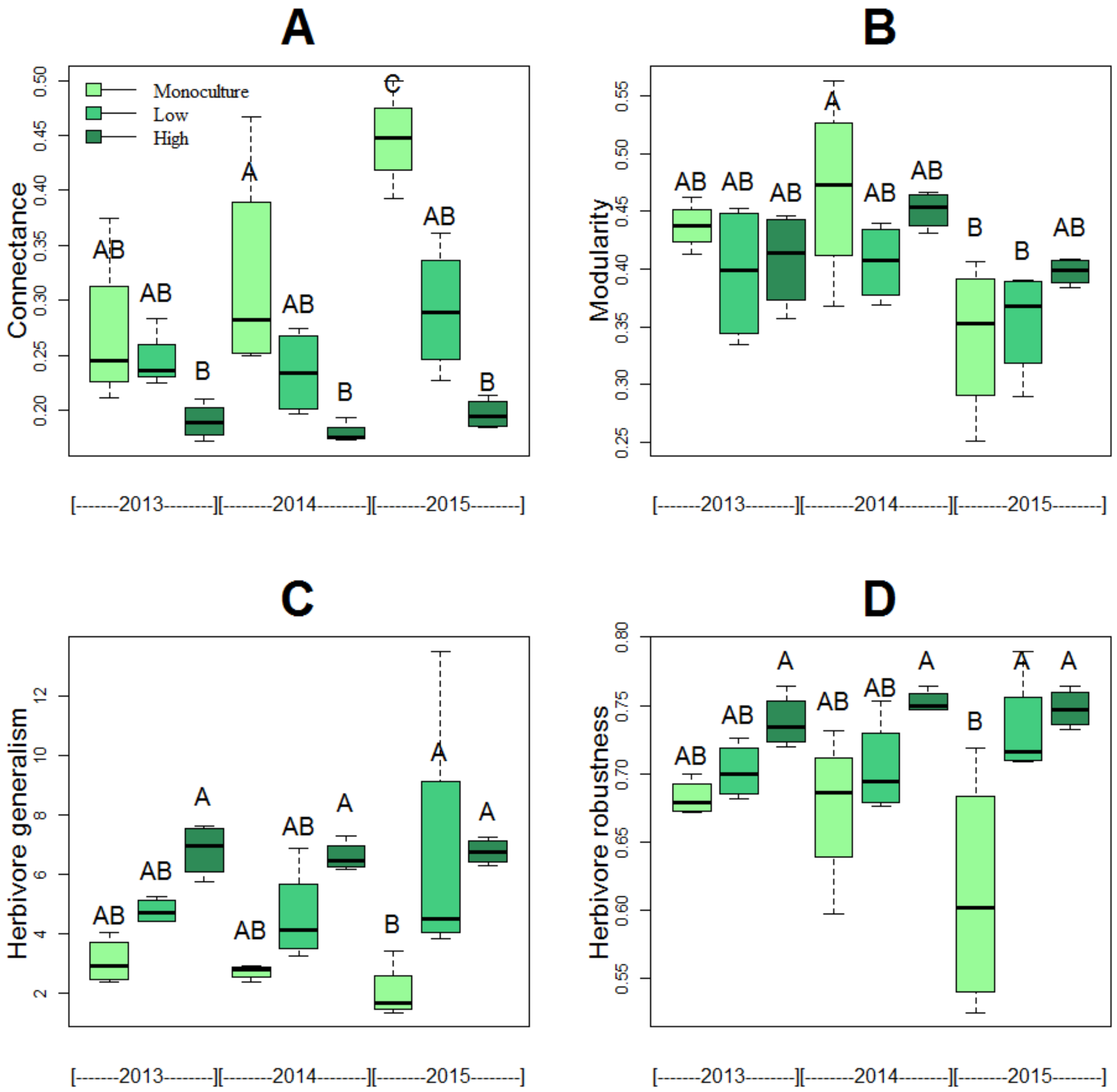

Figure 5-4. Changes in plot-level network structural properties of connectance (A), modularity (B), generality of herbivore diet (C) and herbivore robustness to plant species loss (D) with the three plant diversity treatments and year of sampling. Letters represent the results of Tukey's Honestly Significant Differences test (α=0.05).

Chapter 6 - Conclusion - Environmental variation shapes the network structure of grassland plant and insect interactions

Understanding the origin and long-term persistence of ecological communities, and how stabilizing mechanisms are affected by changing environmental conditions is increasingly critical (Bascompte 2009). Earth's biota and its ecosystem goods and services are under growing pressure from large-scale environmental changes, including the biodiversity crisis, global climate change, increased pressure from invasive species, shifts in biogeochemical cycles, and habitat loss (Dobrowski et al. 2013). Up to 60% of some species groups are predicted to go extinct by 2100 (Pereira et al. 2010). Understanding how environmental gradients affect characteristics of ecological communities such as species diversity and community composition is a longstanding goal of ecology and will improve our ability to predict of how communities will be impacted by global change (Pianka 1966a, Rosenzweig 1995, Mittelbach et al. 2001, Willig et al. 2003, Tilman et al. 2012). In developing predictions about how ecological communities will respond to shifting environmental conditions, it is imperative to understand what properties affect robustness to extinctions in ecological communities, and how those properties are affected by shifting environmental conditions. Understanding how environmental gradients influence ecological network interaction structure is the primary objective of this dissertation. Through study of plant and insect communities across ecological gradients at multiple scales, I identify which ecosystem properties predispose communities to a potential acceleration of extinction cascades.

While biodiversity is often exclusively characterized as species richness, species interactions actually mediate the robustness of ecological communities. Robustness is the capacity of a system to resist collapse following loss of components; for ecological systems, robustness is the capacity of communities to not lose additional species following initial local extinctions (i.e. experience extinction cascades). An ecological network approach provides tools for characterizing species interactions of communities, and has provided evidence that the central goals in the development of ecological networks field is sound, leading to the realization that ecological communities have nonrandom structure with implications for ecosystem stability (Pascual and Dunne 2006). While many studies have elucidated the structure of ecological networks and the consequences of network structure for community robustness (Jordano 1987, Montoya et al. 2006, Rezende et al. 2009, Thébault and Fontaine 2010), few previous studies have examined what relationships, if any, exist between network structural properties and environmental conditions. This dissertation advances knowledge of effects of changing conditions on community structure and robustness through improved understanding of the responses of plant-herbivore (antagonistic) and plant-pollinator (mutualistic) interaction networks to environmental constraints. I characterized ecological network structure across three spatial scales, allowing me to evaluate how gradients of climate, grassland disturbance regime, and plant diversity control the interaction structure of plant-pollinator/floral visitor and plant-herbivore networks. By examining the influence of these ecological gradients on community structure, my research bridges community and ecosystem ecology and improves our ability to predict ecological community responses to future environmental change.

Comparisons between networks of antagonist and mutualistic interactions have rarely been conducted, especially comparisons of changes in network structural properties across ecological gradients. Two fundamental interaction types, plant-pollinator/floral visitor (Chapters 2 & 3) and plant-herbivore (Chapters 2, 4, & 5) interactions were the focus of this dissertation. Pollinating insects are key community members of grassland systems, providing the ecosystem service of pollination of flowering plants. Plant-pollinator communities are a useful system in which to study community persistence because plants and pollinators are directly dependent on one another (i.e., are mutualistic). Plant-herbivore interactions are the building blocks of terrestrial food webs. Herbivores play many key ecological roles including contributing to nutrient cycling, acting as a food source for higher trophic levels, and controlling plant productivity and diversity (Bernays and Chapman 1994, Rohr et al. 2007). Plant-herbivore interactions are trophic and antagonistic, with herbivores directly dependent on their plant hosts but plants not having this direct dependency. Theory predicts that mutualistic and antagonistic networks will exhibit different interaction structures, responses that in turn influence community robustness to extinction cascades.

In a foundational theoretical paper that inspired the initial hypotheses for this dissertation, Thébault and Fontaine (2010) predicted that a modular structure increases the stability of antagonistic networks whereas nestedness increases the stability of mutualistic networks. The hypothesis that nestedness is stabilizing has been since challenged, and the idea that increased generality of interactions in a network is the underlying mechanism of leading to stability in mutualistic networks is a new theme (Blüthgen 2010, Blüthgen and Klein 2011, James et al. 2012, Suweis et al. 2013). I show that nestedness of plant-pollinator/floral visitor communities at the regional scale decreases with increasing temperature variability (Chapter 2), but increases in grasslands not grazed by bison at the landscape scale (Chapter 3). Modularity of plant-herbivore networks at the regional scale decreased with increasing temperature variability (Chapter 2), increased with the presence of bison grazers at the landscape scale (Chapter 4), and increased with increasing plant diversity at the local scale (Chapter 5). An alternative approach to this comparative approach is to use extinction simulation algorithms to calculate the predicted robustness of communities to future species loss (Memmont et al. 2004). Using this approach, the robustness of grassland communities of plant-floral visitor communities was not affected by grassland disturbances (Chapter 3), plant-herbivore increased with the presence of bison grazing (Chapters 4), and plant-herbivore robustness increased with local plant diversity (Chapter 5).

Ecological roles of arthropods and grassland systems are critical components of our biosphere. Arthropods as central components comprise more than half the biodiversity of named species (Price 2002) and yet are rarely the primary focus of conservation efforts. Grasslands are important habitats globally, but many have been lost and/or continue to be threatened by conversion to agricultural lands, fire suppression and urban expansion. North American tallgrass prairie, in particular, is an endangered ecosystem, with only ~4% of native habitat remaining (Samson and Knopf 1994, Hoekstra et al. 2005), making understanding of its dynamics and resilience, effective management, and restoration important conservation objectives. Collectively, the constituent chapters of this dissertation support the broader hypothesis that ecological gradients drive variation in plant-insect interaction network structural properties. Results from my dissertation reveal that network structure of grassland insect and plant communities vary across environmental gradients at multiple scales, including altered network structural properties with climate (Chapters 2 & 4), natural disturbance regimes (Chapters 3 & 4), and plant diversity (Chapter 5). While different network structural properties are predicted to stabilize plant-pollinator and plant-herbivore communities (Thébault and Fontaine 2010), the robustness of these two network types decreased across the same ecological gradients. Across latitudinal gradients, plant and insect communities showed decreased vulnerability under less variable temperature regimes (Chapter 2), while plant-floral visitor networks showed decreased nestedness in grasslands grazed by bison and increased network specialization in frequently burned grasslands (Chapter 3), vulnerability of plant-grasshopper interaction networks decreased in grasslands grazed by bison at the landscape level (Chapters 4), lower plant diversity increased vulnerability of both network types across a regional gradient (Chapter 2), and lower plant diversity increased vulnerability of plant-herbivore in a local scale experiment (Chapter 5). By studying ecological networks across ecological gradients, I identify communities in more variable climates, and grasslands without ungulate grazing with low plant diversity as those with increased susceptibility to extinction cascades. Identifying vulnerable communities can improve management and prediction of future susceptible communities. Overall, these results accentuate the importance of understanding how ecological communities and species interactions are determined by environmental drivers, a critical realization in our era of global change.

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Appendix A - Chapter 2 Supplementary material

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Appendix B - Chapter 3 Supplementary material

Supplemental Figures

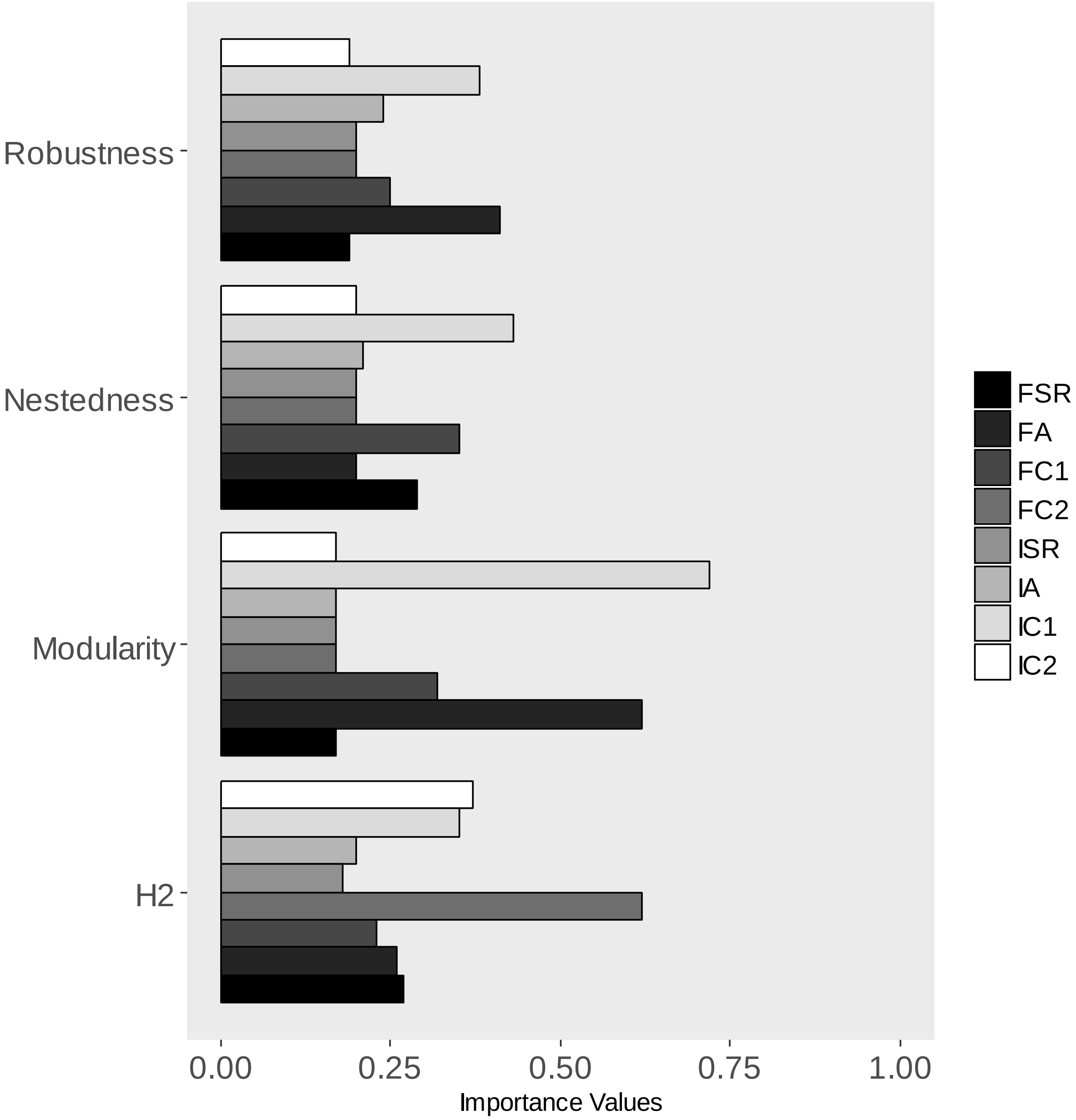

Figure B-1. Relative importance values from all models predicting effects of plant and insect communities on plant-floral visitor network structural properties. Plant and insect community variable abbreviations are listed in Fig. 3-2.

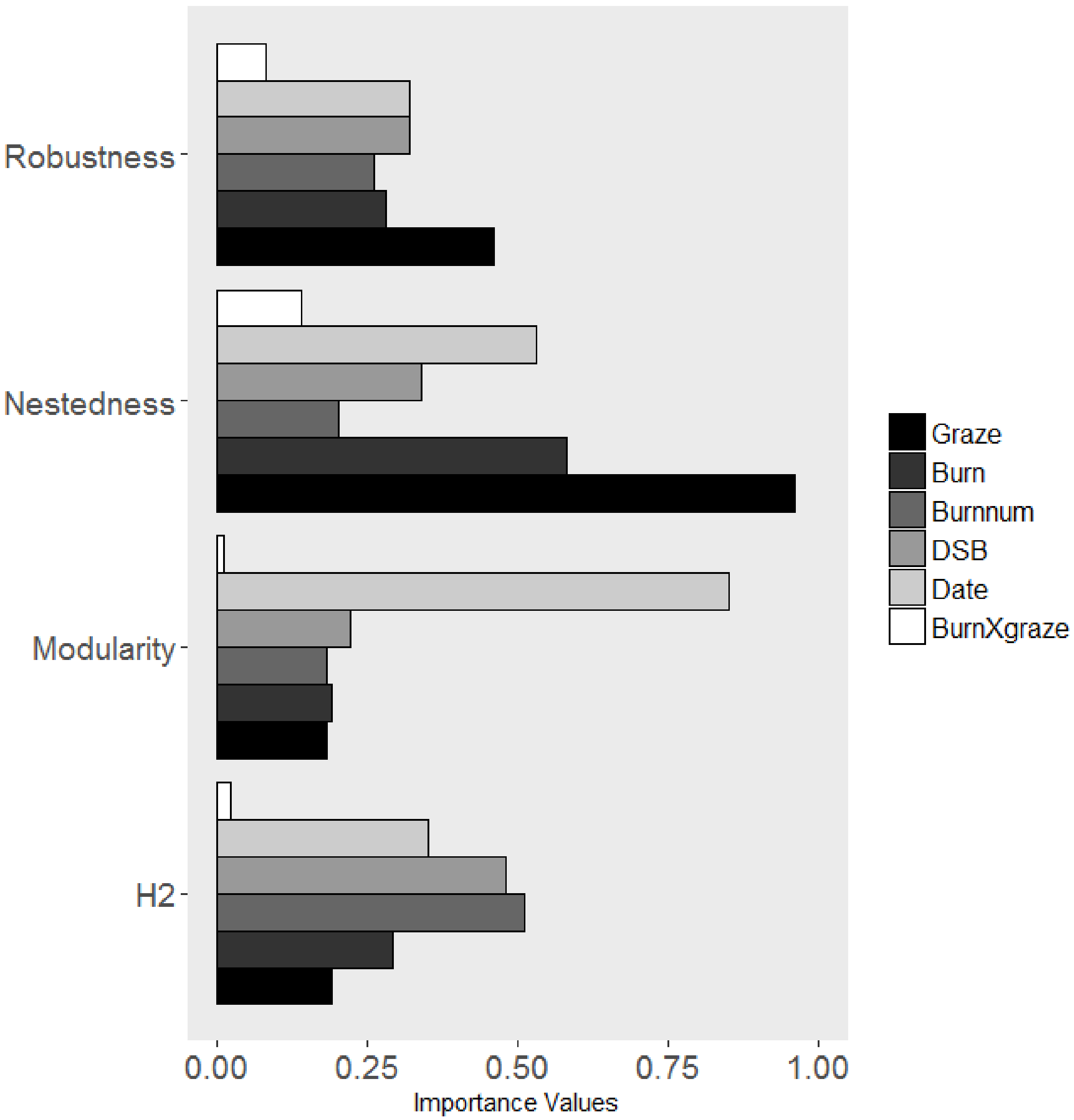

Figure B-2. Relative importance values from all models predicting effects of grassland disturbances on plant-floral visitor network structural properties. Disturbance variable abbreviations are listed in Fig. 3-2.

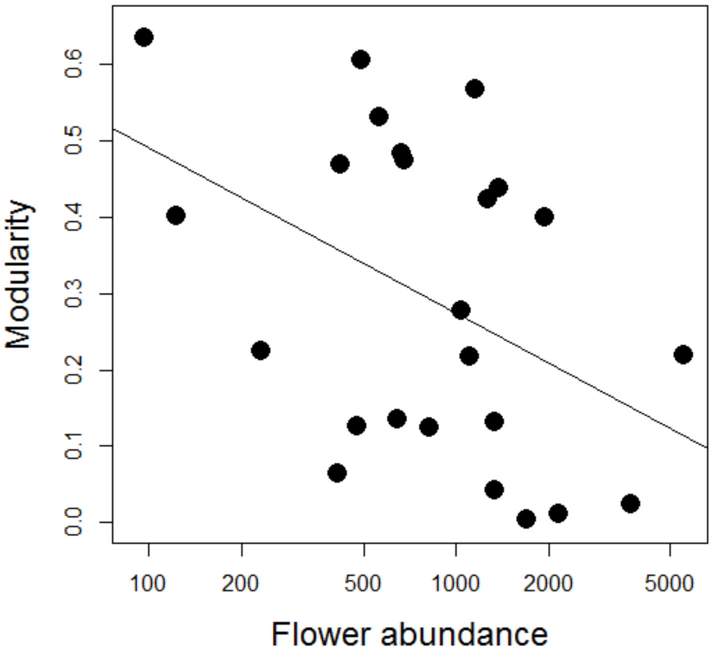

Figure B-3. The only relationship between plant and insect richness and abundance and measures of network structural properties with a relative importance value (RIV) >0.45 for 24 plant-pollinator networks from 12 sites and 2 temporal replications. RIVs for community predictors of network structural properties are given in Appendix B, Fig. B1. Modularity decreased with flower abundance ($F_{1,22}$=5.03, R^2=0.079, *P*=0.035).

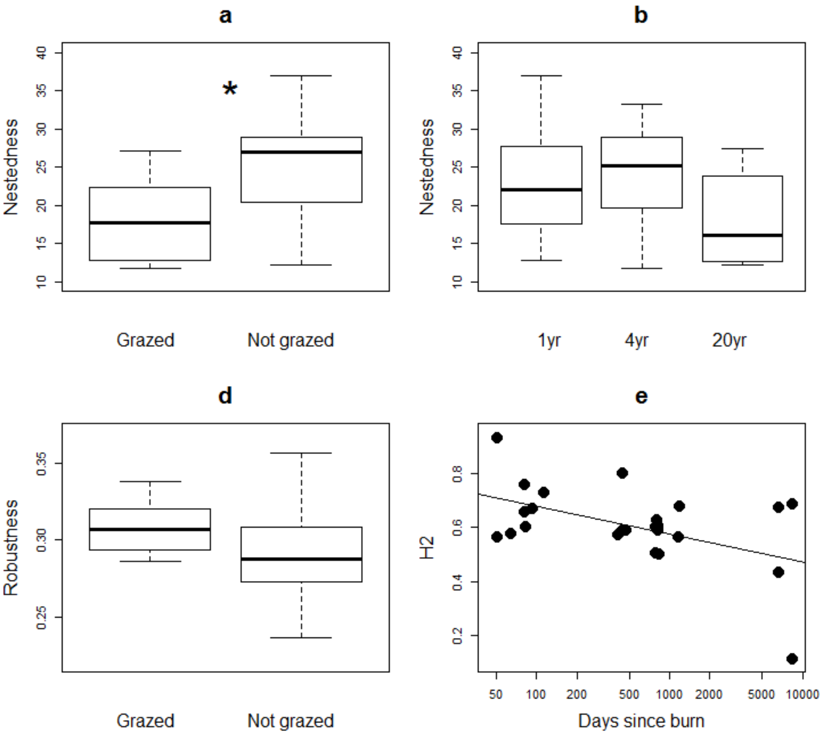

Figure B-4. Relationships between grassland disturbance variables and measures of network structure with relative importance values (RIV) >0.45 and for 24 plant-pollinator networks from 12 sites and 2 temporal replications. RIVs for grassland disturbance predictors of network structural properties can be found in Appendix B, Fig. B2. Nestedness was significantly lower in grazed watersheds (a; t=-2.81, df=20.66, *P*=0.011), was not significantly different across fire return intervals (b; n=24, $F_{2,21}$=1.73, *P*=0.20) and was not significantly different between sampling dates (c; t=1.45, df=21.62, *P*=0.16). Robustness was not significantly different between grazing treatments (d; t=1.42, df=15.56, *P*=0.18). H2 significantly decreased with days since burn (e; $F_{1,22}$=6.02, R^2=0.18, *P*=0.023). Modularity was significantly higher in July compared to June (f; t=-2.57, df=21.75, *P*=0.018).

Appendix C - Chapter 4 Supplementary material

Supplemental Table and Figures

Table C-1. Number of sampled individuals, number of sequences from gut content, and identifying barcode used for each grasshopper species.

Grasshopper species	Individuals sampled	Number of identified sequences	Barcode
Ageneotettix deorum	20	83,507	ACGAGACTGATT
Arphia conspersa	4	2,627	GCTGTACGGATT
Arphia simplex	20	10,065	TGGTCAACGATA
Arphia xanthoptera	20	76,254	ATCGCACAGTAA
Boopedon gracile	20	40,985	GTCGTGTAGCCT
Boopedon auriventris	1	6,599	AGCGGAGGTTAG
Brachystola magna	5	64,474	ATCCTTTGGTTC
Campylacantha olivacea	20	11,819	TACAGCGCATAC
Encoptolophus sordidus	17	53,630	AATTGTGTCGGA
Hesperotettix speciosus	20	56,885	ACCAGTGACTCA
Hesperotettix viridis	20	135,133	GTAGATCGTGTA
Hypochlora alba	20	136,117	TAACGTGTGTGC
Melanoplus bivittatus	20	20,494	CATTATGGCGTG
Melanoplus differentialis	1	26,548	CCAATACGCCTG
Melanoplus femurrubrum	20	10,161	GATCTGCGATCC
Melanoplus sanguinipus	2	77,155	CAGCTCATCAGC
Melanoplus keeleri	20	1,207	CAAACAACAGCT
Melanoplus packardii	20	25,046	GCAACACCATCC
Melanoplus scudderi	20	276	AGTCGTGCACAT
Mermiria picta	20	36,171	CAAATTCGGGAT
Orphulella speciosa	20	29,289	AGTTACGAGCTA
Paratylotropidia brunneri	1	2,001	GCATATGCACTG
Pardalophora haldemani	20	88,460	CACTACGCTAGA
Phoetaliotes nebrascensis	20	44,720	ACCATAGCTCCG
Schistocerca lineata	20	266	TCGACATCTCTT
Syrbula admirabilis	20	15,302	GAACACTTTGGA

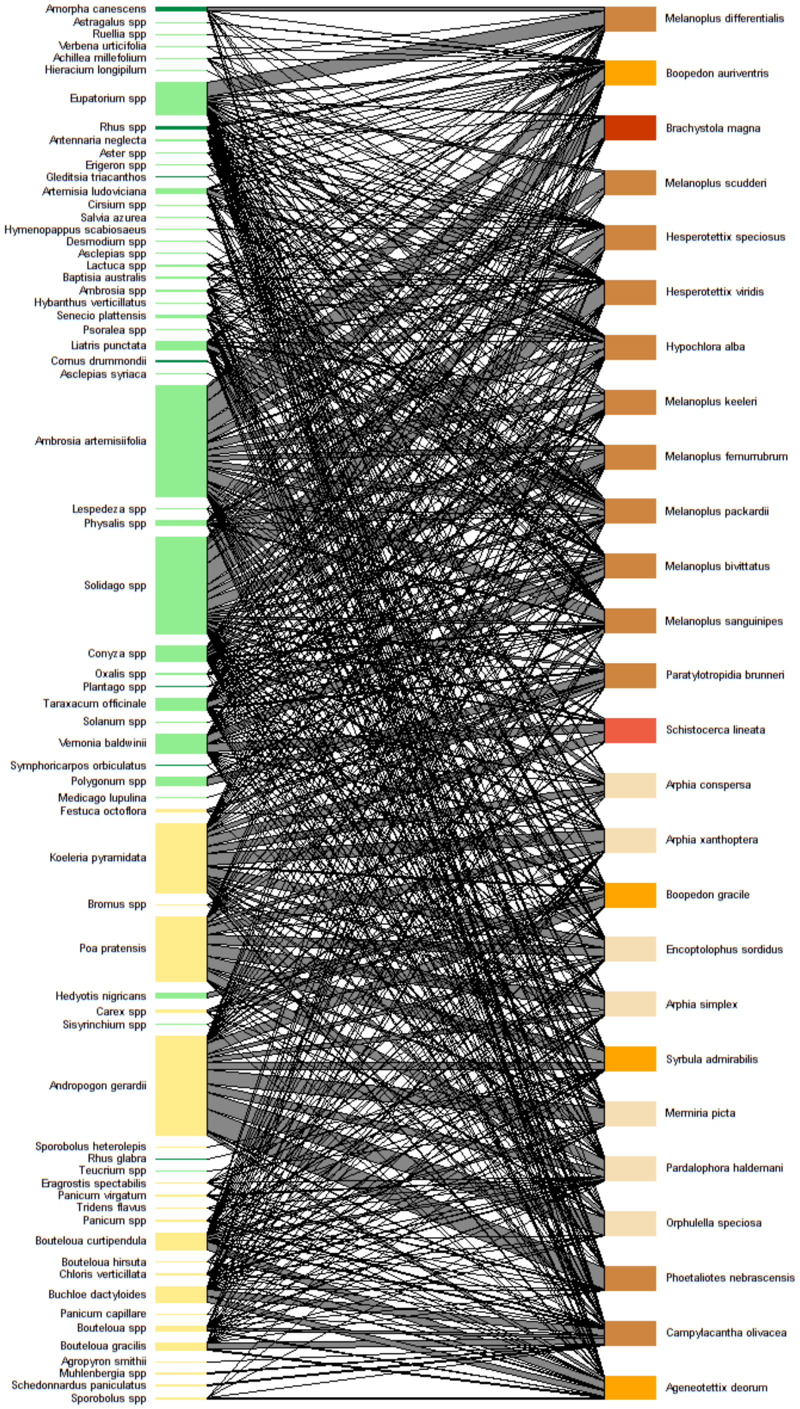

Figure C-1. Full Konza plant-grasshopper network. Bars on the left represent plant species with colors indicating growth form (woody plants=dark green, forbs=light green, and grasses=yellow). Bars on the right represent grasshopper (primarily Acrididae) species with different colors indicating different subfamilies (Cyrtacanthacridinae=salmon, Gomphocerinae=orange, Melanoplinae=brown, Oedipodinae=light tan, and the family Romaleidae=red). Black and gray lines connecting herbivore and plant species represent grasshopper feeding interactions with widths indicating proportion of grasshopper diet.

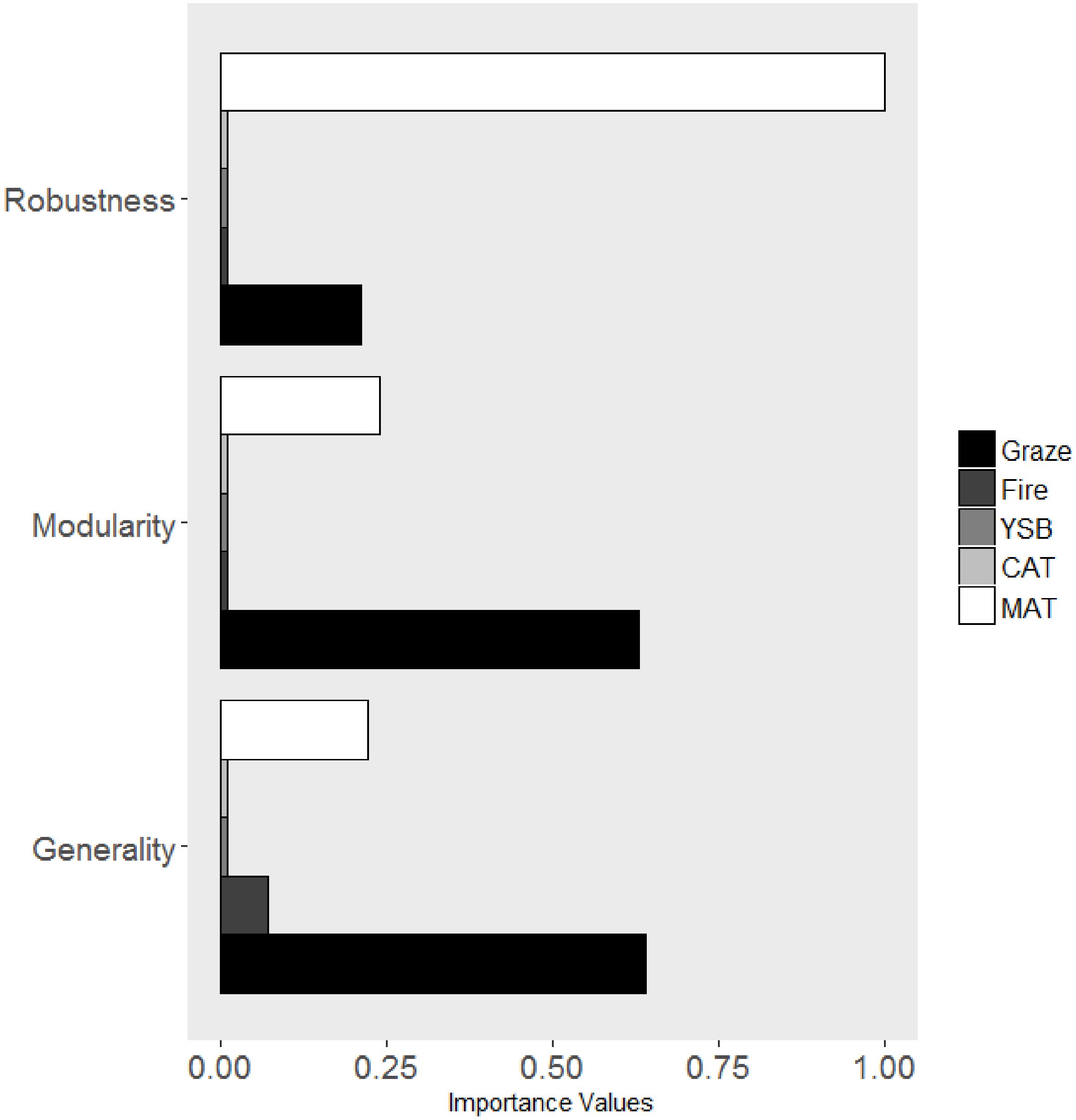

Figure C-2. Relative importance values, a summed and standardized indicator of predictor variable rank across all possible models, for grassland driver predictors (grazing = presence/absence of bison grazers, fire = historical burn interval, YSB = years since last burn, CAP = cumulative annual temperature, MAT = mean annual temperature) of the network structural properties of grasshopper robustness, modularity, and generality of grasshopper diets. Top models with $\Delta AIC_c<2$ are given in Table 4-1.

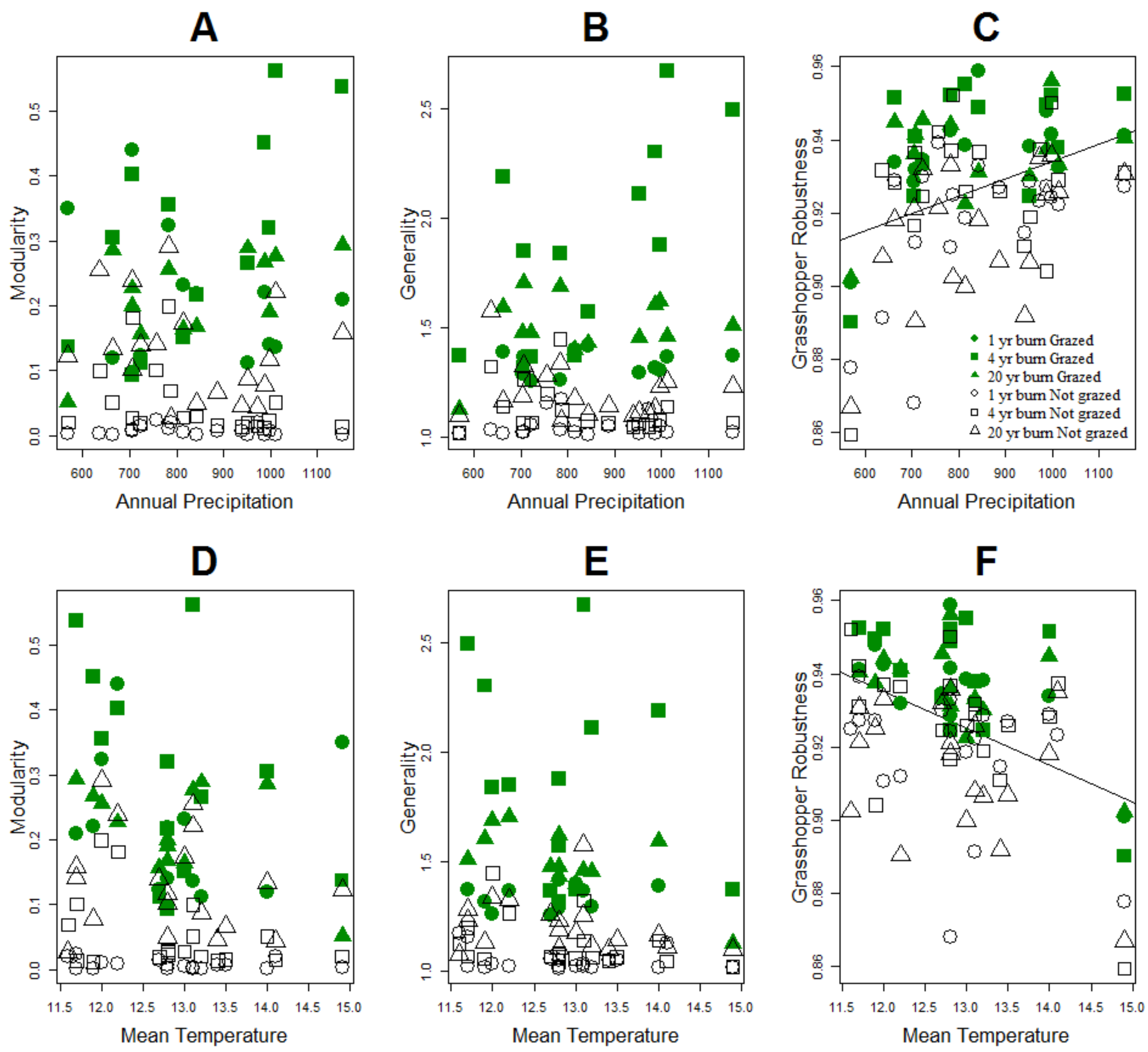

Figure C-3. Regressions for relationships depicted in Fig. 4-3.

Appendix D - Chapter 5 Supplementary material

Supplementary Figures

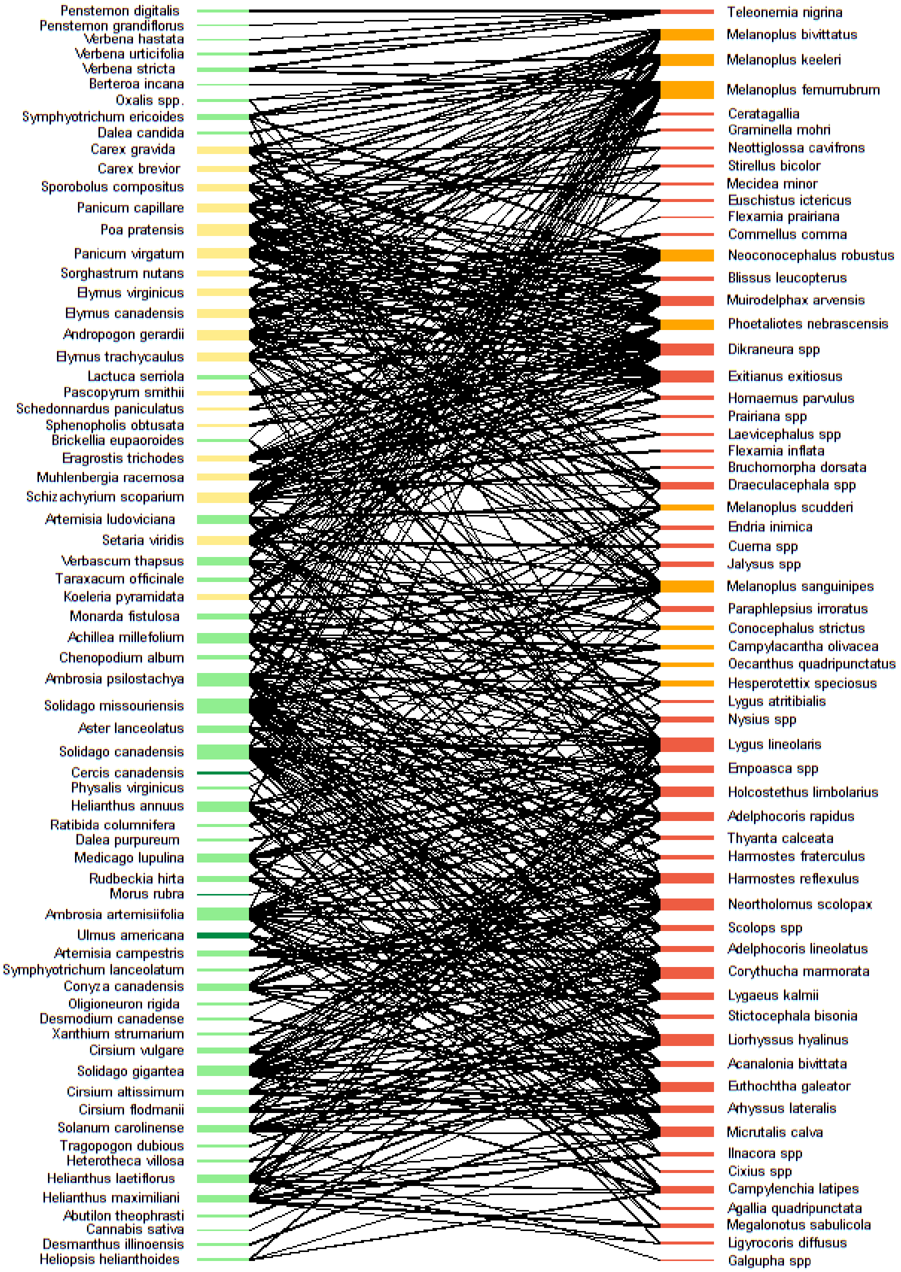

Figure D-1. All interactions (presence/absence) between plant and herbivore species sampled in study plots and found in the literature review. Plant species are represented by bars on the left (yellow bars=grasses, light green bars=forbs, and dark green bars=woody plants. Herbivore species are represented by bars on the right (salmon bars=Hemipterans and orange bars=Orthopterans). Black lines indicate the presence of trophic links.

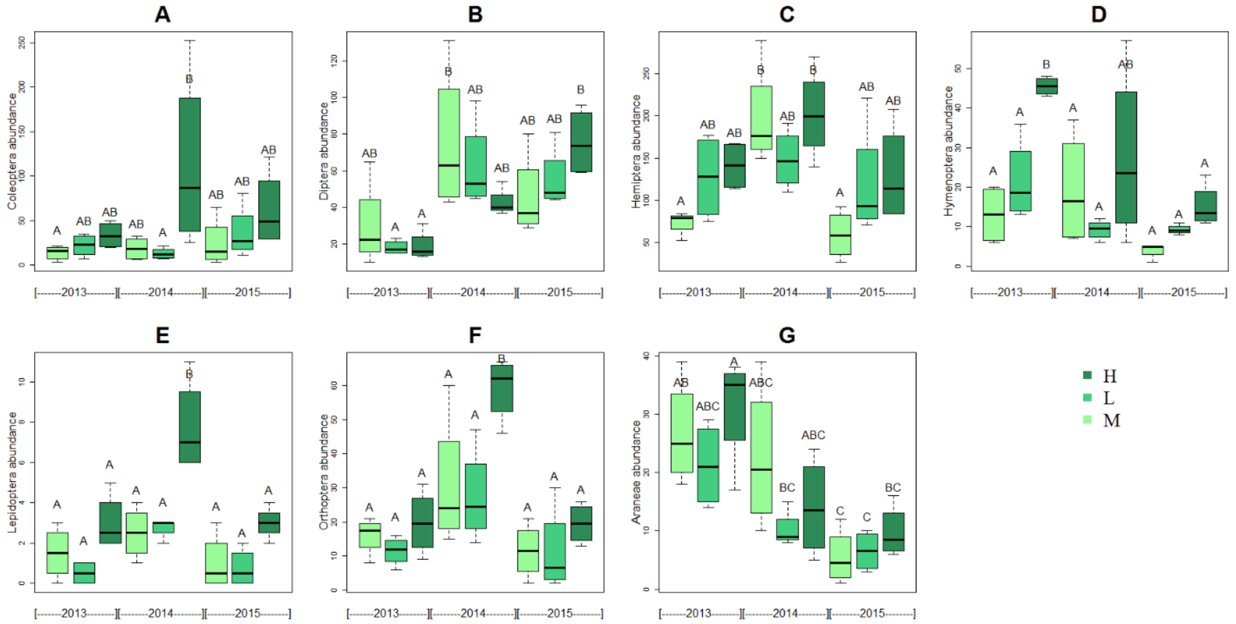

Figure D-2. Changes in abundance of arthropod Orders across plant diversity treatments and years for the seven most abundant orders: Coleoptera (A), Diptera (B), Hemiptera (C), Hymenoptera (D), Lepidoptera (E), Orthoptera (F), and Araneae (G). Plant diversity treatments include high diversity (H, in the darkest green), low diversity (L in medium green) and monoculture treatment (M, in the lightest green). Letters represent the results of Tukey's Honestly Significant Differences test (α=0.05).

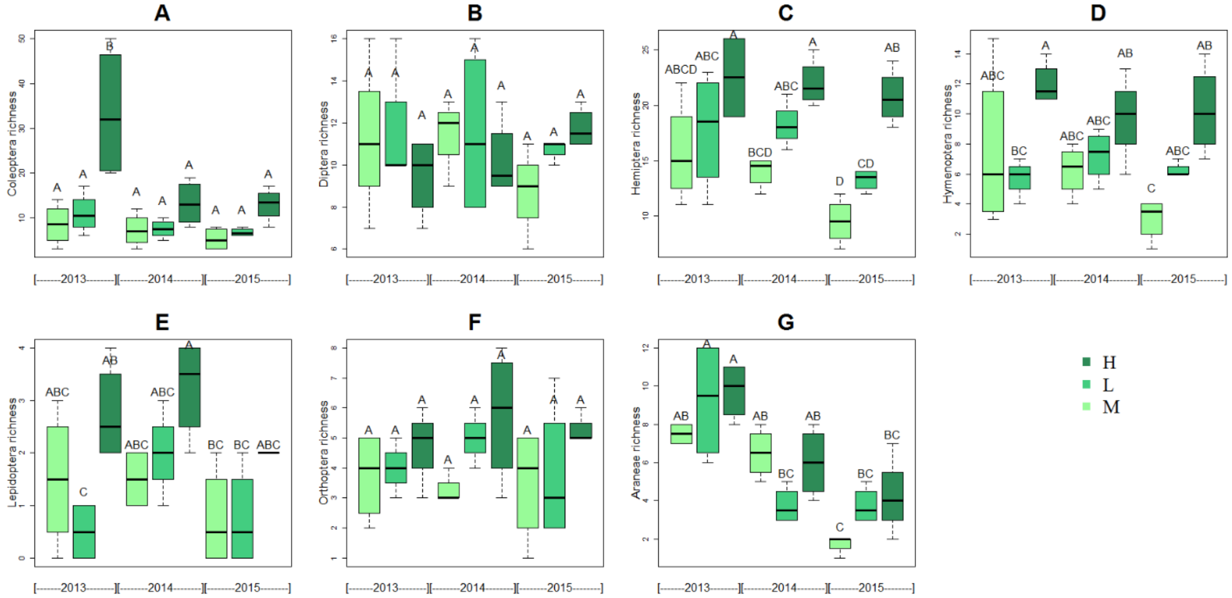

Figure D-3. Changes in the richness of arthropod Orders across plant diversity treatments and years for the seven most abundance Orders: Coleoptera (A), Diptera (B), Hemiptera (C), Hymenoptera (D), Lepidoptera (E), Orthoptera (F), and Araneae (G). Plant diversity treatments include high diversity (H, in the darkest green), low diversity (L in medium green) and monoculture treatment (M, in the lightest green). Letters represent the results of Tukey's Honestly Significant Differences test (α=0.05).

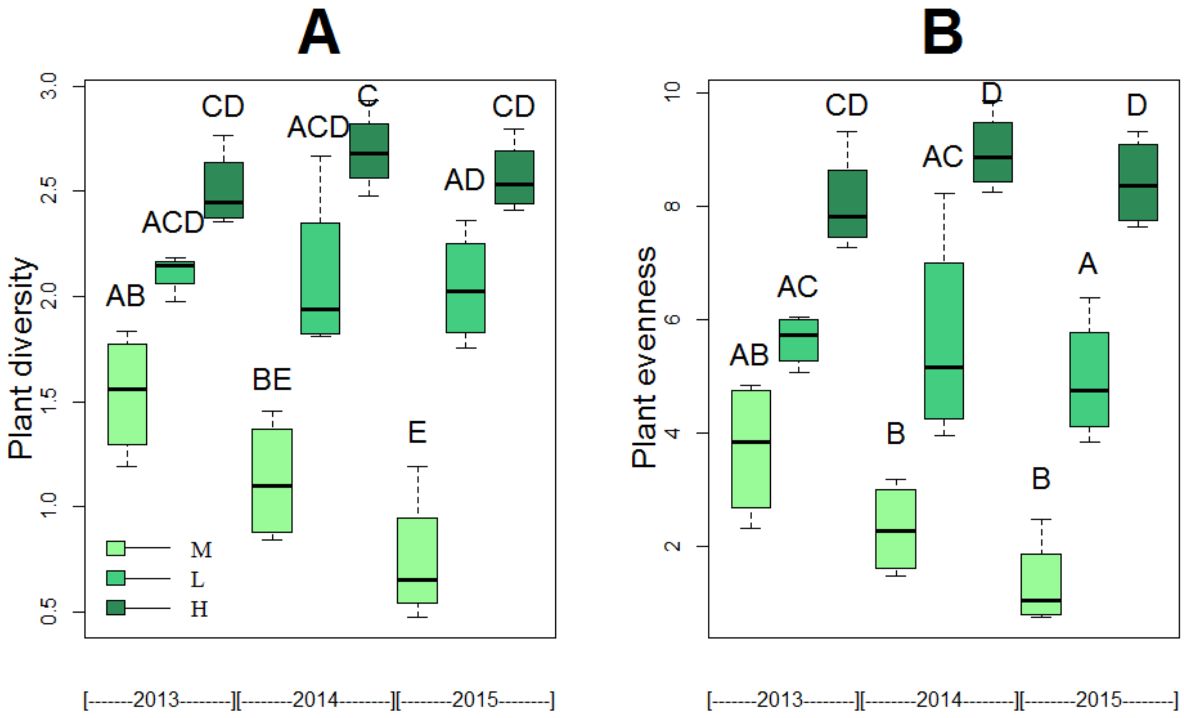

Figure D-4. Changes Shannon's diversity (A) and evenness (B) for the plant community across plant diversity treatments and years of sampling. Plant diversity treatments include high diversity (H, in the darkest green), low diversity (L in medium green) and monoculture treatment (M, in the lightest green). Letters represent the results of Tukey's Honestly Significant Differences test (α=0.05).

Data source references for herbivore diets

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