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Grassland plant community assembly: The role of environmental heterogeneity, evolutionary history, competition, and pollination

by

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Dedication

To Amy for her unending support and for keeping me calm throughout this whole process, and to my parents, Tom and Catherine, who have always supported me in everything, no matter how far off the track I've gone and how long it's taken me to get where I am going.

Abstract

Plant community assembly is not a simple process; any factor that can affect the recruitment or coexistence of individuals can alter the outcome (e.g. nutrients, symbionts). In this thesis, I address a diverse subset of these potential factors, focusing on environmental variation, evolutionary history, competition, and pollination. I begin by testing whether evolutionary history constrains how species respond to 14 environmental factors. From this study, I conclude that evolutionary history has a weak effect on how species respond to changes in their environment, but that it can be important under certain circumstances. Next, I test whether competition is stronger when neighboring species are more related, which is hypothesized to leave a phylogenetic signature on plant communities if competition is important in community assembly. Competition did not increase with relatedness, potentially because competition was diffuse. As such, there was no measurable signature within the community. From these results, I expand existing theory to explore the conditions where competition should leave a phylogenetic signature. The importance of competition in community assembly is expected to increase or remain invariant with productivity, depending on the theory. I tested these ideas using a competition experiment with 22 species, but found competition declined with productivity, which is consistent with theories emphasizing resource supply and demand. Community assembly can also be dictated by recruitment, but few studies address how pollination, an important step in the recruitment process, varies across the community. I used a broad survey to examine whether pollination varied with environmental conditions and a

manipulative experiment to test whether pollination can be predicted by abundance changes. Both flowering and flower visitation were highly dependent on environmental conditions and while they were correlated with abundance, they responded independently to environmental manipulations. This suggests that pollination and potentially seed production may become decoupled from abundance under a variety of conditions. Combined, my results suggest that many processes contribute to community assembly and that each of these processes is only important under certain conditions. More generally, my results cast doubt on the presence of general assembly rules that are applicable beyond the very smallest scales.

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1. General introduction

Community assembly is a complicated process that can be affected by the interaction between multiple species characteristics, the environment, and other organisms (Weiher *et al.* 2011; HilleRisLambers *et al.* 2012). The goal of this thesis is to explore some of the factors that influence plant community assembly within a single grassland plant community. Specifically I investigate how assembly is affected by evolutionary history, competition, environmental variation, and sexual reproduction at the University of Alberta research ranch at Kinsella, Alberta.

1.1. The study site

The Kinsella site ($53^{o}05$ N, $111^{o}33$ W) is an ideal research site for many reasons, not the least of which is that the ranch is one of the largest intact fescue grassland / parkland sites in Canada. The Kinsella site is a 10,000 ha research ranch with large tracts of land that have never been seeded or tilled and a grassland plant community that is species rich and composed almost entirely of species native to the region (J.A. Bennett, unpublished data). Such a pristine grassland community is uncommon these days and presents an excellent opportunity to study grassland community dynamics without many of the human-induced impacts that are changing the structure of grasslands globally (Hoekstra *et al.* 2005).

Research on the plant communities at the site has been conducted for at least 25 years and likely longer (Bailey *et al.* 1990). However, over the last 13 years, an area of approximately 50 ha has been studies intensively by the Cahill

lab group. This body of research has provided a wealth of information from which I developed my research ideas. From this research I learned that related species within the community are often morphologically similar (Kembel & Cahill 2011), which suggested that evolutionary history may influence species function (see Section 1.2). I also learned that competition is mostly belowground (Cahill 2003), but may not have important consequences for community structure (Lamb & Cahill 2008). However, competition can also be very intense and depend on small scale environmental conditions (Lamb *et al.* 2007), which suggested that it may still have important effects within the community (see Sections 1.3 and 1.4). However, competitive outcomes may be influenced by mycorrhizal associations for many species, with potential consequences for pollination (Cahill *et al.* 2008a; see Sections 1.5 and 1.6). Each of these ideas and many more directed my investigations into community assembly and helped me develop the ideas that you will read throughout.

1.2. Evolutionary history and niche conservatism

Plant species vary in their niches, which causes plant populations to respond idiosyncratically to changes in their environment (Path A; Figure 1.1). To help understand how plant populations respond to these changes and how communities are assembled, predictive frameworks have been developed using species traits (e.g. Grime 1977; Westoby 1998). Based upon the idea that related species are more ecologically similar (Darwin 1859) and therefore that niches are conserved, phylogenetic relationships have been used with some success to explain variation in how species respond to both biotic (Burns & Strauss 2011; Reinhart *et al.*

2012) and abiotic (Prinzing 2001; Niinemets & Valladares 2006) elements of their environments (Path B; Figure 1.1). If true, then the phylogenetic relationships among coexisting species may be used to predict the outcome of community assembly (Path C; Figure 1.1); however, there is some doubt about the generality of such a statement (Losos 2008).

For related species to be generally ecologically similar requires a number of conditions to be met. First, functionally important traits must be conserved (Webb *et al.* 2002), yet we know that ecologically relevant traits, at least among plants, are often evolutionarily labile (Cavender-Bares *et al.* 2006; Grime 2006) or plastic (Burns & Strauss 2012). Second, functional convergence (same function, different traits) must be limited, yet we know that there are many ways to cope with a particular challenge (e.g. the evolution of multiple distinct defensive compounds in different lineages; Howe & Jander 2008). Third, for closely related species to be broadly similar, many traits must be conserved, such that there is limited variation in how related species respond to most factors, yet related species often exhibit different responses to many factors. For example, legume species (Fabaceae) fix nitrogen and generally do well in nitrogen poor environments (Craine *et al.* 2002). However, legume species vary in how they respond to herbivory (Ritchie & Tilman 1995) and drought (Pang *et al.* 2011). Thus, these species share some characteristics and part of the niche is conserved, but many aspects of the niche are not conserved. As such niche conservatism is context dependent and most likely rare, suggesting that phylogeny may not be the best predictor of the assembly process.

In Chapter 2, I explore whether or not plant responses to different environmental drivers (e.g. herbivory, nutrients, light, mycorrhizae, etc.) are conserved. Using data from six experiments conducted at my focal field site, I test for niche conservatism in plant responses to a series of ecological factors and factor groupings to test 1) whether niche conservatism is common at the site and 2) whether conservatism is more likely if we consider conserved responses to a group of related factors, rather than to individual factors.

1.3. Evolutionary history and competition

Competition is often inferred to be an important community assembly process by identifying patterns of species co-occurrence that are used as a 'signature' of competition (Diamond 1975; Gotelli & McCabe 2002); if species co-occur less often than expected by chance, then competition is often assumed to have driven the spatial separation of these species. This approach has recently been expanded to incorporate patterns in species traits (e.g. Spasojevic & Suding 2012) and relatedness (e.g. Cavender-Bares *et al.* 2006). Here the expectation is that more similar or closely related species should compete more strongly (Paths D and E; Figure 1.1), causing competitive exclusion and resulting in the community containing species less similar or less related than would be predicted by a null model, which is often referred to as overdispersion (Path F; Figure 1.1). Thus, competition is assumed to be causing these patterns of overdispersion.

Inherent to approaches where patterns of overdispersion are used to infer competition as an assembly process are two assumptions. The first assumption, the "competition-relatedness hypothesis" *sensu* Cahill et al. (2008b), dates back

to Darwin (1859) and assumes that more closely related species compete more strongly. However, evidence supporting this assumption is mixed (Cahill *et al.* 2008b; Burns & Strauss 2011; Violle *et al.* 2011; Best *et al.* 2013) and may depend on the nature of the traits affecting the outcome of competition (Best *et al.* 2013; Cahill 2013). The second assumption is that only competition can lead to phylogenetic overdispersion, yet many processes, such as facilitation (Valiente-Banuet & Verdú 2007) and herbivory (Webb *et al.* 2006) can also result in phylogenetic overdispersion. Further, coexistence theory suggests that competition can leave many different signatures (Mayfield & Levine 2010) and plant competition theory suggests that only certain types of competition are likely to have an effect on community structure (Lamb *et al.* 2009). In Chapter 3, I test these assumptions using data from a published competition experiment conducted in the field (Lamb & Cahill 2008) and a survey conducted at different spatial scales, where competition is expected to be strongest at small spatial scales.

1.4. Competition and environmental variability

Although plant competition is widely regarded as one of the processes affecting community assembly (Path F; Figure 1.1), its relative importance is still debated. This debate is, in part, a result of the variability in the strength of competition across space and time (Path G; Figure 1.1). The most commonly studied, and contentiously debated, factor affecting competition is productivity (e.g. Grace 1991; Cahill 1999; Goldberg *et al.* 1999; Craine 2005; Brooker & Kikividze 2008). There are two main theoretical frameworks used to understand the relationship between competition and productivity, those of Grime (1973,

1979) and those of Tilman (1982, 1988). According to Grime, competition and competitive exclusion are only important for community assembly at high productivity, whereas stress is more important at low productivity (1973, 1979). Conversely, Tilman (1982, 1988) suggested that competition would be stable along productivity gradients, but would switch from competition for soil resources to competition for light as productivity increased. These two theories have dominated the discussion about the role of competition in community assembly. However, while empirical results are mixed, the trend is that competition declines with productivity, which supports neither theory (Goldberg *et al.* 1999).

Other theories have been developed to explain how competition affects plant communities; some of which may explain the decline in competition with productivity. The stress-gradient hypothesis (SGH) predicts that facilitation among plant species will increase with stress, which is often approximated by measuring productivity (Bertness & Callaway 1994; Callaway 1995; Maestre *et al.* 2009). Therefore, its predictions are identical to Grime's in that competition should increase with productivity. However some versions of the hypothesis have produced predictions that show that facilitation may increase, and thus competition decline, over certain ranges of productivity (Bertness & Callaway 1994; Holmgren & Scheffer 2010). Alternatively, by considering the supply and demand of resources (Taylor *et al.* 1990; Davis *et al.* 1998), competition can decline as either net resource supply (supply - demand) (Davis *et al.* 1998) or the ratio of supply to demand (Taylor *et al.* 1990) increases. Thus competition could

potentially decline with productivity, depending on the relationship between productivity, resource supply, and resource demand.

Considering each of these theories, there are predictions of increasing, stable, and declining competition with productivity. In Chapter 4, I investigate these predictions using results from a competition experiment that tested this relationship using a large number of species within a single site. Further, I attempt to address some of the potential causes of disagreement among these theories by differentiating between size and survival using indices of both competitive intensity and competitive importance along gradients of both neighbor biomass and resource availability.

1.5. Pollination and community structure

Although the majority of theory related to plant community assembly focuses on the vegetative abundance of populations within the community, sexual reproduction can potentially play an important role (Path H; Figure 1.1). Relative pollen limitation and thus seed production among members of the community can affect relative propagule pressure and therefore community composition (Knight *et al.* 2005; Sargent & Ackerly 2008; Runquist & Stanton 2013). While there have been many studies examining the effect of seed limitation on plant communities (Clark *et al.* 2007; Aicher *et al.* 2011), few have studied any portion of the process of seed production at the community level (e.g. Cahill *et al.* 2008a; Burkle & Irwin 2010).

In this thesis, I take two approaches to understanding how pollination could affect community assembly. First, in chapter 5, I use an observational

approach to identify the factors that determine which patches of flowers bees choose to forage in. Second, in chapter 6, I use an experimental approach to determine whether changes in local environmental conditions can affect the diversity of plants reproducing sexually.

1.5.1. Bee patch choice

Flower patches that are less frequently visited by bees and other pollinators are more likely to be pollen limited and produce less seed (Knight *et al.* 2005). Given that most seeds do not disperse far from the parent plant (Nathan & Muller-Landau 2000), understanding conditions that influence where pollinators prefer to forage can also identify conditions where seed limitation is less likely (Path I, Figure 1.1). Bees are the most important pollinator in many ecosystems and can have a large influence on the reproductive dynamics of plants (Winfree *et al.* 2011). Thus, understanding the factors affecting their foraging decisions can be potentially important for understanding community assembly. Which flower patches bees choose to visit are in part a function of local flower availability, with flower abundance (Potts *et al.* 2003; Hegland & Boeke 2006) and diversity (Potts *et al.* 2003; Ghazoul 2006) affecting where bees forage. However, bee abundances (Kearns *et al.* 1998; Steffan-Dewenter & Tscharntke 2002) and foraging decisions (Goverde *et al.* 2002; Diekötter *et al.* 2007) are also influenced by local landscape structure and composition. In turn, landscape structure can influence the availability of floral and nesting resources (Roulston & Goodell 2011). Given the interdependence of the local landscape and resource

availability, separating the specific determinants of bee foraging decisions under natural conditions remains challenging.

In Chapter 5, I use structural equation modeling to (1) identify the relative importance of local landscape configuration and the flower community on bee patch use, and (2) determine which aspects of the landscape and plant community most affect bee habitat use at the site.

1.5.2. Environmental variability and the decoupling of vegetative and reproductive responses

Although few studies have examined pollination at the community scale, plants vary in how they allocate resources between reproduction and vegetative growth (Niklas 2004; Fortunel *et al.* 2009), and this allocation can depend on environmental conditions (Niu *et al.* 2008; Bonser & Aarssen 2009). The attractiveness of plants to pollinators also depends on environmental conditions (Becklin *et al.* 2011) and varies among species (Knight *et al.* 2005). If both flowering and pollination depend on environmental conditions and if this varies among species, then environmental conditions should cause differential reproduction across the community (Path I; Figure 1.1). Such changes have potentially large implications for community assembly (Sargent & Ackerly 2008; Runquist & Stanton 2013). Thus, I suggest that any understanding of community assembly is incomplete without some understanding of pollination. Consequently, any estimates of community change without accounting for potential differences in seed production are likely inaccurate in the long term.

In Chapter 6, I test whether vegetative and reproductive responses to changes in environmental conditions are decoupled after experimental manipulation of mycorrhizal fungi, soil nutrients and plant litter (Paths I and J; Figure 1.1). Further, I link the changes in the diversity of species present, flowering, and being visited to treatment-induced changes in the abiotic (light, nitrogen, water, and soil temperature) and the biotic (litter mass, live biomass, relative graminoids abundance, and flowering phenology) environment.

1.6. Thesis outline

This thesis highlights the work I have done over the course of my PhD relating to community assembly (Figure 1.1). In Chapter 2, I investigate how evolutionary history and environmental variation affect niche conservatism and thus community assembly. For Chapter 3, I examine how evolutionary history and niche conservatism influence the effects of competition on community assembly. Similarly, in Chapter 4, I look at the role of environmental variation in influencing the outcome of competition and ultimately its importance in determining community dynamics. In Chapters 5 and 6, I look at the role of environmental variation in pollination, and in Chapter 6, I extend this to identify mechanisms by which environmental variation and pollination can affect community assembly.

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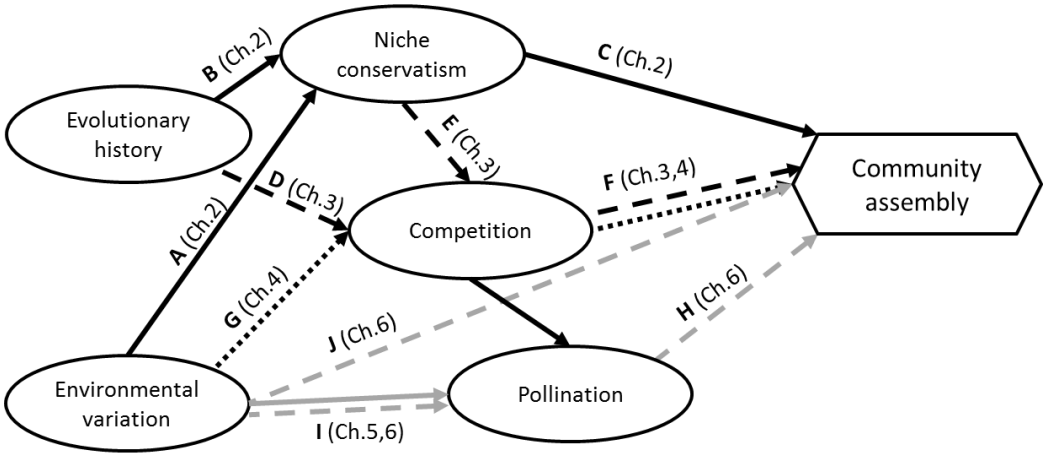

Figure 1.1 The linkages between each of the topics investigated throughout the thesis and their potential role in community assembly. Pathways explored in chapter 2 are shown in solid black lines, chapter 3 - dashed black, chapter 4 – dotted black, chapter 5 – solid grey, chapter 6 – dashed grey.

2. Patterns of niche conservatism vary between monocots and eudicots in response to above- and belowground factors[1].

2.1. Introduction

Plant populations often respond idiosyncratically to changes in their environment (Tilman 1987; Turkington *et al.* 2002). Efforts have been made to identify species characteristics that can be used to develop a predictive framework for changes in the relative abundance of plant populations (e.g. Grime 1977; Westoby 1998). Based upon the idea that related species are more ecologically similar (Darwin 1859), hypothesized patterns of descent (e.g. a phylogeny) have been used with some success in determining how species respond to both biotic (Burns & Strauss 2011; Reinhart *et al.* 2012) and abiotic (Prinzing 2001; Niinemets & Valladares 2006; Willis *et al.* 2008) elements of their environments. Further, many ecological factors differentially affect certain lineages within the community, causing phylogenetic clustering (Verdú & Pausas 2007; Helmus *et al.* 2010). This suggests that phylogeny can be used as a tool to predict species responses to changes in their environment, but for phylogeny to be a useful predictor of ecological responses, the niche must be conserved. However, the prevalence of niche conservatism has been questioned (Knouft *et al.* 2006; Silvertown *et al.* 2006; Losos 2008; Lavergne *et al.* 2010).

Niche conservatism can have multiple definitions (Losos 2008). Here, we define niche conservatism broadly as the tendency of related species to respond similarly to ecological challenges (Wiens & Graham 2005; Wiens *et al.* 2010).

[1] A version of this chapter is in revision. Bennett J.A. and Cahill J.F. Jr. in revision at Perspectives in Plant Ecology, Evolution, and Systematics.

While phylogenetic relatedness is often considered an integrative measure of functional similarity (Webb *et al.* 2002; Mouquet *et al.* 2012), for plants, ecologically relevant traits are often evolutionarily labile (Cavender-Bares *et al.* 2006; Grime 2006) or exhibit plasticity (Berg & Ellers 2010; Burns & Strauss 2012). Further, there are many ways to respond to ecological challenges. For example, defensive compounds are produced using different pathways, but all reduce herbivory (Howe & Jander 2008), and competitive response is associated with many traits representing different ways of coping with reduced resource availability (Wang *et al.* 2010). Additionally, traits may be associated with multiple functions, yet multiple traits may determine a species functional response to a given factor. High volumes of fine roots can increase both nitrogen and water uptake (Craine *et al.* 2003), yet plants require separate traits to cope with low water or low nitrogen environments (Craine 2009). Thus, if a trait is associated with multiple functions, conservatism of that trait does not mean that all of the functions associated with that trait, as multiple traits would need to be conserved for this to be true. Many of the traits necessary to respond to ecological challenges also involve functional trade-offs. As a consequence, plant species may be suited to cope with certain environmental conditions, but not others. Thus, for many reasons, ecological responses are often less conserved than morphological or physiological traits (Prinzing 2001; Losos 2008) and further testing is required to determine the extent of niche conservatism.

When suites of traits appear to confer specific functioning, they have often been grouped into plant functional strategies (Westoby 1998; Reich *et al.* 2003).

Most commonly, plant strategies are associated with responses to resource availability and disturbance, where some species are adapted to quick growth and rapid resource acquisition, while others are adapted to disturbances such as herbivory (Grime 1977; Reich *et al.* 2003; Craine 2009). Responses to both resources and herbivory often vary across broad, phylogenetically distinct functional groups (Coughenour 1985; Lavorel *et al.* 1997; Turkington *et al.* 2002; Niinemets & Valladares 2006), yet the evidence for conservatism of traits representing these plant strategies is mixed (Diaz *et al.* 2004; Brunbjerg *et al.* 2012). While there are a few experimental tests for conservatism of plant strategies, to our knowledge, no studies have tested whether plant responses to multiple factors related to these strategies are conserved.

Plant strategies require coordinated responses to multiple environmental factors, both above- and belowground, and thus that select root and shoot traits co-vary. There is evidence for such covariance (Craine *et al.* 2001; Craine *et al.* 2002), although they may have evolved independently (Kembel & Cahill 2011). Individual root and shoot traits show varying degrees of conservatism (Grime & Mackey 2002; Diaz *et al.* 2004; Kembel & Cahill 2005; Anderson *et al.* 2011; Kembel & Cahill 2011; Comas *et al.* 2012), as do plant responses to various above and belowground factors (Prinzing 2001; Niinemets & Valladares 2006; Silvertown *et al.* 2006). However, it is unclear whether plant responses to either aboveground or belowground factors as groups would be phylogenetically conserved and there are no published experimental studies designed to test these ideas.

Conservatism of plant responses may also be related to the strength of a factor's effect, with the expectation that responses to factors with stronger effects are more likely to be conserved. Across all vascular plant species, much of the trait variation occurs between monocots and eudicots (Craine *et al.* 2001; Kembel & Cahill 2005). This has long been recognized by ecologists, with graminoids and forbs generally treated as distinct functional groups. Functional differences between graminoids and forbs often affect community structure and function (McLaren & Turkington 2010), and, by definition, the evolution of graminoid species, particularly grasses, was a major step in the formation of grassland ecosystems (Linder & Rudall 2005). We should then expect the factors with the greatest effects in grassland communities would be related to the divergence between monocots and eudicots, yet this relationship has not been tested.

To understand patterns of niche conservatism in response to multiple ecological factors and the relationship between niche conservatism and community dynamics, we synthesized the results of six experiments conducted in a single grassland system within the Aspen Parkland eco-region of Canada. In total, 14 abiotic and biotic treatments were applied: aboveground insecticide; belowground insecticide; contact fungicide; drought; fixed interval watering; high intensity clipping; litter removal; low intensity clipping; nitrogen addition; nitrogen, phosphorus and potassium (NPK) addition; shading; systemic fungicide; variable interval watering; and warming. From population responses to these factors, we tested for niche conservatism (as measured by phylogenetic signal) in responses to each individual factor and in responses to four groups of factors

representing resource, herbivory, aboveground, and belowground factor groupings. Further, we evaluated the relationship between niche differentiation at a deep node, representing divergent responses between monocots and eudicots, and community dynamics.

2.2. Materials and methods

2.2.1. Site description

All experiments occurred at the approximately 5000 ha University of Alberta research ranch at Kinsella, Alberta, Canada (53°05'N, 111°33'W). Research occurred in three fields located in different areas of the ranch totalling 100 ha. The fields used are unseeded and unbroken and represent a savannah type habitat with mixed grass prairie (primarily *Hesperostipa curtiseta* (Hitchc.) Barkworth, *Poa pratensis* L. and *Festuca hallii* (Vasey) Piper) interspersed with stands of aspen (*Populus tremuloides* Michx.). Though historically lightly grazed by cattle, grazing was halted for the duration of each experiment. For a more thorough site description see Lamb (2008).

2.2.2. Data selection

Data were taken from six separate multi-year multi-factorial experiments, containing a total of 14 factors (Table 1). Here, we limit our analyses to main effects, though we recognize complex interactions among this number of factors can occur. We measure species responses to a given factor as the ratio of relative abundance for each species in control versus treatment plots. Due to the difficulties inherent in estimating species-specific biomass of co-occurring grass species and the ambiguity of identifying individuals when most species are clonal,

relative abundance was estimated using percentage vegetative cover, rather than biomass or numbers of individuals. Percentage cover is commonly used to assess relative change within herbaceous plant communities (Tilman 1987; Lamb & Cahill 2008). Specifically, our abundance estimates were the mean of three cover estimates taken over the growing season from $0.25m^2$ sub-plots within each larger control or treatment plot. Changes in relative abundance were calculated as the log response ratio of abundances (ln(treatment/control)) for each species within each block of each treatment-control combination. The log response ratio was used instead of percent change to normalize responses (Hedges *et al.* 1999).

The overall mean change in abundance and 95% confidence intervals for each factor were estimated using species-specific mean responses to each treatment in a set of mixed models in SPSS (v. 19.0). Only species for which we could calculate the standard error for a given factor were included. Initially, each model included treatment as a fixed effect and the calendar year the data was collected, the duration of the experiment, experiment identity and species identity as random effects with the residuals weighted by the inverse of the standard error for each species within each factor. In the final model, we retained only species identity among the random effects as the other random effects were redundant and explained no additional variation, resulting in a Hessian matrix that was not positive definite.

2.2.3. Niche conservatism

Our definition of niche conservatism – related species respond similarly to ecological factors – is broad and our approach is holistic in its focus on population

outcomes, rather than trait-focused measures of plant anatomy or physiology. To measure niche conservatism, we decomposed the variation in how species responded to a series of ecological factors across a molecular phylogeny (Pavoine *et al.* 2010). This method assesses trait (or in this case population response) diversity among all the species descending from each branch of each node of the phylogenetic tree, measured as quadratic entropy, a Euclidean distance-based diversity index (Rao 1982). This information is used to generate both a visual display of where divergence occurred along the tree and uses randomization procedures to determine if there is significant phylogenetic signal. The randomization tests for these analyses indicate whether response variation is skewed towards a single node, a few nodes, the root, or the tips. Responses were considered to be conserved if the tests for phylogenetic signal indicated that there was significant variation at one or a few nodes that represent deep branches within the phylogeny (e.g. among classes, orders, families, or tribes).

To construct the molecular phylogeny, we sampled 146 species across 35 families found at the study site (see Appendix 1). Ninety-six specimens were collected directly from the ranch, while 48 additional taxa were sampled from herbarium specimens at the University of Alberta Vascular Plant Herbarium (ALTA, table A1). A 1400bp section of the ribulose-bisphosphate carboxylase gene (*rbcL*) was amplified and sequenced to construct the phylogeny using standard techniques. Phylogenetic relationships among focal taxa were inferred using Bayesian inference and maximum likelihood (see Appendix 1 for complete methods). Although we only sampled one gene, sequence variation in *rbcL* was

sufficient to resolve relationships such that the topology was consistent with published angiosperm phylogenies with relatively strong support. In addition, few polytomies are present except amongst close relatives within Poaceae and Asteraceae (see Appendix 1). As these polytomies are near the tips of the phylogenetic tree, they should have little effect on our analyses (Swenson 2009)

To quantify phylogenetic signal, we pruned the full phylogenetic tree to include only species for which we had a response value for a given factor. In total, we had 14 pruned trees which we used in the subsequent analyses. We then used updated version of the R scripts from Pavoine et al. (2010) provided by the author in the ade4 package in R (Chessel *et al.* 2004). A full description of these methods can be found in Appendix 1. Each test for phylogenetic signal was conducted for each individual factor with both ultrametric and non-ultrametric trees. The results were similar, and thus we only present those using the non-ultrametric tree.

Niche conservatism in relation to resource, herbivory, aboveground, and belowground factors was assessed by classifying each individual factor into these groups (Table 2.1). Tests conducted on responses to resource and herbivory factors tested whether plant strategies (Grime 1977; Reich *et al.* 2003) were conserved. Tests using responses to above- and belowground factors tested whether the phylogenetic conservatism seen for many root and shoot traits (Kembel & Cahill 2005; Cahill *et al.* 2008b; Anderson *et al.* 2011; Kembel & Cahill 2011; Comas *et al.* 2012) resulted in conservatism in species responses to aboveground and belowground factors.

To estimate responses to each of the factor categories, we standardized the direction of effect such that each factor was expected to negatively affect population growth (Table 2.1). For example, the effects of water addition were made negative, whereas drought was left as is. These values were then used in two separate models, one for resource and herbivory groupings and one for aboveground and belowground groupings. Only species with at least three response values in a category were included in the models. For both models, we initially included factor category and species identity as fixed effects with factor identity nested within factor category, experimental duration, calendar year of harvest, and experiment identity included as random effects. However, we only retained one random effect in both final models, factor identity nested within factor category, as it was the only random effect that explained any variation. From each model, we estimated the marginal mean for each factor category and the mean for each species within the factor category. These species-specific category means were then used in our phylogenetic analyses.

To explore the importance of functional differences between graminoids and forbs for community dynamics, we correlated those functional differences with mean population responses. We measured functional differences as the proportion of quadratic entropy associated with the node representing monocot-eudicot divergence on the phylogeny. We then correlated these values with mean population responses and with the absolute value of the population response using the Pearson correlation coefficient.

2.3. Results:

As expected, species varied in their responses to the individual factors, with only high intensity clipping and shading causing significant net change across populations (Figure 2.1). Population responses to individual factors were generally not evolutionarily conserved (Table 2.2); significant evidence of niche conservatism was found only in 2 of 14 factors (systemic fungicide application and low intensity clipping (Figure 2.2, Table 2.2)). Variation in plant responses to systemic fungicide addition was skewed towards a single node differentiating asterids, which mostly responded negatively, from the other core eudicots, which generally showed positive responses (Figure 2.2A). Conversely, variation in responses to low intensity clipping was skewed towards multiple nodes representing variation within the Asteraceae, within the Poaceae, and between monocots and eudicots, where monocots increased following clipping and eudicots were on average neutral (Figure 2.2B).

In contrast to results from individual factors, there was evidence of broad conservatism in response to factor categories. Specifically, we found evidence for niche conservatism in response to the group of belowground factors, but not to groups of aboveground, top-down, or bottom-up factors (Table 2.2, Figure 2.3a). Variation in species responses to belowground factors was significantly skewed towards a single node corresponding to a split between monocots and eudicots (Figure 2.3b), where monocots declined strongly in response to belowground stresses and eudicot responses were variable, but on average positive.

Variation in responses between monocots and eudicots was significantly correlated with the mean population responses ($r = 0.632$, $P = 0.015$), but not the absolute strength of the effect ($r = 0.329$, $P = 0.183$). In general, when factors resulted in positive effects for the majority of populations (low intensity clipping, high intensity clipping, and variable interval watering), there were divergent responses between monocots and eudicots (Figure 2.4). Conversely, the factors with strong negative effects on most populations (shade and drought) affected monocots and eudicots evenly (Figure 2.4).

2.4. Discussion:

Plant species varied in their population responses to the different individual factors, but these responses showed only occasional evidence of niche conservatism. The results of previous studies on ecological responses and environmental niches have been inconsistent as well, with some studies showing strong conservatism (Prinzing 2001; Willis *et al.* 2008; Burns & Strauss 2011; Reinhart *et al.* 2012), others weak conservatism (Niinemets & Valladares 2006; Thuiller *et al.* 2011), mixed conservatism (Cahill *et al.* 2008b), or no conservatism (Cavender-Bares *et al.* 2004; Silvertown *et al.* 2006). There are many reasons for niche conservatism to be variable, including the niche axis considered, its relationship to local environmental conditions, the nature of the species pool, and the need to adapt to a diverse set of selective forces (Grime 2006; Losos 2008; Prinzing *et al.* 2008). We suggest that functional convergence needs to also be considered. Ecological challenges have many solutions (e.g. mycorrhizae or root traits for nutrient acquisition (Lambers *et al.* 2008)), and thus

there is a high likelihood of functional convergence even if traits are conserved. Our finding of limited niche conservatism, despite trait conservatism within the studied grassland site (Kembel & Cahill 2011) supports this concept, although our results also suggest other mechanisms.

In the current study, niche conservatism was primarily related to differences between monocots and eudicots; monocot abundances were lower when experiencing belowground stresses and higher following simulated herbivory, whereas eudicot responses were more variable. Both these results are consistent with previous findings. Belowground traits and root foraging vary broadly between monocots and eudicots (Grime & Mackey 2002; Kembel & Cahill 2005), while graminoids have long been known to respond positively to grazing (Coughenour 1985). More interestingly, belowground responses are conserved as a group, whereas only responses to grazing are conserved aboveground. As traits related to gathering soil resources (e.g. adventitious root growth and high root allocation) and regrowth following grazing (e.g. basal meristem and high root allocation) have largely been conserved across graminoids and most monocots (Coughenour 1985; Chase 2004), it suggests that it is the effect of the environment on these traits that causes the differences in how aboveground and belowground responses are conserved. In this system, belowground insect suppression had minimal effect, causing belowground responses to be driven by belowground resource responses. Having a large root system already in place is going to be advantageous following resource pulses, regardless of the nature of the resource. Conversely, both shading and clipping had large effects on

population abundances and there are known trade-offs between shade and herbivory tolerance (McGuire & Agrawal 2005). This suggests that there are fewer functional trade-offs in root than shoot traits, which is why we see responses to belowground factors conserved as a group, but not responses to aboveground factors.

Despite differences in responses to belowground factors and grazing between monocots and eudicots, grouped resource and herbivory responses were not conserved. Selective forces related to productivity are expected to cause convergent trait evolution, while the variable nature of disturbances are expected to cause divergent trait evolution (Grime 2006). We cannot eliminate convergent evolution as the mechanism behind the lack of niche conservatism in resource responses, yet there are also trade-offs between belowground resource capture (high root allocation) and shade tolerance (high shoot allocation) under conditions of nutrient and water limitation (Valladares & Niinemets 2008). Thus we suggest it is a combination of trade-offs and convergence that limits conservatism of resource responses. However, trade-offs alone could have limited conservatism of herbivory responses. There are obvious resource allocation trade-offs between herbivory tolerance and resistance (Agrawal & Fishbein 2006) which could limit conservatism. Further, insect herbivory is variable in its form (Crawley 1989) and although grasses may be adapted to grazing (Coughenour 1985), it seems unlikely that any species would be well adapted to all forms of herbivory.

Divergence in monocot and eudicot responses was not associated with the absolute strength of a factor's effect, but rather just with strong positive effects,

while the factors with strong negative effects affected both groups equally. This pattern suggests that, of the individual factors measured, the evolution of the ability to respond positively to grazing and, to a lesser extent, large pulses of water is important in maintaining graminoid dominance in grasslands. Further it is consistent with the proposed importance of seasonal drought and grazing in the evolution and maintenance of grassland ecosystems (McNaughton 1985; Linder & Rudall 2005; Strömberg 2011).

Of the factors which were conserved, only systemic fungicide, which suppressed mycorrhizae (Cahill *et al.* 2008a), did not vary between monocots and eudicots. Here, we found variation between asterids and other core eudicots, whereas other recent studies have found variation among grass tribes (Reinhart *et al.* 2012). There were differences in both methodology (e.g. inoculation vs. suppression, greenhouse vs. field) and species pools between the two studies that make comparison difficult without further work. However, it does suggest that there are phylogenetic functional groups in mycorrhizal response, but that these groups vary contextually.

2.4.1. Synthesis

Niche conservatism in response to individual factors appears to be rare within this grassland community, despite morphological similarities among related species. The multitude of ways for species to respond to challenges combined with functional trade-offs in a spatially and temporally heterogeneous environment likely preclude conservatism of the 'response niche' from becoming too common. However, we found deep conservatism in response to a broad group

of belowground factors. This is consistent with conservatism of traits (e.g. specific root length) that are important in multiple belowground functions (e.g. resource uptake), but suggests that niche differentiation occurs in response to individual factors (e.g. specialized on nitrogen or water). Further, the fact that conservatism happened at the basal node for plants within this community, suggests that these niche elements played a strong role in the original diversification of these lineages within grasslands for it to have been conserved for such a long period of time.

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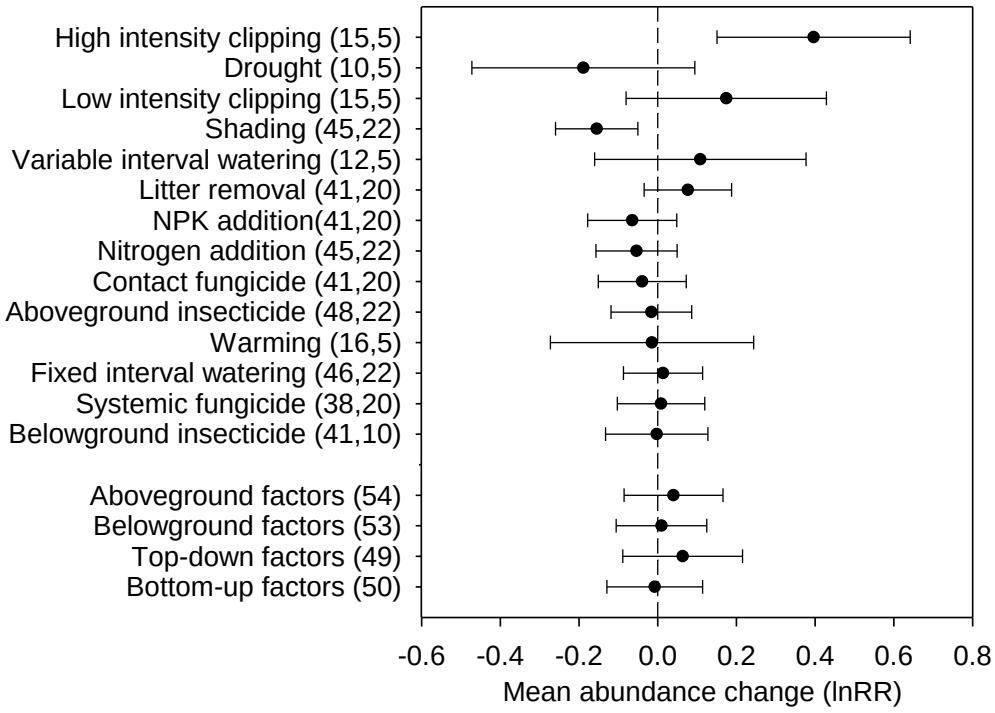

Figure 2.1 Average change in relative abundance following manipulation of various individual factors and factor categories applied to a native grassland. Factor and factor category effects are separated by the solid line and arranged in descending order of the absolute value of the average response. Dots represent the estimated marginal mean of the log response ratio with error bars showing the 95% confidence intervals of that estimate. Numbers following factor names represent the number of species measured followed by the number of replicates for individual factors and the number of species measured for factor categories.

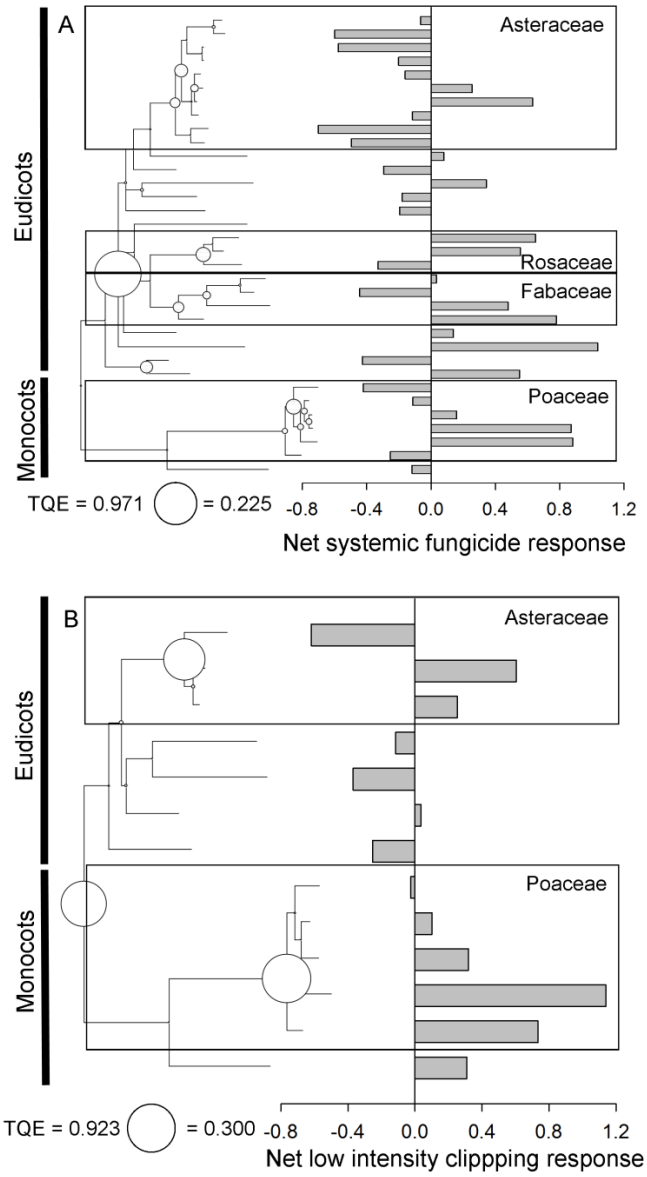

Figure 2.2 Phylogenetic signal in plant species' responses to (A) contact fungicide application and (B) low intensity clipping depicted graphically as response diversity decomposed across a community phylogeny. TQE is the total quadratic entropy (response diversity) and the size of the circle at a given node represents the proportion of entropy concentrated at that node, which corresponds to the amount of divergence at that node. The bar graphs on the right of each panel show the response of species at that tip location to that factor, with monocots and eudicots separated by the bar on the left and the major plant families in boxes of each panel.

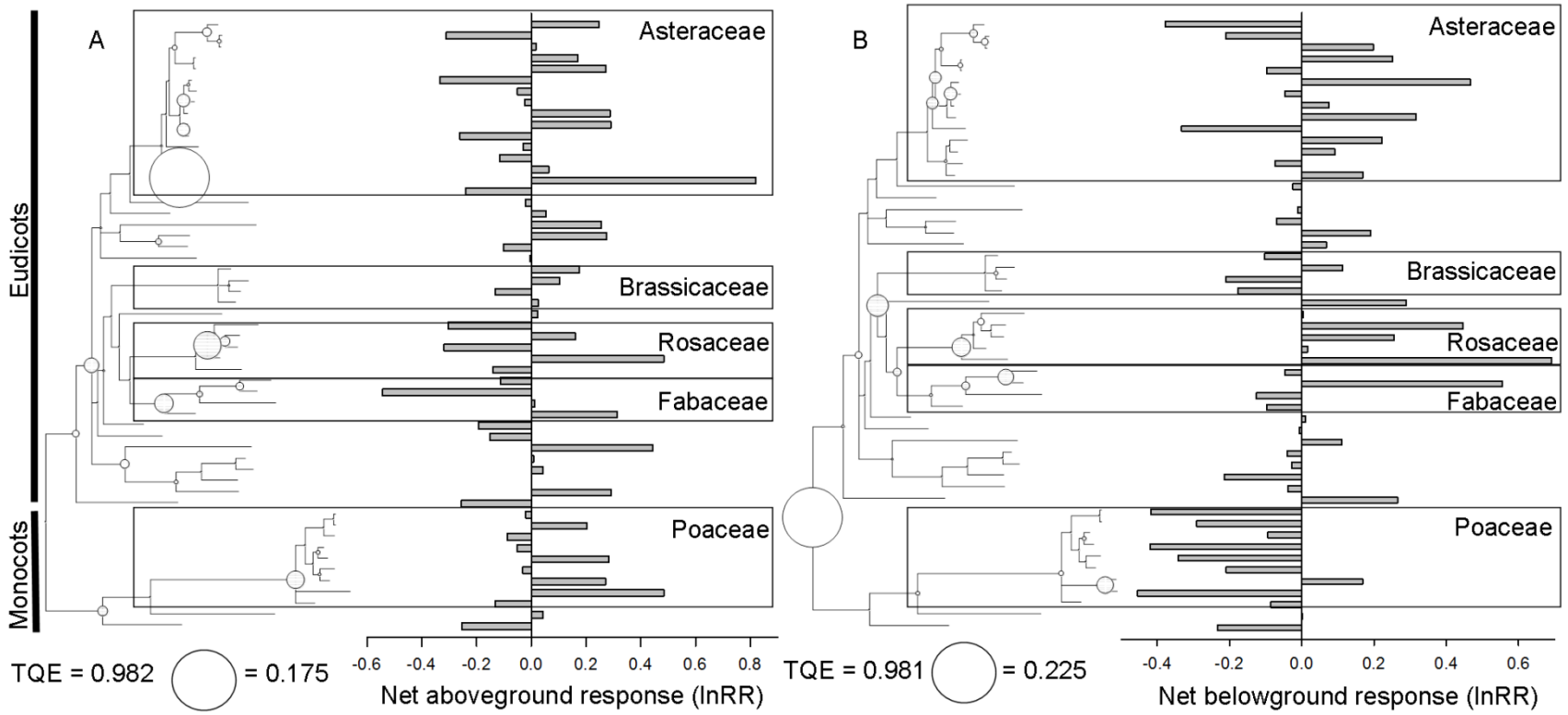

Figure 2.3 Phylogenetic signal in plant species' responses to (A) aboveground and (B) belowground factors. The size of the circle at a given node represents the contribution of that node to total diversity in responses. The bar graphs show the average response of the species at that tip location on the tree to either aboveground or belowground stresses and disturbances. Monocots and eudicots are shown along the left hand side of panel A and the major grassland plant families are enclosed within boxes.

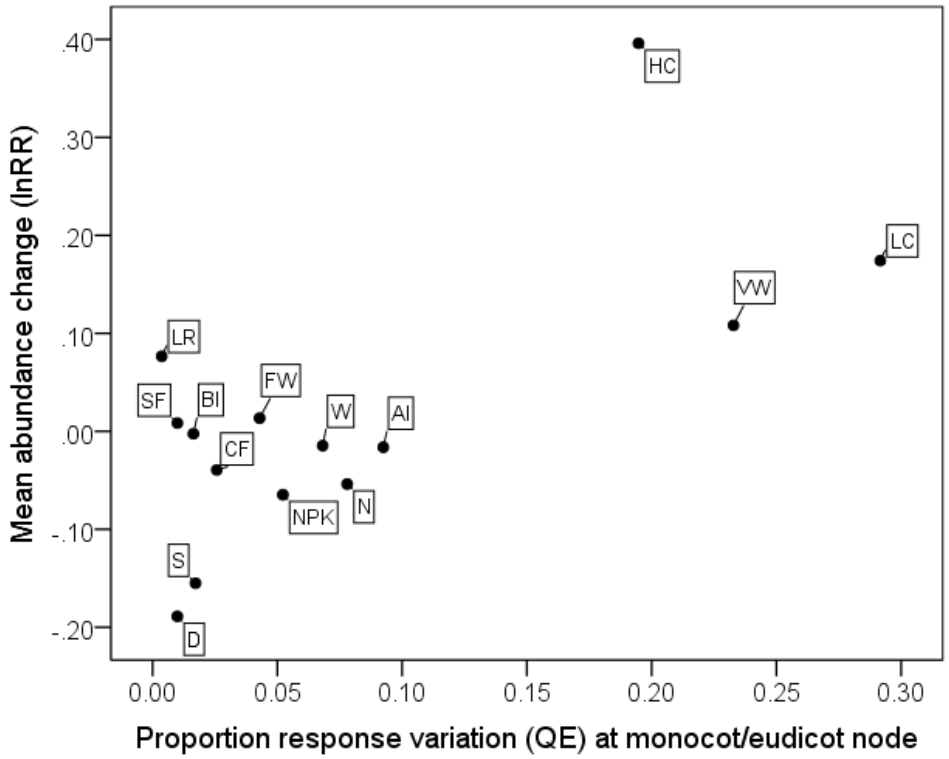

Figure 2.4 Variation between monocot and eudicot responses to 14 factors and their average effect size across the community. Factor names are abbreviated as follows: AI – aboveground insecticide, BI – belowground insecticide, CF – contact fungicide, D – drought, FW – fixed interval watering, HC – high intensity clipping, LC – low intensity clipping, LR – litter removal, N – nitrogen addition, NPK – NPK addition, S – shade, SF – systemic fungicide, VW – variable interval watering, and W – warming.

Table 2.1 Meta data for each factor included in the analysis.

Factor*	Category†	Harvested	Length (yrs)	Species	Blocks	Dir. ¶	Methods
Above. insecticide	A,H	2003/2005	2/3	48	22	+	(Coupe *et al.* 2009; Clark *et al.* 2012)
Below. insecticide	B,H	2005	3	41	10	+	(Coupe *et al.* 2009)
Contact fungicide	U,U	2010	3	41	20	?	§
Drought	B,R	2010	3	10	5	−	(White *et al.* 2012)
Fixed watering	B,R	2005	3	46	22	+	(Lamb 2008)
High clipping	A,H	2010	3	15	5	−	(White *et al.* 2012)
Litter	A,U	2010	2	41	20	?	**
Low clipping	A,H	2010	3	15	5	−	(White *et al.* 2012)
Nitrogen	B,R	2005	3	45	22	+	(Lamb 2008)
NPK	B,R	2010	2	41	20	+	††
Shading	U,U	2005	3	45	22	−	(Lamb 2008)
Systemic fungicide	U,U	2005	3	38	20	?	(Cahill *et al.* 2008a)
Variable watering	B,R	2010	3	12	5	+	(White *et al.* 2012)
Warming	U,U	2010	3	16	5	?	(White *et al.* 2012)

* High and low refer to the intensity of clipping; above and below refer to aboveground and belowground; fixed and variable refer to the interval of watering. † Factors are classified as aboveground (A) or belowground (B) and herbivory (H) or resource-based (R). Factors we could not classify are categorized as unknown (U). ¶ Factors were classified as having a positive (+), negative (−) or unknown (?) hypothesized direction of effect. § Rovral® (Bayer) was applied to half the plots at a rate of 0.36 g/m^2 active ingredient (iprodione) every two weeks. ** Litter was raked each spring in all plots, replaced in control plots and disposed of in litter removal plots. †† Fertilizer was added as 3-4 month slow release 14:14:14 nutrient pellets (Osmocote®, Scotts) each spring at a rate of 5.22 g NPK / m^2.

Table 2.2 Phylogenetic signal in individual factors and factor categories shown as the significance of skewness to a single node, few nodes, or towards the roots or tip.

			Significance of skewness (p)*		
Factor class	Factor type	# Species	Single node†	Few nodes‡	Root/tip¶
Individual	Aboveground insecticide	40	0.53	0.397	0.278
	Belowground insecticide	35	0.506	0.934	0.229
	Contact fungicide	33	0.788	0.621	0.469
	Drought	9	0.056	0.14	0.248
	Fixed interval watering	41	0.918	0.987	0.412
	High intensity clipping	12	0.913	0.894	0.847
	Litter removal	34	0.952	0.445	0.553
	Low intensity clipping	13	0.635	**0.042**	0.278
	Nitrogen addition	42	0.679	0.112	0.566
	NPK addition	33	0.521	0.737	0.508
	Shading	39	0.65	0.882	0.679
	Systemic fungicide	34	**0.033**	0.668	0.591
	Variable interval watering	10	0.968	0.906	0.309
	Warming	10	0.884	0.989	0.254
Aggregated§	Aboveground	54	0.149	0.592	0.435
	Belowground	53	**0.029**	0.628	0.292
	Top-down	49	0.099	0.456	0.359
	Bottom-up	50	0.188	0.244	0.59

* Values significant at $\alpha = 0.05$ are bolded. † Single node skewness refers to situations where a single node (branching point) on the phylogenetic tree accounts for most of the variation in plant responses. ‡ Similarly, few nodes skewness refers to situations where a small number of nodes can explain variation in plant responses. ¶ Root/tip skewness occurs when most of the variation in plant responses can be explained by either deep branches in the tree or by variation among the tips of the tree. § Responses to aggregated categories of factors represent model estimated mean responses by individual species to all factors that fit in that category (Table 1).

3. Competition and phylogenetic overdispersion: An experimental test of the relationship[2]

3.1. Introduction

Competition occurs in most natural communities; however, there have been contentious debates regarding its role in community assembly and how we determine that role. Among the most contentious methods for identifying competition as being important in community assembly has been the search for a 'signature' of competition in patterns of co-occurrence, where negative co-occurrence patterns ("checkerboards") are used to infer evidence that competition structures the community (Diamond 1975; Connor & Simberloff 1979; Connell 1980; Gotelli & McCabe 2002). Such approaches are still used (e.g. Maestre *et al.* 2008), but can also incorporate patterns in species traits (e.g. Stubbs & Wilson 2004) and relatedness (e.g. Cavender-Bares *et al.* 2006). Though this general approach assumes that competition causes these patterns of dispersion, support for the assumption is typically theoretical rather than empirical.

A core theoretical justification behind the search for a signature of competition dates back at least to Darwin (1859), who states that related species should be more similar and thus compete more strongly with each other (the "competition-relatedness hypothesis" sensu Cahill *et al.* 2008). Central to the competition-relatedness hypothesis is the assumption that there is trait conservatism within a lineage, such that more closely related species will also be more similar (Webb *et al.* 2002). A related idea, the theory of limiting similarity

[2] A version of this chapter has been published. Bennett, J.A., Lamb, E.G., Hall, J.C., Cardinal Mc-Teague, W.M. and Cahill, J.F. Jr. 2013. Ecology Letters 16, 1168-1176.

(MacArthur & Levins 1967), holds that species can coexist only when they are below a certain threshold of niche overlap; above that value one species will be competitively excluded. Combined, these tenets of modern community ecology suggest that in a community in which competition is strong, there should be exclusion of some species, such that those which persist will be ecologically distinct and the community will be overdispersed (more dispersed than expected by chance) in niche space (MacArthur & Levins 1967) and with respect to phylogeny (Webb *et al.* 2002) . Thus, if competition structures communities, co-occurring species should be less similar or less related than expected by chance, which can theoretically be detected using null-model analysis (Weiher *et al.* 1998; Webb *et al.* 2002).

Recently, the component assumptions of the competition-dispersion relationship have been questioned. Empirical support for the competition-relatedness hypothesis is mixed, with studies finding evidence for (Burns & Strauss 2011; Violle *et al.* 2011) and against (Cahill *et al.* 2008; Best *et al.* 2013), depending on whether species characteristics associated with competition are conserved (Violle *et al.* 2011) or not (Best *et al.* 2013). Further, it has been suggested that competitive outcomes may be tied to specific character states, rather than mean or maximum values, and such character states are often a function of the environment and not necessarily heritable (Cahill 2013). Competition in the field is also diffuse, occurring between many different individuals of multiple species, both closely and distantly related species. Such diffuse competition can dilute the effects of individual neighbors (Thorpe *et al.*

2011) and cause complicated competitive dynamics, such as indirect facilitation (Levine 1999). Thus, it remains unclear what the expected effect of mean relatedness should be on the outcome of diffuse competition, although, borrowing from invasion ecology, communities that are more related on average should compete more strongly with the focal plant (Procheş *et al.* 2008).

Additionally, the idea that competition (and only competition) can lead to phylogenetic overdispersion is also being challenged. Recent theoretical work suggests that for competition to leave a signature of overdispersion is contingent upon how competitive and niche differences are related to phylogeny (Mayfield & Levine 2010). In the field, there is also evidence that facilitation (Valiente-Banuet & Verdú 2007) and herbivory (Webb *et al.* 2006) can both result in phylogenetic overdispersion among species within a community. This later issue relates directly to Connell's (1980) classic idea, 'the ghost of competition past'; without evidence that competition is driving the observed patterns, then the pattern does not support any particular process. Thus, we suggest that due to this empirical and conceptual ambiguity, understanding how competition affects phylogenetic community structure requires measurement of competition itself.

Interactions among individuals, such as competition, are expected to influence community assembly at small spatial scales where competing species share a resource pool (Huston 1999). As spatial scale increases, so does the environmental heterogeneity of the conditions included within the 'community' sample; although individuals may be functionally connected through gene flow, they are unlikely to have direct competitive encounters. Field tests of the

competition-overdispersion relationship have exploited this principle such that competition is expected to cause overdispersion at small scales, with environmental filtering causing clustering at larger scales (Cavender-Bares *et al.* 2006; Kraft & Ackerly 2010). However, a meta-analysis suggests that plot area is unassociated with the likelihood of finding significant phylogenetic community structure, although studies using very small scales are rare (Vamosi *et al.* 2009). If we cannot find strong evidence of overdispersion at the scales where plants are most likely to interact ($0.01m^2$ - $0.25m^2$), then it would suggest that overdispersion as a signature of competition is rare and that such processes are unimportant in community assembly.

Here we test two of the assumptions inherent in using phylogenetic overdispersion as a signature of competition: (1) that competition is stronger among closely related species and (2) that phylogenetic overdispersion is related to the strength of competition. Further, we test for the signature of competition using a more common method by looking at variation in phylogenetic community structure across spatial scales varying from 0.01 m^2 to 10,000 m^2.

3.2. Materials and methods

3.2.1. Study site

The study occurred in an unbroken and unseeded 50 ha section of native prairie at the University of Alberta research ranch at Kinsella, Alberta, Canada ($53^o05'N$, $111^o33'W$). The field site is a savannah type habitat with mixed grass prairie interspersed with stands of aspen (*Populus tremuloides*). Graminoids comprise most of the biomass at the site, but eudicots comprise > 70% of the

species (Lamb & Cahill 2008). Primary productivity at the site is co-limited by water and nitrogen (Lamb *et al.* 2007), although competitive intensity is more closely linked to water availability (Lamb & Cahill 2008). Competition is generally intense and reduces plant growth by approximately 90 % during seedling establishment (Lamb *et al.* 2007; Bennett & Cahill 2012) and 50% in established plants (Lamb & Cahill 2008). However, there is substantial variation in the actual strength of competition experienced at any particular location, including substantial evidence for facilitation (Bennett & Cahill 2012).

3.2.2. Phylogeny construction

We used the molecular community phylogeny outlined in Appendix 1 which sampled 146 species across 35 families found at the study site. In brief, a 1400bp section of the ribulose-bisphosphate carboxylase gene (*rbcL*) was amplified and sequenced to construct the phylogeny using standard techniques. We pruned tips from the larger phylogeny if they were not present in the current community dataset. This resulted in two pruned phylogenies containing 89 species for the survey and 53 species for the competition experiment (experimental details below). Both pruned phylogenies were well resolved and contained few polytomies. Polytomies were all near the tips, which should have little effect on our analyses (Swenson 2009). For species for which we had no phylogenetic information, we substituted congeners otherwise absent from the data set (four species total). Some species were cryptic unless flowering, making differentiation among congeners difficult. These congeners (three species pairs)

were pooled and assigned the identity of the most common species for phylogenetic analyses.

3.2.3. Competition experiment

We used the data from Lamb and Cahill (2008) to test whether phylogenetic overdispersion increases with the strength of competition. The strength of competition was assessed using 20 pairs of established plants for the 12 most abundant species at the site. Plant pairs were selected as similarly sized plants of the same species separated by approximately 1m. Neighbors were removed around one plant in each pair and half of the pairs were subjected to a nitrogen addition treatment at a rate of $5.4 g/m^2$. Competition was estimated as the log response ratio (Hedges *et al.* 1999) comparing the relative growth rate of plants grown with neighbors to plants grown without neighbors. Community composition was measured as percent cover in $0.25 m^2$ quadrats centered on the target plant grown with neighbors. More detailed methods on the experiment can be found in the original manuscript (Lamb & Cahill 2008).

3.2.4. Competition-relatedness

To test whether relatedness between the target plant and the community influenced the strength of competition, we calculated two indices representing the mean phylogenetic distance between the target plant and their competitors, one unweighted (MPD.t) and the other abundance weighted (MPD.t.a) using the picante package (Kembel et al. 2010). Phylogenetic distances were calculated as the pairwise distances among species using the cophenetic function in R and we averaged these distances for all species present in that community sample.

Abundance weighting was done by calculating these distances by their relative abundance within the community. We then used these estimates in factorial mixed models with competition intensity as the response variable which included nitrogen treatment and either MPD.t or MPD.t.a as fixed effects in SPSS (v.20.0). Both models included focal species as a random factor.

3.2.5. Competition-overdispersion

To estimate phylogenetic community structure, we calculated unweighted and abundance weighted estimates of the net-relatedness index (NRI) and nearest-taxon index (NTI) (Webb *et al.* 2002) from the community composition data using the picante package (Kembel *et al.* 2010) in R. We use NRI.a and NTI.a to denote the abundance weighted estimates. NRI measures the degree of relatedness among all members of the community, thus placing greater relative emphasis on deeper branching than NTI, which measures the relatedness of the nearest neighbor for all members of the community and places greater relative emphasis on the tips of the phylogeny (Webb *et al.* 2002). We determined whether phylogenetic structure departed from random using 1000 randomizations following an occurrence weighted null model (independent swap; Gotelli 2000). This null model algorithm is considered to be conservative (Kraft & Ackerly 2010); however, this algorithm minimizes error rates, especially in communities where there is phylogenetic signal in species abundances (Kembel 2009). To test whether competition intensity affected phylogenetic community structure, we ran a series of separate mixed models with NRI, NTI, NRI.a, and NTI.a as response variables. Each model included a factorial combination of competition intensity

and nitrogen treatment as fixed factors and focal species as a random factor in SPSS (v.20.0).

Competition is not the only process that can lead to overdispersion and many processes can cause affect phylogenetic structure (Cavender-Bares *et al.* 2009; Mayfield & Levine 2010). Therefore, we tested whether total soil nitrogen, soil moisture, estimated annual incident radiation, and light penetration to the soil influenced overdispersion in the community (see Lamb & Cahill 2008 for measurement methods details). From these measurements, we extracted two principal components using principal components analysis (see Appendix 9) and used these components in four separate general linear models to test for effects on NRI, NTI, NRI.a, and NTI.a.

3.2.6. Phylogenetic signature and spatial scale

We tested whether phylogenetic community structure varied with scale by measuring phylogenetic structure in 98 2 x 2 m (4 m^2) plots. Plots were selected to maximize variability in micro-topography and plant community composition. Within each plot, we assessed community composition at two smaller spatial scales, where we would expect the effects of competition to be most intense. For each plot we assessed species composition in fifteen randomly placed 0.01 m^2 quadrats and in one central 0.25 m^2 quadrat. Species composition for the whole plot (4 m^2) was assessed as the presence or absence of species in any of the smaller quadrats. To create larger sized areas, where we expected environmental filtering to be operating, we grouped neighboring plots based on their spatial proximity. For example, when we paired plots, we only created pairs between

plots that were separated by 35.3 m at most (the hypotenuse of a 25 m by 25 m plot), such that each plot pairing was said to represent 625 m^2. We repeated this process such that 3 plots grouped represented 50 x 50 m or 2,500 m^2 and 4 plots 100 x 100 m or 10,000m^2. For these larger areas, we determined community composition as all species present within each plot within that group. From these measures of community composition, we estimated NRI and NTI using the Picante package (Kembel *et al.* 2010) in R. We used bootstrapped one-sample t-tests to determine if the average phylogenetic community structure differed from zero at each spatial scale for NRI and NTI separately in SPSS (v20.0).

To try to explain some of the variation in phylogenetic community structure, we also measured nitrate, ammonium, phosphate, soil pH, soil texture, soil moisture and slope within each of the 4 m^2 plots (see Appendix 9). Similar to the competition experiment, we extracted three principal components from these variables and used them to explain variation in NRI and NTI at the individual plot scale (4 m^2) using general linear models.

3.3. Results

3.3.1. Competition relatedness

Contrary to theoretical predictions, we found no evidence that competition increased in intensity with higher mean relatedness between focal plants and their neighbors. Competition intensity was unrelated to both MPD.t and MPD.t.a, nor was this relationship altered by nitrogen addition (Fig. 3.1; see Table 9.3).

3.3.2. Competition-overdispersion

Competition was not associated with increased phylogenetic overdispersion, nor did this relationship vary as a function of nitrogen addition; there was no relationship between competition intensity and phylogenetic community structure whether we used NRI, NTI, NRI.a, or NTI.a as our metric (Fig. 3.2; see Table S4). Communities did tend to become more overdispersed along principal component 1 (Fig. 3.4), which was related to low incident radiation and low light penetration (see Appendix 9) and suggests a primary role of light. However, the relationship explained relatively little of the total variation and was only significant for NRI (R^2 = 0.038, $F_{1,189}$ = 7.048, P = 0.009) and NRI.a (R^2 = 0.055, $F_{1,189}$ = 9.764, P = 0.002; see Table 9.5 for full results).

3.3.3. Phylogenetic signature and spatial scale

We found mixed results regarding the relationship between phylogenetic structure and spatial scale of observation. At the 0.01m^2 scale, where we expect interactions to be the most intense, communities were not overdispersed for NTI, but were for NRI (Fig. 3.3; Table 3.1), even though NTI is expected to be the statistic best able to detect the effects of competition (Kraft *et al.* 2007). Further, even for NRI at 0.01 m^2, the mean difference from zero was quite small (> -0.1), and we did not detect significant phylogenetic structure for any individual plots at that scale (Table 3.1). Similarly, at the 0.25 m^2 scale, mean phylogenetic community structure was random (Fig. 3.3; Table 3.1). Given that we know competition occurs, yet we found little evidence for a phylogenetic signature at such small scales, this suggests that competition does not cause a detectable

phylogenetic signature at the site. However, consistent with previous predictions (Cavender-Bares *et al.* 2006), phylogenetic clustering increased with spatial scale for both NRI and NTI, although communities were significantly clustered only at the 4 m^2 scale (Fig. 3.3; Table 3.1). However, each spatial scale was quite variable and showed evidence of both clustering and overdispersion (Table 3.1). For the 4 m^2 scale, principal component 2 explained some of this variation for both NRI ($R^2 = 0.066$, $F_{1,92} = 4.167$, $P = 0.044$) and NTI ($R^2 = 0.060$, $F_{1,92} = 4.577$, $P = 0.035$; see Table 9.5 for full results). Communities became more phylogenetically clustered along the component (Fig. 3.4), which was associated with clay content, water retention, and pH (see Appendix 9). This suggests that niche conservatism in response to poorly-drained, high-clay, basic soils caused phylogenetic clustering.

3.4. Discussion

Competition is often inferred to be the mechanism by which communities become overdispersed (Webb *et al.* 2002; Cavender-Bares *et al.* 2009). However, empirical support is lacking, and despite the experimentally verified presence of strong competition in this system (Lamb & Cahill 2008; Bennett & Cahill 2012), we found no evidence that competition caused phylogenetic overdispersion, nor did we find consistent evidence for such overdispersion at any scale. While we do not imply that competition cannot cause phylogenetic overdispersion in communities, our data suggest that overdispersion is not a necessary outcome of competition. At a minimum, this implies that continued use of the competition-overdispersion assumption needs to be 'field-tested' before being applied to a

given system. Current understanding of plant competition and coexistence theory may offer some insight into when overdispersion is more, or less, likely an outcome of competition. Following these theories, whether we can detect overdispersion should depend on (1) the type of competition, (2) the importance of relatedness, and (3) the nature of trait conservatism (Fig. 3.5).

3.4.1. The nature of competition

Within the competition literature, it is widely accepted that the nature of competition can vary depending on whether plants are competing aboveground for light, or belowground for soil resources (Casper & Jackson 1997; Schwinning & Weiner 1998), and that these differences can affect the likelihood of competitive exclusion. When competing for light, larger plants can overtop smaller plants and pre-empt light interception, and competition for light is size asymmetric because the relative benefit of being large is disproportional to the size difference (Schwinning & Weiner 1998). Conversely, belowground competition is size symmetric, meaning that resource capture is proportional to size. Due to these differences in symmetry, shoot competition is much more likely to cause competitive exclusion and will have a greater impact on plant community structure than root competition (Lamb *et al.* 2009). For competition to leave a phylogenetic signature, competitive exclusion must occur and competition must be stronger than other factors influencing community assembly (Mayfield & Levine 2010). Therefore, in systems where root competition dominates, such as this one (Lamb & Cahill 2008) and many others (Casper & Jackson 1997), competition is unlikely to leave a strong signature (Fig. 3.5).

However, when light was more limiting, we did find that phylogenetic dispersion increased, which is consistent with the concept of competition for light promoting overdispersion.

3.4.2. The importance of relatedness

For patterns of overdispersion to be caused by competition, competition must also be stronger between related species (Webb *et al.* 2002). Embedded within this is a further assumption that competition occurs in a pairwise fashion. In studies considering pairwise interactions, competition tends to be stronger among related species (Valiente-Banuet & Verdú 2007; Burns & Strauss 2011), but these relationships are sometimes weak (Cahill *et al.* 2008) and can depend on the taxonomic group and taxonomic scale being considered (Cahill *et al.* 2008). While pairwise competition is important in structuring some plant communities (Kelly & Bowler 2005; Valiente-Banuet & Verdú 2007), competition within many plant communities is diffuse (Wilson & Keddy 1986). Our results suggest that in grassland communities with no dominant species and primarily diffuse competition, relatedness has little effect on the strength of competition.

Theoretical predictions about how relatedness should influence the outcome of diffuse competition are less clear than for pairwise interactions. Occupancy of similar niche space by related species is predicted to reduce the likelihood of invasion (Procheş *et al.* 2008). However, just as relatedness does not always affect the strength of competition, relatedness is not always a good indicator of invasion success (Procheş *et al.* 2008). Theory suggests that this may be related to dominance structure within the community. In systems with a

dominant species, relatedness to the dominant should be important, but when a dominant is lacking, diffuse competition should reduce the importance of relatedness (Thorpe *et al.* 2011). In natural settings, there are many other interactions between co-occurring plants, many of them indirect, that may reduce the importance of competition between related species (Beltrán *et al.* 2012). For example, suppression of a shared competitor can lead to indirect facilitation (Levine 1999), while sharing mycorrhizae can alter competitive outcomes (Moora & Zobel 1996). Such interactions are common in natural systems and are likely to reduce the importance of relatedness in determining the outcome of competition and thereby reduce or eliminate any potential phylogenetic signature of competition. Thus, in communities where diffuse competition dominates, we should not expect competition to leave a measurable phylogenetic signature (Fig. 3.5).

3.4.3. Trait conservatism

Recent theoretical advances suggest that the conservatism of niches and competitive abilities is important in determining the phylogenetic signature of competition within a community (Mayfield & Levine 2010; Fig. 3.5). If most strong competitors come from a single lineage (competitive ability is conserved) and there are niche differences are not conserved, then competition will cause clustering. Conversely, if niche differences are conserved, but competitive abilities are not, then competition will be stronger among related species that share similar niches, and competition will cause overdispersion. If both niche and

competitive abilities are conserved, then the signature of competition depends on the relative importance of competitive ability and niche differences.

Empirical results seem to support the importance of niche conservatism in determining the degree of dispersion. In microcosms containing protist communities, niche differences were conserved and competition caused overdispersion (Violle *et al.* 2011); in contrast, in mesocosms containing amphipod communities, niche differences were not conserved and competition was unrelated to phylogenetic community structure (Best *et al.* 2013). In herbaceous plant communities theory suggests that niche differences are likely to be conserved, while competitive ability differences are more likely to be convergent (Grime 2006), which should lead to competition causing overdispersion (Mayfield & Levine 2010). However, despite evidence of niche conservatism in relation to multiple environmental drivers (Bennett *et al.* in review), we found no evidence of phylogenetic overdispersion at the site, despite strong competition.

Often forgotten in the discussion of trait conservatism and phylogenetic signature within communities is the fact that it is often the differences in trait states and not mean or maximum trait values that influence the outcome of competition (Cahill 2013). For example, it does not matter how tall you can get, it matters how tall you are. Such trait states can vary as a function of competition or environmental conditions, obscuring patterns of trait conservatism (Burns & Strauss 2012). Thus, conservatism of mean or maximum trait values does not

necessarily impact the phylogenetic signature within communities, as it is the expressed trait state that that determines coexistence or competitive exclusion.

3.4.4. Spatial scale and signature detection

Phylogenetic community structure is expected to change with spatial scale, with interspecific interactions leading to a signature of overdispersion at small spatial scales and environmental filtering leading to clustering at larger scales (Cavender-Bares *et al.* 2006; Vamosi *et al.* 2009; Kraft & Ackerly 2010). At the small spatial scales used in the study, interspecific interactions are certainly occurring between species, but we found little evidence for a consistent pattern of overdispersion. Overdispersion was weak and only detected at the $0.01m^2$ scale, but likely only due to the large sample size (N = 1462). These results are similar to others where near random means were found across scales and only large sample sizes enabled detection of significant clustering (Kembel & Hubbell 2006); similarly, the authors concluded that there was likely no real evidence for clustering. Further, overdispersion was only significant for NRI, although NTI is expected to be the more powerful statistic for detecting limiting similarity (Kraft *et al.* 2007). The relative power of NTI and NRI varies as a function of multiple variables, making it difficult to identify why the results differed between the two statistics (Kraft *et al.* 2007; Kraft & Ackerly 2010). Our support for clustering at larger spatial scales is slightly more robust. We feel confident that the significant clustering at the 4 m^2 scale is a real trend, given that both indices responded and the relatively low sample size (N = 98). It is also likely that the larger spatial scales would have been significant if replication was higher. As such, we support

the prediction that clustering will increase with spatial scale (Cavender-Bares *et al.* 2006).

Heterogeneity in phylogenetic signature was high, with evidence for both clustering and overdispersion at spatial scales varying from very small to quite large. Heterogeneity in environmental conditions such as soil type has been shown to influence the signature detected (Kembel & Hubbell 2006). Further, we found that clustering was more common in exposed environments with high light penetration and in waterlogged, basic soils. Combined, this suggests that many factors can influence the signature detected and that the spatial scale of these processes is also quite variable. Further, it suggests that spatial heterogeneity in the relative influences of competition and environmental filters is common place and highlights the need to understand this heterogeneity if we are to develop general principles of phylogenetic community assembly.

3.4.5. Synthesis

Although competition is widely assumed to cause phylogenetic overdispersion, tests of this assumption are rare. We found little evidence that competition drives patterns of overdispersion, at least in systems similar to the one used in this study. Further, we suggest that this system is not that dissimilar from many other plant communities where competition is primarily diffuse and belowground. Therefore, we think it unlikely that competition will often lead to a detectable signature of overdispersion. Adding to the predictions laid out by Mayfield and Levine (2010), we suggest a number of extra conditions that must be met for competition to lead to a detectable signature of overdispersion (Fig. 5).

Further, our data suggest that, while competition driven overdispersion may happen, it is spatially heterogeneous and obscured by other processes, often rendering it undetectable. Given the relatively small proportion (2-12 %) of plots that show significant phylogenetic structure in ours and other studies (e.g. Kembel & Hubbell 2006; Kraft & Ackerly 2010), we suggest that studies focus on experimentally determining the factors driving this heterogeneity, rather than searching for general trends.

3.5. References

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Table 3.1 Results of bootstrapped one-way t-tests determining whether or not phylogenetic community structure deviates from zero.

Index*	Area	N	Est. Mean	Est. S.E.	P†	Min.	Max.	–‡	+‡
NRI	**0.01**	**1462**	**-0.097**	**0.010**	**0.001**	-0.92	1.48	0	0
	0.25	98	-0.011	0.036	0.781	-0.84	0.94	0	0
	4	**98**	**0.108**	**0.038**	**0.004**	-0.87	1.18	0	0
	625	31	0.207	0.171	0.239	-1.78	2.09	1	2
	2500	27	0.098	0.175	0.599	-1.87	2.39	1	2
	10000	19	0.191	0.229	0.419	-1.80	2.40	1	1
NTI	0.01	1462	0.012	0.011	0.290	-1.15	1.11	0	0
	0.25	98	0.022	0.041	0.586	-0.98	0.95	0	0
	4	**98**	**0.212**	**0.055**	**0.001**	-0.93	1.97	0	3
	625	31	0.169	0.166	0.321	-2.09	2.08	1	3
	2500	27	0.176	0.167	0.298	-1.33	1.97	0	1
	10000	19	0.062	0.162	0.708	-1.11	1.69	0	1

* NRI denotes the net relatedness index and NTI the nearest taxon index (Webb *et al.* 2002). † Values in bold are significant at $\alpha = 0.05$. ‡ (+) represents the number of cases exhibiting significant clustering and (−) significant overdisperison at $\alpha = 0.05$.

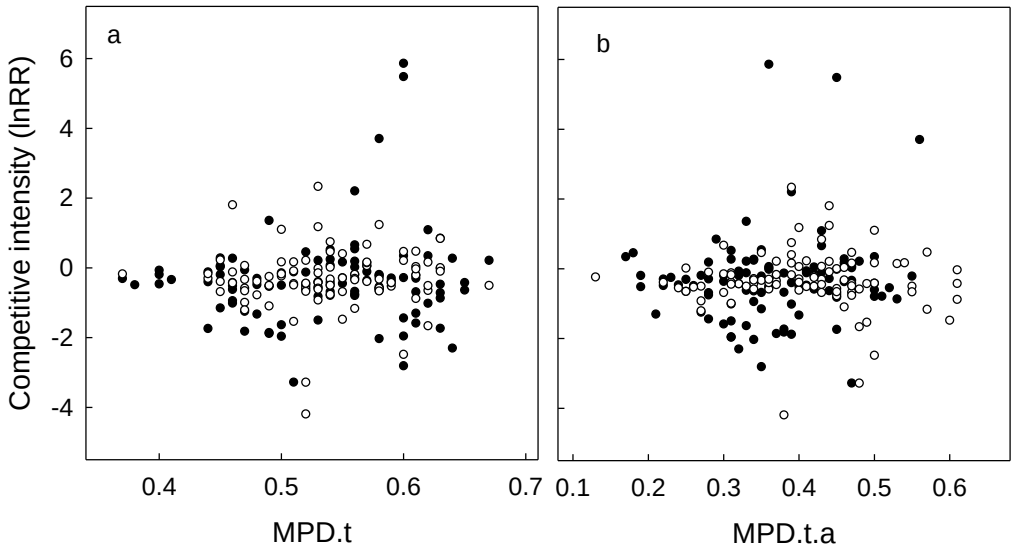

Figure 3.1 The relationship between the mean phylogenetic distance from neighbors to the target plant and competition intensity in control (filled circles) and nitrogen addition (open circles) plots using (a) unweighted and (b) abundance weighted estimates. No significant relationship was found. Here, lnRR refers to the log response ratio (Hedges *et al.* 1999).

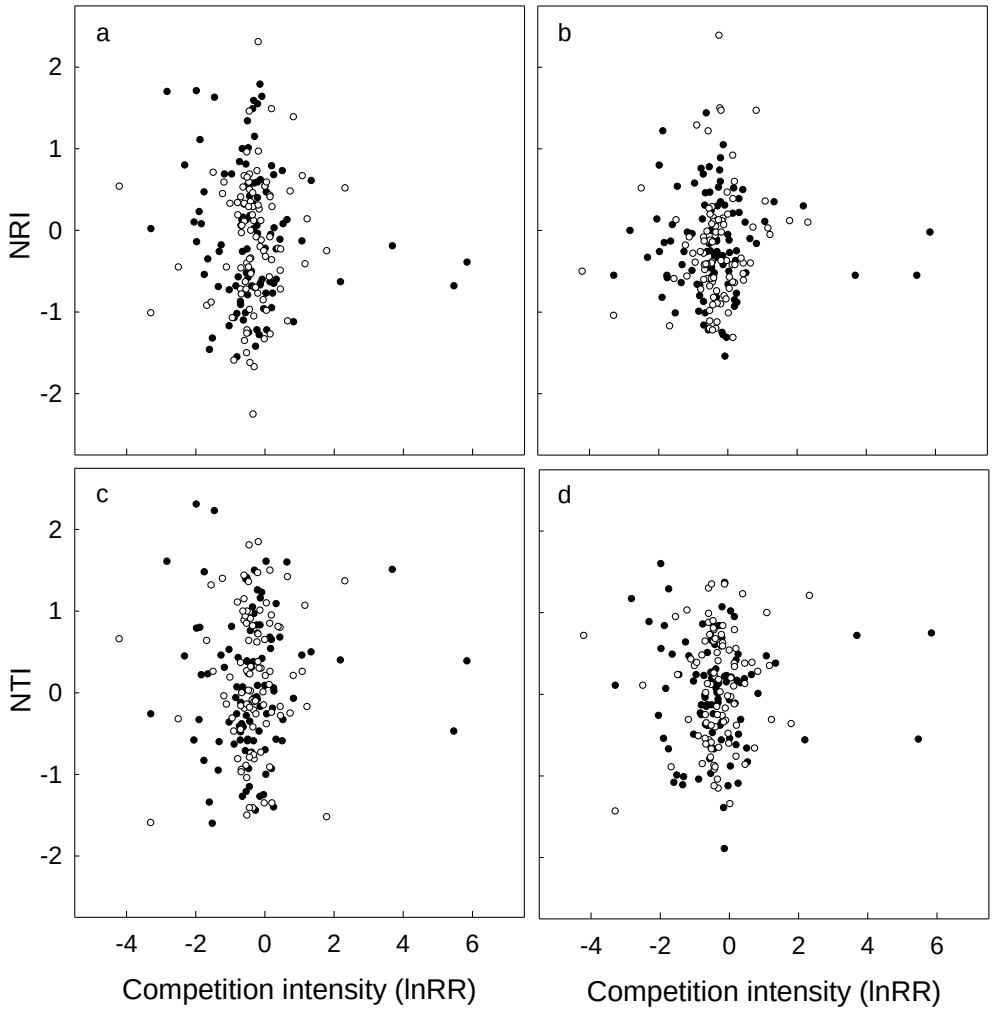

Figure 3.2 The relationship between competition intensity and phylogenetic community structure measured as (a,b) the net relatedness index (NRI) and (c,d) the nearest taxon index (NTI) in control (filled circles) and nitrogen addition (open circles) plots. Plots (a) and (c) show unweighted estimates of NRI and NTI respectively, while plots (b) and (d) show abundance weighted estimates. No significant relationships were found. Here, lnRR refers to the log response ratio (Hedges *et al.* 1999).

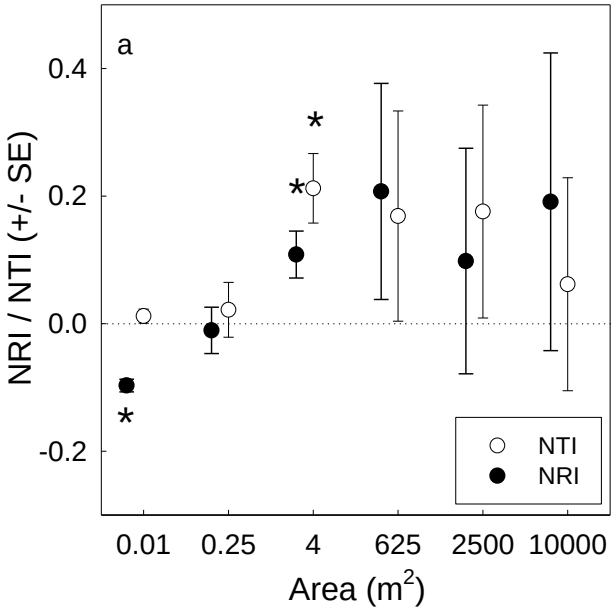

Figure 3.3 Mean phylogenetic community structure measured as the net relatedness index (NRI, filled circles) and the nearest taxon index (NTI, open circles) at six different spatial scales. Means significantly different from zero are marked with asterisks.

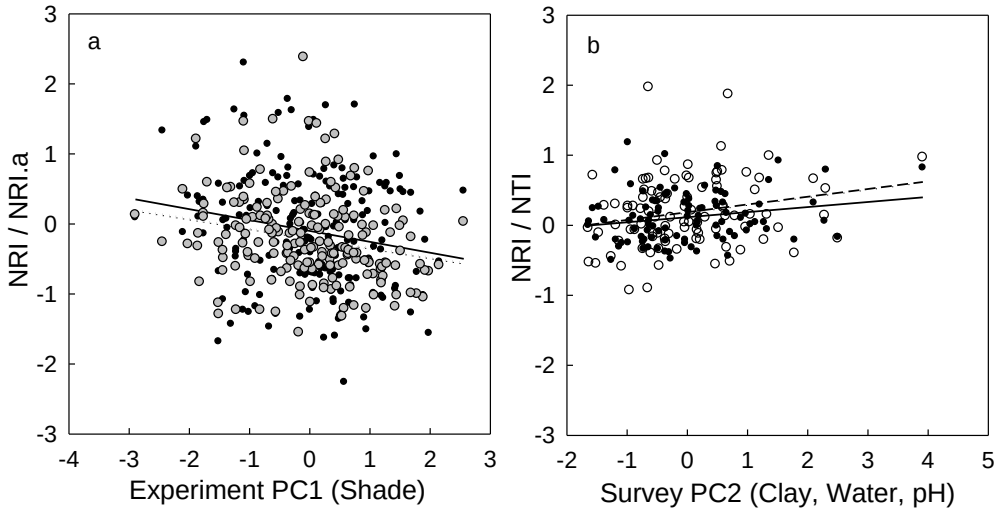

Figure 3.4 The relationships between environmental filters and phylogenetic community structure for the net relatedness index (NRI, black circles – solid lines), abundance weighted NRI (NRI.a, grey circles – dotted line) and the nearest taxon index (NTI, open circles – dashed line). For the competition experiment (a), both NRI and NRI.a declined (became more overdispersed) with principal component 1 (PC1) which represents increasing shade at ground level. In the spatial survey (b) both NRI and NTI increased (became more clustered) with principal component 2 (PC2) which represents soils becoming poorly drained with elevated pH.

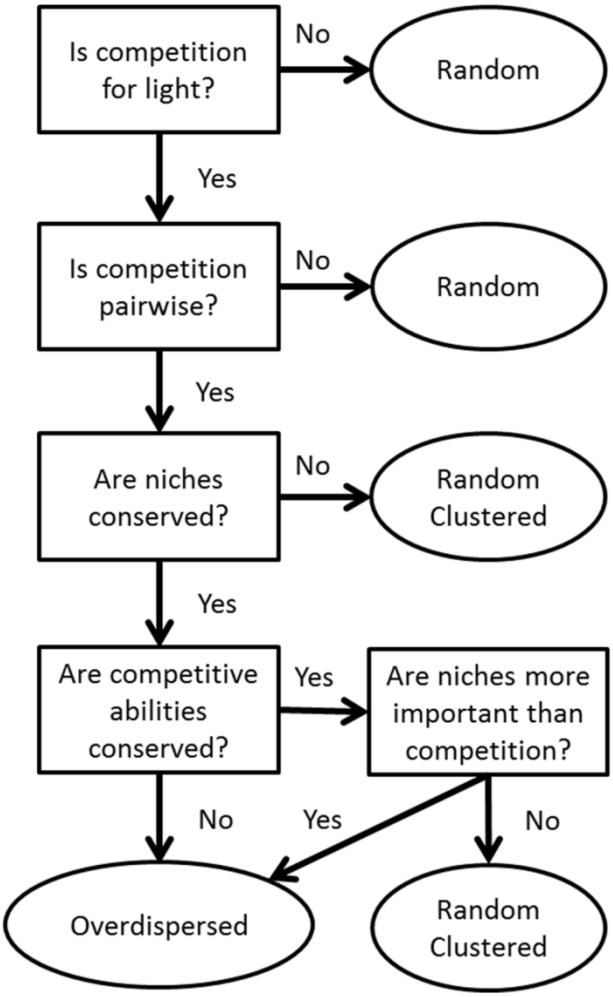

Figure 3.5 The hypothesized model showing the conditions that must be met for competition to yield a detectable phylogenetic signature of overdispersion. The conditions are shown in boxes and predicted phylogenetic signatures in bubbles. Competition must be pairwise and for light, while niches, but not competitive abilities, must be conserved for overdispersion to occur. However, if competitive abilities are conserved, the influence of niches on community structure must be stronger than competition. Pathways dealing with the conservatism of competitive abilities and niches are modeled after the predictions of Mayfield and Levine (2010).

4. Evaluating the relationship between competition and productivity within a native grassland[3]

4.1. Introduction

Plant competition is regularly seen as a factor influencing the structure of natural communities. However, the intensity of competition is not constant through space or time (Tilman 1988; Davis *et al.* 1998; Goldberg *et al.* 1999; Grime 2001; Craine 2009), and understanding the factors that influence its intensity is critical to understanding the assembly of plant communities. One factor that plant ecologists have long focused on is the relationship between plant competition and productivity. This issue has been studied for so long and so intensely that it is a common topic in introductory ecology textbooks (e.g. Stiling 2002; Cain 2008; Ricklefs 2008; Molles & Cahill 2011).

Textbook authors often present competition-productivity relationships in the context of the theories of Grime (1973, 1977, 1979), Newman (1973), and Tilman (1982, 1988). Many aspects of the disagreements amongst these authors are presented elsewhere (Grace 1991; Brooker *et al.* 2005; Craine 2005). In brief, Grime (1973, 1977, 1979) asserted that competition and competitive exclusion is only important in structuring communities at high productivity, with stress more important at low productivity. Newman (1973) suggested that competition was strong at both low and high productivity, though it switched from root to shoot competition as resources increased. However, Newman (1973) agreed that it was likely that only shoot competition would reduce species diversity, a prediction

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consistent with later experimental work (Hautier *et al.* 2009; Lamb *et al.* 2009). Tilman (1982, 1988) also suggested the intensity of competition would be invariant along productivity gradients, but expected competition to be an important structuring force for communities at all levels of productivity (Tilman 1988). Thus these sets of theories make separate predictions about when competition will be strong, and when it will be important for structuring communities along productivity gradients (Table 1).

Debate and data focused on competition-productivity (C-P) relationships have appeared in the scientific literature for over twenty years (e.g. Grace 1991; Wilson & Tilman 1991; Campbell & Grime 1992; Wilson & Tilman 1993, 1995; Twolan-Strutt & Keddy 1996; Peltzer *et al.* 1998; Cahill 1999; Goldberg *et al.* 1999; Craine 2005; Craine 2007; Grime 2007; Tilman 2007; Wilson 2007; Brooker & Kikividze 2008), including experimental efforts that have spanned continents (Reader *et al.* 1994). One of the first meta-analyses in ecology was focused on this issue (Goldberg et al. 1999). They found the intensity of competition for plant survival and growth *declined* with productivity; an outcome that supports none of the theories described above. These unexpected and synthetic empirical results do not appear with the same frequency as the original theories in modern textbooks, even though another meta-analysis found similar results (Maestre *et al.* 2005). Despite the fact that declining competition with productivity appears to be relatively common, these results are often disputed (Lortie & Callaway 2006; Maestre *et al.* 2006), while Grime and Tilman typically

remain the starting point for new theory on this topic (e.g. Craine 2005; Michalet *et al.* 2006).

One such expansion of Grime's theory, the stress-gradient hypothesis (SGH), relates facilitation to stress (Bertness & Callaway 1994; Callaway 1995; Callaway & Walker 1997), where the level of stress within a community is often approximated by measuring productivity (Brooker *et al.* 2005; Maestre *et al.* 2009) and facilitation is measured using metrics similar to those used to study competition. Thus, in practice, SGH studies are empirically identical to C-P studies, although the research focus is more often on the outcome of pair-wise interactions rather than effects on community structure (Maestre *et al.* 2009). SGH is often only applied to low productivity areas, with the primary prediction being that facilitation should be most common (Bertness & Callaway 1994; Maestre *et al.* 2009), intense (Callaway & Walker 1997) or important (Maestre *et al.* 2009; Malkinson & Tielbörger 2010) at high to intermediate stress and thus that competition should be lowest in unproductive areas. Attempts have been made to apply this hypothesis to more productive areas (Holmgren & Scheffer 2010), including an additional, yet little explored, aspect of the initial SGH which predicts that associational defences can cause facilitation to increase with productivity when herbivore pressure is intense (Bertness & Callaway 1994). Thus the predictions of SGH are multi-faceted and can be consistent with the predictions of Grime, Newman, Tilman, or the results presented by Goldberg, depending on the range of productivity encountered.

Alternate hypotheses regarding the relationship between competition and productivity focus not on the biomass of neighbours, but on the supply and demand of resources (Taylor *et al.* 1990; Davis *et al.* 1998). In these theories, it is either difference between supply and demand (Davis *et al.* 1998) or the ratio of supply and demand (Taylor *et al.* 1990) that determines the outcome of competitive interactions, with competition declining as either net resource supply (supply - demand) or the ratio of supply to demand increases. Thus in both these cases, competition can decrease with productivity. Although these theories are rooted in the foundations of plant ecology (Weaver & Clements 1938), they do not receive as much attention as theories relating to the work of Grime and Tilman.

C-P relationships can be expected to vary as a function of many factors. For example, plant survival and growth may respond differentially to the presence of neighbours (Wilson 1993; Goldberg & Novoplansky 1997; Howard & Goldberg 2001; Maestre *et al.* 2009), and may have differential effects on species exclusion (Howard & Goldberg 2001). These differences are rarely addressed by theory (Miriti 2006; Maestre *et al.* 2009), though they have been addressed empirically (Davis *et al.* 1998; Goldberg *et al.* 1999; Schiffers & Tielbörger 2006). Additionally, theory predicts different relationships for competitive *intensity* versus competitive *importance* (Grace 1991; Brooker *et al.* 2005; Craine 2005; Grime 2007; Brooker & Kikividze 2008; Maestre *et al.* 2009). Intensity is typically defined as the absolute magnitude of an effect (Grace 1991; Grace 1995), while the assessment of importance is highly variable and there is much

contention among researchers about how it should be measured. For this manuscript, we will measure importance as the magnitude of competition relative to maximum plant performance (Grace 1991; Brooker *et al.* 2005; Kikvidze *et al.* 2011). We recognize competitive importance can also be viewed in terms of the ultimate effects of competition on population growth rates (Freckleton *et al.* 2009), competitive exclusion and community structure (Lamb & Cahill 2008; Damgaard & Fayolle 2010). When differentiating between these definitions of importance, we will refer to them as relative and demographic importance respectively. However, demographic importance is difficult to measure in a perennial plant community given the long life spans of some plants and as such we cannot test them with the data at hand (but see Lamb & Cahill 2008 for a potential method of looking at community consequences of competition).

Finally, though C-P relationships are typically presented as an aspect of a community, they may be highly variable among species within a community. To date, most experimental studies of competition have used one or a small number of *phytometers*, species intended to be representative of the entire community (e.g. Wilson & Tilman 1993; Belcher *et al.* 1995; Twolan-Strutt & Keddy 1996; Davis *et al.* 1998; Brooker *et al.* 2005), with exceptions including studies such as Wilson and Tilman (1995) with eight species and Callaway *et al.* (2002) with more than sixty. What is lacking are experimental studies with a large number of species measuring multiple responses within a single community - allowing a true test of the overall relationship within that community.

The aim of this paper is to investigate the predictions of each of the aforementioned theories as outlined in Table 4.1 for the plant community within a single site using a large number of species. We attempt to address some of the potential causes of disagreement among empirical results by differentiating between size and survival using indices of both competitive intensity and competitive importance among plots naturally varying in both neighbour biomass and resource availability.

4.2. Methods

4.2.1. Study site and species

The study occurred in an unbroken and unseeded 50 ha section of native prairie at the University of Alberta research ranch at Kinsella, Alberta, Canada ($53^{o}05$'N, $111^{o}33$'W). The field site is a savannah type habitat with mixed grass prairie interspersed with stands of aspen (*Populus tremuloides).* Standing crop within the prairie area naturally varies from 100 to 800 g/m^2, with *Hesperostipa curtiseta* and *Festuca hallii* being the most common species at low to moderate productivity, switching to *Poa pratensis* and *Galium boreale* at moderate to high productivity (J.A. Bennett, unpublished data). The site is co-limited by water and nitrogen (Lamb *et al.* 2007), although competitive intensity is more closely linked to water availability (Lamb & Cahill 2008). Competition is generally intense; often reducing plant growth by approximately 90 % during seedling establishment (Haag *et al.* 2004; Lamb *et al.* 2007) and 50% in established plants (Cahill 2003b; Lamb & Cahill 2008). Neighbour effects on seedling survival are typically near

neutral (Cahill 2003b; Haag *et al.* 2004), except during extended drought, when competition greatly increases seedling mortality (Cahill 2003a).

The site receives an average of 418 mm total precipitation annually, which includes 155 mm of snow and rain from first snowfall through spring and 217 mm rain over the summer months (June through August). However, there is never an average year in a continental grassland, and precipitation was approximately 75% of average both leading up to and during the experiment. Daily temperatures over the experimental period were similar to the thirty year average, with mean daily temperatures of 15.2 ^{o}C, average highs of 22.3 ^{o}C and lows of 8.0 ^{o}C. Though the site has historically been grazed by cattle, grazing had been halted four years prior to the onset of the experiment and did not occur for the duration of the study. Insect herbivory has little effect on the plant community at this site (Coupe *et al.* 2009), while grazing by free-ranging ungulates (e.g. deer) and small mammals is infrequent (J.A. Bennett, personal observation).

We recognize that the range of productivity within our study is smaller than would be found in a transcontinental study (Reader *et al.* 1994; Callaway *et al.* 2002). However, none of the C-P theories except the SGH (Callaway & Walker 1997; Maestre *et al.* 2009) suggest they operate only under specific ranges of productivity, and even the SGH has been extended into more productive environments (Bertness & Callaway 1994; Holmgren & Scheffer 2010). We also recognize that non-linearities in relationships (Belcher *et al.* 1995; Kikvidze *et al.* 2011) would be difficult to detect using narrow productivity ranges.

Twenty-two plant species were selected for the experiment, including four annual species and eighteen perennial species (Table 4.2). Perennial species were chosen to be representative of the species naturally occurring across the range of productivity within this grassland. Annuals are uncommon at the site, yet are present and could respond to competition differently than perennials (Gomez-Aparicio 2009). Due to limited seed availability for the annual species present at the site, annual species were chosen from the regional pool and according to seed availability. Combined, these 22 species represent approximately 25% of the total vegetative cover and 15% of the total vascular species richness at the site.

4.2.2. Experimental design

Twenty replicate blocks, each consisting of two 2m x 2m plots, were established in the summer of 2008. To test for the effect of neighbours on plant growth and survival, one plot of each pair was randomly assigned a 'neighbours removed' treatment and the other a 'neighbours intact' treatment. Neighbour removal was initially accomplished through a combination of mowing and the application of a broad spectrum herbicide (Round-up®, Monsanto), and maintained by applying herbicide to non-target plants using a paint brush and hand weeding as needed. These plots were surrounded by a 0.5 m vegetation free buffer zone at the edge of which roots were severed to a depth of 0.1 m to minimize interactions with vegetation surrounding the plots. This edge was re-cut on an approximately monthly basis. As the removal of vegetation in neighbours removed plots resulted in the removal of much of the litter layer, neighbours intact plots were also raked to remove an equivalent portion of the litter layer.

Standing biomass was low at the time of transplanting, minimizing the amount of damage to aboveground plant structures; however, we kept raking intensity low to reduce damage to the soil surface and existing vegetation.

Each plot was divided into sixty-four 0.25 m by 0.25 m cells in an 8x8 grid. In total there were 20 blocks × 2 plots × 64 cells for a total of 2560 planting locations in this experiment. One seedling of a given species was transplanted into each cell such that each species had two to three individuals in each plot. Species' positions within the grid were assigned randomly for each block, so that the identity of the planted neighbour was consistent between plots within the block, but varied among blocks. This design ensured that, if competition occurred between seedlings that we planted, any neighbour-specific competitive effects (sensu Goldberg 1990; Goldberg 1996) on target plant performance would remain consistent within blocks, but that species-specific responses would not be confounded by the identity of the planted neighbour when comparing across species and across blocks.

Seedlings were started in the greenhouse and transplanted into the field at the beginning of June 2009 at approximately four weeks of age. All seedlings at the time of transplanting had at least their first two true leaves, although most had at least four leaves. When transplanting, a 2 cm wide and 5 cm deep circular hole was made using a soil step sampler and the seedling was inserted along with the propagation soil. The narrow hole diameter was chosen to minimize damage to surrounding roots, although some trampling of the surrounding vegetation did occur. To increase establishment success, plots were watered with approximately

2 L/m^2/day for the first 5 days following seedling transplanting and 1 L/m^2/day for the next 5 days, but received no supplemental water after 10 days. All seedlings that died within the first ten days of the initial transplanting were replaced with new individuals from the trays grown in the greenhouse. Seedling mortality was monitored for all plants approximately biweekly following the initial replacement period for transplants, with percent survival of transplants calculated in late August 2009 after 13 weeks of growth.

4.2.3. Plant growth

To measure biomass and estimate growth of annual plants, all annuals were clipped in early August 2009 to avoid mass loss due to seed dispersal. Plants were then dried at 65^{o}C for at least 72h and weighed. This study is intended to persist long-term, and thus destructive measures of perennial plants could not be taken. Instead, perennial growth was estimated using species-specific biomass regressions and plant measurements taken in late August 2009, prior to senescence. For these plants, we measured the maximum width (w_1), width perpendicular to maximum (w_2), and height (h) of each plant. We took the same measurements on a second smaller set of plants also grown with and without neighbours, and clipped these plants for the development of our biomass regressions. For 3 of the 18 species, survival of these plants was too low ($N < 5$) to estimate biomass and these species were removed from all analyses concerning plant growth. We used backwards step-wise regression to estimate biomass with ln(biomass) as the dependent variable and ln(basal area), ln(height), and ln(flowering stems) as independent variables. For *Bouteloua gracilis,* our step-

wise regression model selected only ln(flowering stems), causing plants without flowers to be underestimated; therefore we removed flowering stems from our starting model, which still gave acceptable results ($R^2 = 0.919$). For *Hedyserum alpinum*, we were unable to obtain a suitable regression ($P > 0.05$); therefore we removed *H. alpinum* from analyses related to growth. The full set of equations and the regression results can be found in Table 4.3. Similar model selection analyses using mixed models and small sample AIC (AIC_c) selected identical parameters and gave identical coefficient estimates as the regression approach. We chose not to harvest roots or monitor root growth due to our desire to avoid destructive sampling and the inherent logistical issues of having more accurate measures of root biomass in the neighbours removed than the neighbours intact treatment (Cahill 2002). However, our estimates of shoot growth should provide adequate estimates of the total effect of neighbours on plant performance, which presumably is a proxy for fitness (Cahill 2002).

4.2.4. Productivity estimation

Aboveground net primary productivity was estimated as standing biomass in grams dry weight /m^2 (g/m^2) for each block at peak biomass in late July 2010. We could not harvest biomass from within the plots with neighbours intact as it would disrupt the long term goals of the study. Therefore, vegetation was clipped in four 0.1 m by 1 m quadrats surrounding the block, with individual quadrats placed on the north, south, east and west sides of each block. Samples were then sorted to remove dead material, dried at 65^oC for at least 72h, and weighed. Values for the individual quadrats ranged between 130 and 630 g/m^2; however,

productivity is naturally spatially heterogeneous at the site. We therefore used the average biomass of the four quadrats as our estimate of productivity for that block. Averaging among the quadrats restricted the range of productivity to 225 - 460 g/m2. This could underestimate the absolute range of productivity between blocks, but should still represent the relative differences among blocks. We recognize that given the range of productivity covered, our test is not a definitive test of the associated theories; however, it is a test of the relationship between competition and productivity for this site.

Soil moisture was measured in both neighbours intact and neighbours removed plots using a ML2x – ThetaProbe Soil Moisture Sensor (Delta-T Devices) in late May 2010. Within each plot soil moisture was measured 5 times, once in each corner and the center. Within neighbours removed plots, care was taken to avoid sampling within the immediate vicinity of a seedling. Soil moisture in neighbours removed plots approximates the moisture retention capacity of the soil (gross water supply), whereas soil moisture where neighbours are intact approximates difference between supply and demand (net water supply) (Davis *et al.* 1998). We did not calculate the ratio of supply to demand as suggested by Taylor *et al.* (1990) because we did not directly measure demand. Similarly, we chose not to estimate demand using the method laid out by Davis *et al.* (1998) due to potentially confounding instances when neighbours increase water availability as seen in studies of facilitation (Bertness & Callaway 1994). We recognize that the effect of the plant community on soil moisture in late May is less than can be expected in mid-July, but soil moisture measurements in July

were not taken. However, among other plots at the field site within the same growing season, net water supply in May was highly correlated with net water supply in July (r = 0.782, $P < 0.001$).

Although neighbour biomass can be expected to vary with water availability, this correlation is not perfect. Across a survey of 100 sites, net water supply was correlated with standing crop (r = -0.321, P = 0.003; J.A. Bennett, unpublished data). However, this does not explain the majority of the variation in standing crop. In this particular study, standing crop was uncorrelated with either net (r = 0.146, $P = 0.538$) or gross water supply (r = 0.178, $P = 0.453$), although net and gross water supply were highly correlated (r = 0.697, $P = 0.001$). This suggests that other factors are potentially limiting to plant growth, including, but not limited to, nitrogen (Lamb *et al.* 2007). Determining these factors and their role in competition is the subject of future research.

4.2.5. Competition metrics

Competition can differentially affect survival and growth (Goldberg & Novoplansky 1997; Goldberg *et al.* 1999; Howard & Goldberg 2001). Further, both survival and growth are important components of fitness (Goldberg *et al.* 1999; Howard & Goldberg 2001; Aarssen & Keogh 2002; Neytcheva & Aarssen 2008), with species-specific competitive effects on seedling growth being a strong predictor of species' abundances in the field (Howard & Goldberg 2001). Thus we estimated the effect of competition for both survival and size separately.

The choice of response metric can influence the form of the C-P relationship (Grace 1995; Goldberg *et al.* 1999; Hedges *et al.* 1999; Brooker &

Kikividze 2008; Rees *et al.* 2012). We chose the log response ratio (lnRR) to estimate competitive intensity (Cahill 1999; Goldberg *et al.* 1999) as it is an unbiased estimate of the effect of competition that is usually normally distributed and symmetrical around zero (Hedges *et al.* 1999; Rees *et al.* 2012). The ratio was calculated such that positive response ratios indicate competition and negative ratios indicate facilitation:

$$\ln RR = \ln\left(\frac{NR}{NI}\right)$$

From the response ratios, we classified interactions as positive, neutral or negative to determine interaction frequency. Interactions were classified as positive if neighbours increased plant survival or size by greater than 10% (lnRR < -0.0953) and negative if neighbours reduced survival or size by greater than 10% (lnRR > 0.0953). All other interactions were classified as neutral.

We calculated the relative importance of competition using the importance index (I_{imp}) (Seifan *et al.* 2010), modified so that competition would be positive and facilitation negative:

$$Iimp = \frac{(NR - NI)}{|NR - NI| + |NR - N\max|}$$

This index was chosen as it is symmetrical around zero for competitive and facilitative interactions although there have been some concerns made regarding its utility in some situations (Rees *et al.* 2012). Here N_{max} refers to the maximum performance of a given species in either the neighbours removed or neighbours intact treatments. These indices were calculated for each species within each block except under specific conditions. For survival, indices were not calculated

for a given species within a specific block if mortality was complete for that species within that block. Similarly, indices were not calculated for size if mortality for a given species reached 100% in either neighbours intact or neighbours removed plots within that block.

4.2.6. Statistical analyses

To determine if the effects of competition on survival and size differed, we used two mixed models, one for competitive intensity and one for competitive importance. These models included plant response measure (survival vs. size) as a fixed factor and species and block as random factors, with either competitive intensity or importance as the response variable in SPSS (v18.0). All plant response measures for a given species were pooled at the plot level prior to analysis; survival represents the proportion of seedlings that survived the year and size represents the average size of surviving individuals within that plot.

To test whether neighbour standing crop, gross water supply, or net water supply were associated with changes in competitive intensity or importance for either plant survival or growth, we used twelve mixed models specifying different independent and response variables in SPSS (v18.0). For each independent variable (standing crop, gross water supply, net water supply) we ran four models: lnRR – survival, I_{imp} – survival, lnRR – growth, and I_{imp} – growth. Although gross water supply is not technically a measure of productivity, it represents potential productivity. Thus for ease of comparison, we will refer to it as a measure of productivity. Variation in competition-productivity (C-P) slopes among species was accounted for with a random interaction between species and productivity

that allowed the C-P slopes to vary randomly by species. Estimation method (regression or weighed) was also included as a random effect for these analyses.

To test for changes in the frequency of interaction types (competitive, neutral or facilitative) across the range of biomass and resource availability found for both plant size and survival, we used six generalized linear mixed models with PROC GLIMMIX in SAS (v9.2) specifying the multinomial distribution. Interaction type was used as the response variable with either neighbour biomass, gross water supply, or net water supply as continuous fixed effects and species as a random effect.

4.3. Results

Across all species and blocks, plants were 17.3 times larger in no competition plots than in competition plots, whereas survival was 1.2 times higher with neighbours (55% survival) than without (47% survival). This resulted in variation in the magnitude of competitive intensity ($F_{1,614}$ =420.85, $P < 0.001$, Fig. 4.1 A) and importance ($F_{1,610}$ = 49.92, $P < 0.001$, Fig. 4.1 B) between survival and size. The magnitude of competitive intensity was comparable to other competition studies on seedlings conducted at the site for both size and survival (Haag *et al.* 2004; Lamb *et al.* 2007), suggesting these results are 'typical' for this location.

Both competitive intensity and importance declined with increasing neighbour biomass when considering seedling survival (Fig. 4.2 A,C, Table 4.4); however neither gross nor net water supply significantly affected competitive effects on seedling survival (Table 4.4). For seedling growth, both competitive

intensity and importance declined with increasing gross water supply (Fig. 4.2 B,D, Table 4.4), but neither neighbour biomass nor net water supply had a significant effect (Table 4.4).

Across all species, facilitative interactions were common for plant survival (45%), but rare for size (14%). The remaining neighbour effects on survival were both competitive (30%) and neutral (25%), whereas, neighbours were largely competitive when measuring growth (85%), and rarely neutral (<1%). Echoing the declines in competitive intensity and importance, the frequency of competitive effects on survival decreased with standing crop, with a concurrent increase in the frequency of facilitative effects (Table 4.4). Interaction frequency was not found to change for survival with either gross or net water supply. Given the rarity of non-competitive effects on plant growth, it is unsurprising that the relative frequency of competitive and facilitative interactions on growth was consistent irrespective of the productivity estimate used (Table 4.4).

4.4. Discussion

Across all models, we found that competitive intensity declined with productivity, but that the type of productivity measurement that was associated with this decline depended on the plant response. However, we found no evidence of increasing competitive effects across the range of productivity used as predicted by Grime (1973, 1979) and parts of the SGH (Bertness & Callaway 1994; Callaway & Walker 1997; Maestre *et al.* 2009; Kikvidze *et al.* 2011). This was consistent regardless of the competition metric, plant response, or productivity measurement used. As competition was neither intense nor relatively

important at high productivity for either plant survival or size, we suggest that these results are also not consistent with an increase in the likelihood of competitive exclusion as predicted by Newman (1973), at least not over this range of productivity.

Given that we found a decline in competitive intensity with at least one measure of productivity for both plant growth and survival, we find little support for Tilman (1982, 1988) either. However, we did find that competition was invariant when measuring plant growth and standing crop as well as survival and gross water supply. This could be construed as support for Tilman's prediction, but given that C-P relationships were so often negative, the support is marginal at best.

As previously mentioned, we found little support for the most common prediction of the SGH: that facilitation will increase with increasing stress (Bertness & Callaway 1994; Callaway & Walker 1997; Michalet *et al.* 2006; Maestre *et al.* 2009; Holmgren & Scheffer 2010), nor did we see evidence for a hump-shaped distribution of facilitation along a stress gradient (Maestre *et al.* 2009; Holmgren & Scheffer 2010). It is possible that a hump-shaped relationship would appear if our range of productivity covered lower productivity areas. However, across the range of productivity measured, facilitation of survival always decreased with stress and we found no evidence of net facilitation in relation to plant growth at any productivity level. Our results are consistent with the earliest version of the SGH (Bertness & Callaway 1994) which predicted that facilitation could increase with productivity under high herbivore pressure due to

associational defences which lead to a reduced risk of herbivory (Bertness & Callaway 1994; Callaway & Walker 1997). Although this mechanism is likely active at the site to some extent, it is unlikely to be dominant as herbivory remains low when cattle grazing is not active (Coupe *et al.* 2009, personal observation). However, other mechanisms of facilitation including protection from light damage and desiccation (Callaway 1995) could have led to this pattern (see below).

Although the SGH cannot explain the decline in competitive effects on plant growth and survival with productivity found in this system, these results are consistent with separate meta-analyses (Goldberg *et al.* 1999; Maestre *et al.* 2005). Both our results and the meta-analyses are at least partially consistent with theories that account for variation in both resource supply and demand (Taylor *et al.* 1990; Davis *et al.* 1998; Arii & Turkington 2001). The decline in competition with productivity we observed is consistent with his predictions, but we did not find a relationship between competition and net resource supply as predicted by Davis (Davis *et al.* 1998). This suggests that the supply and demand theory is also unable to predict the outcome of competition in this grassland community. However, we cannot rule out this theory because our measurements of net resource supply were not perfectly timed. Although net resource supply is correlated between early and mid-growing season, this correlation is not perfect and it is possible that a relationship exists between net resource supply and competition at peak biomass.

There are other pieces of evidence that suggest that resource supply and demand are important in determining the relative effects of competition. Nutrient uptake is not perfectly efficient (Yuan *et al.* 2006) and light is not always limiting at high productivity (Abrams 1995; Dickson & Foster 2011). This can result in increasing net nutrient availability at high productivity (Yuan *et al.* 2006), which does not necessarily coincide with an increase in aboveground competition (Abrams 1995; Dickson & Foster 2011), and can cause a decrease in total competition with productivity. Of course an increase in aboveground competition is going to be dependent on the range of productivity explored. However, even if at higher productivity, competition does increase, this would suggest that the relationship between competition and productivity would have to be non-linear for this site.

We also found a number of differences between plant survival and growth which are consistent with previous findings; neighbours tend to have neutral to positive effects on plant survival and competitive effects on plant growth (Wilson 1993; Dyer *et al.* 2001; Howard & Goldberg 2001; Maestre *et al.* 2009). We also found that neighbour effects were associated with resource supply for growth and standing crop for survival. These differences between seedling survival and growth are consistent with the concept that environmental effects on plant-plant interactions can vary depending on the life history stage of the plant (Goldberg & Novoplansky 1997; Miriti 2006; Schiffers & Tielbörger 2006; Maestre *et al.* 2009); however, this aspect of theory is not fully developed.

Some have hypothesized that neighbour effects can become more competitive as plants grow, in part due to differences in resource requirements (Miriti 2006). In the current study, the association between resource supply and competitive effects on growth suggests that neighbour effects on growth are largely determined by resources. Resource interactions in mild environments are thought to be mostly negative (Maestre *et al.* 2009), which explains the large competitive effects on growth. However, seedling survival can be facilitated through a number of mechanisms including reduced probabilities of desiccation, photoinhibition and herbivory (Callaway 1995). These mechanisms do not necessarily affect available resources, which may explain why seedling survival increased with standing crop and not resource supply. In some cases, this can lead to an increase in facilitation of seedling survival with productivity (Goldberg *et al.* 1999, this study), perhaps because low productivity sites have too little vegetative cover to provide these benefits to seedlings, making facilitation more likely at higher productivities.

Neither our results nor those of the meta-analyses (Goldberg *et al.* 1999; Maestre *et al.* 2005) fully support any of the major theories, suggesting that new theory regarding the relationship between plant-plant interactions and environmental gradients should be developed. These theories should incorporate both competition and facilitation as both occur simultaneously within most sites, and should also account for the effects of multiple environmental gradients on different life history components. Some work has been done in this direction

(Craine 2009; Maestre *et al.* 2009), although these theories must become more mechanistic and explicit in their predictions.

4.5. References

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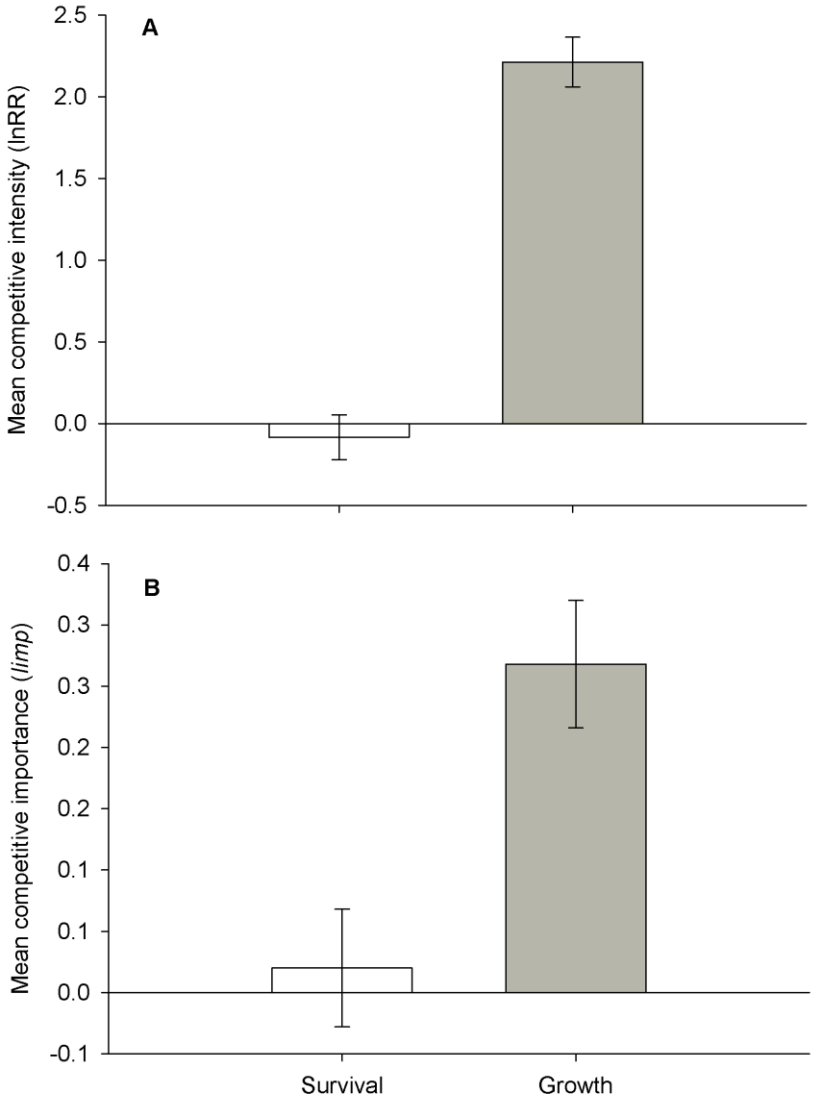

Figure 4.1 Mean competitive intensity (A) and competitive importance (B) for seedling survival and size. Responses were calculated such that competition is represented by positive values and facilitation by negative values. Means represent the average of all species and error bars indicate one standard error. Note that the y-axes in the two panels use different scales.

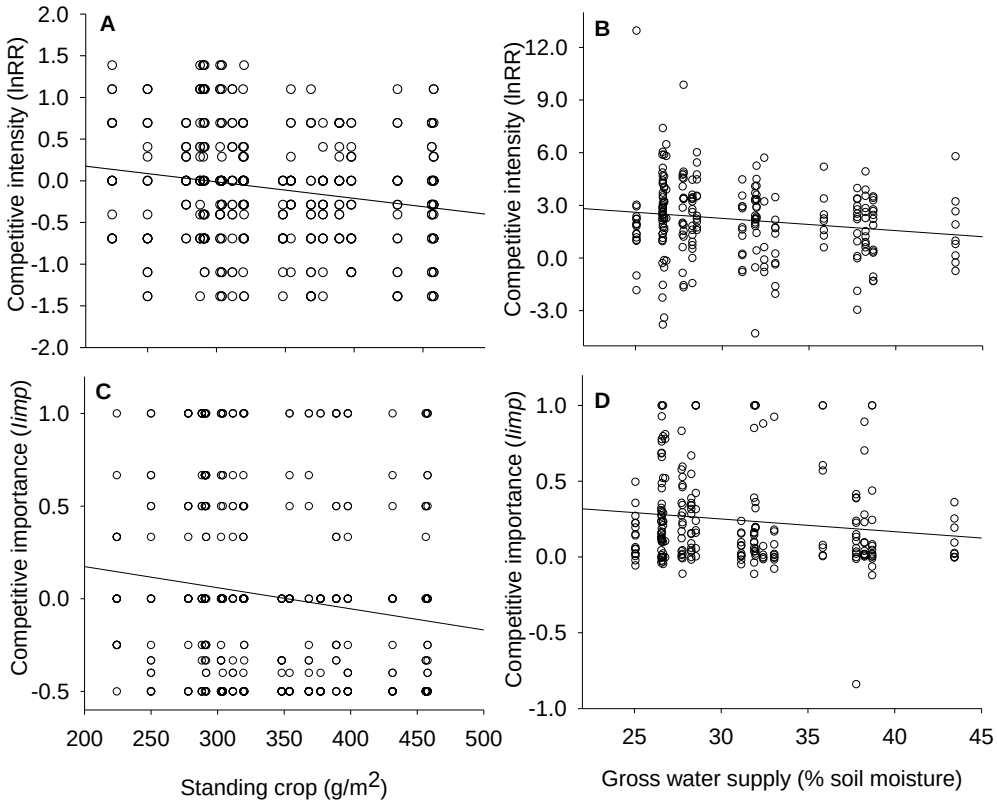

Figure 4.2 Changes in competitive intensity and importance with productivity. Competitive intensity (A) and relative competitive importance (C) decline as a function of standing crop for survival. Similarly, competitive intensity (B) and importance (D) decline for plant growth with gross water supply. Horizontal solid lines denote zero on the y-axis. Values above this line are competitive and below this line are facilitative. Dashed lines represent best fit lines. Each panel has a different scale for the y axis and that x axes are the same for A and C and for B and D.

Table 4.1 C-P relationship predicted for each major theory and the Goldberg meta-analysis.

Response metric	Gradient	Grime	SGH	Newman	Tilman	Goldberg	Davis	This study
Intensity	Standing crop/Gross supply	+	+	0	0	−	0/−	−
	Net supply						−	0
Competitive frequency	Standing crop/Gross supply		+					−/0
Relative importance	Standing crop/Gross supply	+	+			−		−
Demographic importance	Standing crop/Gross supply	+	+	+	0	−		

Note: Predictions are based on Grime (Grime 1973, 1977, 1979), Bertness and Callaway (Bertness & Callaway 1994), Maestre *et al.* (Maestre *et al.* 2009), Newman (Newman 1973), Tilman (Tilman 1982, 1988), Goldberg *et al.* (Goldberg *et al.* 1999), and Davis et al. (Davis *et al.* 1998). Cells containing a + mean that we expect increasing competition along the gradient, cells with a 0 mean that we expect a non-significant relationship, and cells with a − mean we expect a negative relationship. If a cell is left blank, then that particular metric or gradient does not apply to that theory. The column labelled this study refers to our findings and will be explained further in the results and discussion.

Table 4.2 A list of species used within the experiment by growth form and family.

Life history	Family	Species	Frequency
Annual	Brassicaceae	*Capsella bursa-pastoris* (L.) Medik.	<1
		Lepidium densiflorum Schrad.	<1
	Chenopodiaceae	*Chenopodium album* L.	6
		Monolepis nuttalliana (Schult.) Greene	<1
Perennial	Apiaceae	*Zizia aptera* (A. Gray) Fernald	2
	Asteraceae	*Artemisia ludoviciana* Nutt.	57
		Gaillardia aristata Pursh	7
		Heterotheca villosa (Pursh) Shinners	<1
		Solidago missouriensis Nutt.	69
		Symphyotrichum laeve (L.) Á. Löve & D. Löve	40
	Campanulaceae	*Campanula rotundifolia* L.	52
	Fabaceae	*Hedysarum alpinum* L.	5
	Lamiaceae	*Monarda fistulosa* L.	4
	Linaceae	*Linum lewisii* Pursh	<1
	Poaceae	*Bouteloua gracilis* (Kunth) Lag. ex Griffiths	28
		Bromus inermis Leyss.	26
		Elymus trachycaulus (Link) Gould ex Shinners	85
		Nassella viridula (Trin.) Barkworth	<1
		Poa pratensis L.	95
	Rosaceae	*Drymocallis arguta* (Pursh) Rydb.	12
		Geum triflorum Pursh	23
	Scrophulariaceae	*Penstemon gracilis* Nutt.	14

Note: Frequency of occurrence was determined by a 2009 survey of 100 2×2m plots spread across the field site. Values of <1 denote plants that are known to occur at the field site, but were not observed within the plots.

Table 4.3 Biomass regression coefficient estimates and significance tests.

	Regression coefficients				Regression results			
Species	**Intercept**	**ln(height)**	**ln(flowers)**	**ln(basal area)**	**Adjusted R^2**	**F**	**df**	**P**
Zizia aptera (A. Gray) Fernald	-6.76			1.11	0.964	240.61	1,8	<0.001
Artemisia ludoviciana Nutt.	-8.03			1.33	0.944	153.23	1,8	<0.001
Gaillardia aristata Pursh	-8.92			1.49	0.953	182.42	1,8	<0.001
Solidago missouriensis Nutt.	-11.01	3.85			0.645	15.54	1,7	0.006
Symphyotrichum laeve (L.) Á. Löve & D. Löve	-7.65		0.47	1.14	0.977	193.27	2,7	<0.001
Campanula rotundifolia L.	-11.01		-1.48	2.10	0.892	29.964	2,5	0.002
Monarda fistulosa L.	-9.14	1.14		0.95	0.991	343.13	2,4	<0.001
Bouteloua gracilis (Kunth) Lag. ex Griffiths	-6.981			1.00	0.903	56.99	1,5	0.001
Bromus inermis Leyss.	-7.01	1.1	0.48	0.54	0.993	437.36	3,6	<0.001
Elymus trachycaulus (Link) Gould ex Shinners	-11.53	3.32			0.986	572.31	1,7	<0.001
Nassella viridula (Trin.) Barkworth	-8.95	1.86		0.56	0.97	147.94	2,7	<0.001
Poa pratensis L.	-8.21	1.44		0.68	0.966	39.21	2,6	<0.001
Drymocallis arguta (Pursh) Rydb.	-7.22	0.62	2.41	0.76	0.971	67.17	3,3	0.003
Geum triflorum Pursh	-8.23			1.43	0.979	416.72	1,8	<0.001
Penstemon gracilis Nutt.	-7.17		2.39	1.08	0.995	527.78	2,3	<0.001

Note: For each species, if a particular regression coefficient was removed from the regression model by backward step-wise regression, then it is left blank in the table below. For *Bouteloua gracilis*, ln(flowers) was not included in the regression model as it caused underestimation of biomass for plants without flowers.

Table 4.4 Results of mixed model analysis of competition productivity relationships.

Plant response	Competition metric	Response variable	F	d.f.	P
Survival	Intensity (lnRR)	Gross water supply	0.69	1,306	0.408
		Net water supply	0.65	1,362	0.422
		Standing crop	**18.00**	**1,250**	**<0.001**
	Importance (Iimp)	Gross water supply	0.61	1,359	0.434
		Net water supply	0.12	1,392	0.728
		Standing crop	**12.71**	**1,384**	**<0.001**
	Interaction frequency	Gross water supply	1.07	1,381	0.301
		Net water supply	0.49	1,381	0.486
		Standing crop	**16.92**	**1,381**	**<0.001**
Biomass	Intensity (lnRR)	**Gross water supply**	**6.55**	**1,225**	**0.011**
		Net water supply	0.15	1,226	0.700
		Standing crop	0.42	1,221	0.516
	Importance (Iimp)	**Gross water supply**	**4.35**	**1,223**	**0.038**
		Net water supply	0.19	1,222	0.666
		Standing crop	0.02	1,225	0.880
	Interaction frequency	Gross water supply	0.32	1,205	0.573
		Net water supply	0.39	1,205	0.536
		Standing crop	0.91	1,205	0.342

Note: Significant results are in bold.

5. Plant community and local landscape characteristic effects on bee habitat use in a temperate savannah

5.1. Introduction

Pollinators are experiencing population declines across the globe (Kearns *et al.* 1998; Biesmeijer *et al.* 2006), threatening crop production and reproduction of wild plants (Potts *et al.* 2010). Many factors appear to be driving this decline, although habitat loss is thought to be of primary importance (Potts *et al.* 2010). In temperate regions, grasslands and savannahs often support the greatest abundance and diversity of bees (Grundel *et al.* 2010), though they are among the most threatened habitats globally (Hoekstra *et al.* 2005; Caro & Sherman 2011). Biodiversity conservation strategies of many temperate regions focus on the maintenance of natural and semi-natural areas to serve as reservoirs of diversity (Albrecht *et al.* 2007; Öckinger & Smith 2007; Sjödin *et al.* 2008; Concepcion *et al.* 2012). The density of semi-natural areas can have strong effects on the abundance and diversity of pollinators (Steffan-Dewenter *et al.* 2002; Jauker *et al.* 2009), but we know less about how pollinators use these areas.

Understanding how animals use habitat can improve conservation by reducing conflicts with other land uses and improving reserve design (Caro 2007; Berger-Tal *et al.* 2011). Only a small percentage of temperate grasslands and savannahs are protected (Hoekstra *et al.* 2005) and many of the areas being used for pollinator conservation are human influenced or created (Albrecht *et al.* 2007). Furthermore, some of these semi-natural areas also serve as rangeland (Öckinger & Smith 2007) and are managed for forage production (Fuller 1987; McCartney

1993). Thus, understanding how pollinators use the landscape is necessary to help design land management practices that are beneficial for pollinator conservation, while maintaining other land use practices.

Among pollinators, bees (series Apiformes within the superfamily Apoidea) are the group most adversely affected by human land-use and are also the most important pollinator in many ecosystems (Winfree *et al.* 2011). Land-use intensity, landscape composition and structure can all affect bee abundance and diversity (Kearns *et al.* 1998; Steffan-Dewenter & Tscharntke 2002; Brosi *et al.* 2008). These habitat effects are typically measured at the landscape scale, but can also affect bee activity at finer scales ranging from 5 to 50 m (Goverde *et al.* 2002; Diekötter *et al.* 2007). At the landscape scale, the effects of habitat alteration are thought to be primarily through changes in the distribution of floral and nesting resources (Roulston & Goodell 2011). Within the landscape, these resources are tied to spatial heterogeneity in both resource availability and landscape context, which can vary at fine scales (Auerbach & Shmida 1987). Thus habitat quality for bees is likely to vary at a fine scale. However, given the interdependence of the local landscape and resource availability, separating the specific determinants of habitat quality remains challenging.

Furthermore, different bee species vary in the scale at which they forage (Greenleaf *et al.* 2007) and in their habitat requirements (Murray *et al.* 2012). Differences in the scale of response among bees is primarily related to differences in body size (Greenleaf *et al.* 2007), which affects both foraging distances and energetic requirements (Kremen *et al.* 2007). Bumblebees have large bodies

relative to other groups of bees and have both large foraging distances and high energetic requirements. They are sensitive to habitat loss (Winfree *et al.* 2009; Bommarco *et al.* 2010) and respond behaviorally to habitat fragmentation at fine scales (Goverde *et al.* 2002). Smaller bees are also sensitive to change (Winfree *et al.* 2009; Bommarco *et al.* 2010), but have shorter flight distances (Greenleaf *et al.* 2007) and may respond to different cues within the landscape (Murray *et al.* 2012). This suggests that habitat usage is likely to differ among bee groups and should be accounted for in bee conservation efforts.

Here, we address the relative effects of local landscape characteristics and the plant community on bee habitat use by monitoring bee visitation within plots across a native remnant of the Aspen Parkland. We use structural equation modeling to 1) identify the relative importance of local landscape configuration and the flower community on bee habitat use, 2) determine which aspects of the landscape and the plant community affect bee habitat use and 3) determine if these factors vary between bumblebees and other bees at the site.

5.2. Materials and methods

5.2.1. Site description and study design

The study occurred in an unbroken and unseeded 50 ha section of native prairie at the University of Alberta research ranch at Kinsella, Alberta, Canada ($53^{o}05'$N, $111^{o}33'$W) within the Aspen Parkland eco-region. The field site is one of few remaining native grassland areas within the eco-region. These grasslands are managed through prescribed burning and grazing to reduce encroachment by woody plants (Fitzgerald & Bailey 1984; Bailey *et al.* 1990), resulting in a

landscape that is a mosaic of mixed grass prairie, shrubland and aspen stands (*Populus tremuloides*). However, all grazing was halted for the duration of the study.

In May 2009, 100 2m x 2m plots were established spread across 50 hectares. The study area was limited in size by the intensity of the sampling protocols (see below), although it is within the range of sizes used for pollination studies (Öckinger & Smith 2007; Bommarco *et al.* 2010; Sabatino *et al.* 2010). Plots were placed within both grassland and shrubland sites and were positioned to maximize variation in the local plant community and micro-environment. Minimum distances between plots varied from 4m to 75m, depending on spatial heterogeneity in the plant community. For each plot, we quantified the local landscape characteristics, vegetative productivity, the flower community and floral visitation. Of the 100 initial plots, 86 plots contained flowers belonging to species for which we observed insect visitation. The other 14 plots are not considered in this analysis.

5.2.2. Local landscape characteristics

Local landscape characteristics were quantified as the minimum distance to the nearest tree (tree distance), forest cover and proportion unforested area dominated by shrubs (proportion shrubland). Tree distance represents the distance to the forest edge (solitary trees were excluded) and was measured using a 30m measuring tape if the distance was less than 30m or with a Bushnell Sport 850 range finder when greater than 30m. Geographic position was determined for each plot using a Garmin GPSMap handheld GPS unit. These data were imported

into ARCGIS (v. 10) and layered over a recent aerial photo of the field site. We then traced the borders between each of the three habitat types (forest, shrubland, and grassland) and determined the cover for each habitat type within non-nested 5, 10, 20, 40, and 80m radius buffers around each plot. Initial analysis suggested that the local habitat types within the 10m and 20m buffers had the largest effects on bee visitation and diversity, so only these distance buffers were used for further analysis. From these measurements, we determined forest cover and proportion shrubland at both the 10m and 20m scale.

Forest cover represents the proportion of the local habitat that is low quality foraging habitat for much of the year, but has abundant dead wood which is used as a nesting resource for many bee species (Grundel *et al.* 2010; Roulston & Goodell 2011). Therefore a positive association with forest cover may represent the need for nesting resources while a negative association may represent the need for foraging resources. Proportion shrubland represents habitat with high available foraging resources that may also supply woody debris as nesting resources.

5.2.3. Plant community

The favorability of site conditions for plant growth was assessed by clipping standing live plant biomass, which we use as an estimate of local vegetative productivity. Biomass was clipped in late July 2009, at peak plant biomass, in a $0.1m^2$ strip, dried for at least 72h at 65^oC, and weighed. The flower community was quantified by counting the number of flowering stems per species every two weeks from mid-June to the end of August. Flowering species were

considered insect pollinated if we observed insects visiting flowers of that species. Insect pollinated plants generally flower from early May until early September, but the spring was dry and flower densities were low, so we delayed the onset of the study until conditions improved. Similarly, few plots had flowers in September, so monitoring was terminated. Flowering stem counts were pooled to determine the flower community across the summer. If a flowering species was present in a plot in more than one count, we used the maximum number of flowering stems for that species as our estimate of its abundance within that plot. From these counts of flowering stems, we generated three indices of the flower community: the total number of flowering stems (flower abundance), the number of species flowering (flower richness), and an estimate of community composition (flower community).

For each plot, flower abundance was estimated by summing flower abundances across species and flower richness by counting the number of flowering species. Flower community composition is represented by three ordination axes generated by non-metric multidimensional scaling on a presence-absence matrix of the flower community using the Sorensen distance measure (PC-Ord v 6.0). The final stress of the ordination was 19.6, with the three axes cumulatively explaining 68% of the variation in community composition. The first two axes of the ordination can be seen in Fig. 2. Axis 1 represents a gradient from woody to herbaceous vegetation (low to high scores) in productive areas near forest edge, whereas Axis 2 represents a gradient from low to high

productivity (low to high scores) in open grassland habitats. Axis 3 is not shown as it had no relationship with floral visitation (Fig. 3).

5.2.4. Floral visitation

Floral visitation was quantified through direct observation. Each plot that contained insect pollinated flowers was observed for 12 minutes every two weeks from mid-June to the end of August. In total, plots were observed for 2616 minutes over the course of the summer. All observation periods were between 10:00 and 17:30 as this corresponds to observed periods of bee activity. Floral visitors were initially identified as morphospecies on the wing and we recorded the number of visits and the identity of the flowering species visited within the 12 minute time interval. To aid in taxonomic identification of morphospecies, bees were captured using a combination of netting and pan-trapping over the course of the summer. From these collections, specimens considered representative of our morphospecies were identified to genus or to species if possible using a combination of published keys (Laverty and Harder 1988, Packer 2007, Koch et al 2012) and expert opinion. These observations were summed across the summer, to calculate four indices of patch use by bees: 1) total bee visitation, 2) total bee richness, 3) bumblebee visitation and 4) non-bumblebee visitation.

5.2.5. Structural equation modeling

We used structural equation modeling to separate the direct and indirect effects of local landscape characteristics on bee habitat use (for a description of structural equation modeling and its applications see Grace 2008). As each of our measures of bee response is potentially affected by the same plant community and

landscape characteristics, we used the same model structure for each bee response (Fig.1). We hypothesized that landscape characteristics would affect bee habitat use directly, through edge effects (Chacoff & Aizen 2006) and nesting resource availability (Grundel *et al.* 2010; Roulston & Goodell 2011), and indirectly through effects on the quality of the site for plant growth and ultimately the flower community.

Landscape characteristic effects on site quality for plant growth were modeled as a direct effect of tree distance on vegetative productivity (Fig. 1), as areas near forest edge often have favorable microclimatic conditions for plant growth (Harper *et al.* 2005). Tree distance was also modeled as directly affecting forest cover and proportion shrubland at both 10m and 20m scales (Fig. 1). We included correlated error terms between the two scales for both habitat type variables and between forest cover and proportion shrubland within each of the scales as they both refer to change along the gradient from grassland to shrubland to forest. Tree distance and each of these habitat variables were then directly connected to our bee response variable (Fig. 1) to test our hypotheses regarding their effect on bee use of the plot. To achieve multivariate normality, tree distance and forest cover were transformed by natural logarithm prior to analysis.

Vegetative productivity was hypothesized to have both direct and indirect effects on bee habitat use. Increasing nutrient and water availability generally increases primary productivity at the site (Lamb *et al.* 2007), but can also increase floral rewards available per flower (Cartar 2004; Baude *et al.* 2011). Given these relationships, we assume that rewards increase with productivity, although we did

not measure floral rewards directly. Therefore, we included a path between vegetative productivity and bee response (Fig. 1) to represent the effect of floral rewards on bee habitat use (Roulston & Goodell 2011).

We also included a path between productivity and flower abundance to represent the effect of local resource availability on flower production (Burkle & Irwin 2010) and between productivity and flower richness to represent the positive relationship between productivity and diversity expected given the range of productivity in the study (Tilman *et al.* 1996). Correlated error terms were included between flower abundance and flower richness as sites that maintain a large number of flowers generally support flowering throughout the summer and therefore more flowering species. Both high flower abundances (Potts *et al.* 2003b; Hegland & Boeke 2006) and flower diversity (Potts *et al.* 2003b; Ghazoul 2006; Ebeling *et al.* 2008) can attract more bees and may attract a higher diversity of bees. Therefore, flower abundance and flower richness were modeled as affecting bee habitat use, with flower abundance natural logarithm transformed and flower richness square root transformed prior to analysis.

Each flower composition ordination axis was included in the model as a separate variable. Flowering species also vary in their attractiveness to bees (Rasheed & Harder 1997; Cahill *et al.* 2008) and the number of bee species that visit them (Waser *et al.* 1996). Therefore, we modeled each flower axis as potentially affecting bee habitat use. Plant community composition can be strongly influenced by local site conditions; however, we had no reason to hypothesize which site conditions would be associated with any of the ordination

axes in particular, so we did not specify any relationship between these variables initially. In the final model, a path was included between productivity and flower axis 1 (Fig. 1) as it was suggested by modification indices (Grace 2006) and any influence of productivity on the flower community was thought to be causal given the importance of productivity in structuring plant communities (Grime 2001). Correlated error terms were also included between each of the flower composition axes and other landscape and flower community variables as suggested by modification indices to improve model fit (Fig. 1). However, prior to their inclusion in the model, we evaluated each correlation to ensure they were ecologically plausible.

Each of our bee response variables was transformed prior to analysis; total bee visitation, bumblebee visitation, and other bee visitation were all natural logarithm transformed, while bee richness was square root transformed. All structural equation modeling was done using Amos within SPSS (v. 18.0).

5.3. Results

5.3.1. Flower abundances and bee visitation

In total, the plots contained 2045 flowering stems belonging to 40 species of insect pollinated plants (see Appendix 3), with an average of 6.0 stems/m^2 (S.D. 4.3; range 0.3 – 61.3) and 1.0 plant species/m^2 (S.D. 0.6; range 0.3 – 3). We observed a total of 616 visits by bees to these flowers (see Appendix 3) belonging to 24 different morphospecies (corresponding to an estimated 44 species across at least 14 genera; see Appendix 3). This represents a mean of 7.2 visits (S.D. 17.2; range 0 – 121) belonging to 1.1 bee morphospecies (S.D. 1.7; range 0 – 9) per

plot. Of the bee visits 288 were by bumblebees (5 morphospecies, approximately 8 species) and 328 were by other bee genera (19 morphospecies, at least 36 species).

5.3.2. Overall model fit

Given the identical model structure used for each of the response variables, model fit did not vary across models. Model fit was deemed acceptable as we achieved multivariate normality and the chi-square test was non-significant ($\chi^2_{36} = 34.67$, $p = 0.532$). Using the landscape and patch quality variables we measured, we were able to explain around 50% of the variation in total bee visits, bee species, and other bee visits (Fig. 5.3a,b,d), but explained under 40% of the variation in bumblebee visits (Fig. 5.3c). We present only standardized effects of directional paths here for ease of comparison among factors. However, both the unstandardized effects and correlations among error terms can be found in supplementary materials (see Appendix 3).

5.3.3. Plant community effects

Both total bee visitation and bee richness were higher when flowers were abundant (Fig. 5.3a,b), which is consistent for both bumblebees (Fig. 5.3c) and other bees (Fig. 5.3d). No other factors had consistent effects in all four models, although a number of similarities were found between total bee visitation and richness. Both total visitation and richness were greater in plant communities containing tall herbaceous species typically found near forest edges (flower axis 1; Fig. 5.3a,b) and in communities containing species typical of productive open areas (flower axis 2; Fig. 5.3a,b). Plant community type also affected habitat use

by bumblebees, but not other bees (Fig. 5.3d), where bumblebees preferentially visited tall herbaceous communities near forest edges (flower axis 1; Fig. 5.3c). Flower richness only affected total bee visitation, but the effect was opposite of expected, with bee visitation declining with increasing flower richness (Fig. 5.3a).

Primary productivity had limited direct effects on bee habitat use, with productivity having a moderate positive effect on both total bee richness (Fig, 5.3b) and non-bumblebee visitation (Fig. 5.3d). However, productivity had positive indirect effects on all bee responses, primarily through effects on flower abundances (Table 5.1). This effect was weaker for bumblebees as they responded less strongly to flower abundance (Table 5.1, Fig. 5.3c)

5.3.4. Local landscape characteristics

Local landscape characteristics had relatively weak direct effects on total bee visitation and richness, with forest cover having a negative effect at the 10 m scale and a positive effect at the 20 m scale (Fig. 5.3a,b). These effects of forest cover were strong for non-bumblebees (Fig. 5.3d), but bumblebees did not significantly respond to forest cover (Fig. 5.3c), reducing the strength of this effect when averaged across all bees. However, bumblebees did preferentially use habitat closer to forest edges as seen in the negative relationship with tree distance (Fig. 5.3c).

Tree distance also had a number of indirect effects on bee responses (Table 5.1), although these effects are often in different directions. When trees were further away, there was an increase in all bee responses through a decrease in forest cover within 10m of the plot (Table 5.1); however, this effect was weak

for bumblebees as they did not significantly respond to changes in forest cover (Fig, 5.3c). Conversely, total bee visitation, bee richness and non-bumblebee visitation increased when trees were closer through increases in both forest cover and productivity (Table 5.1).

5.3.5. Relative factor importance

Across all bee responses, flower abundance had the largest total effect on bee habitat use (Table 5.2). This effect was consistent for total bee visitation, bee richness and non-bumblebee visitation. Tree distance had the largest total effect for bumblebees and the second largest effect overall (Table 5.2), despite having indirect effects that both increased and decreased bee habitat use (Fig. 5.3, Table 5.1). Primary productivity had the next largest effect (Table 5.2) as it had generally positive direct and indirect effects (Fig. 5.3, Table 5.1). Both forest cover variables and the first flower cover axis also had fairly large effects, although these effects were not consistent across bee responses (Table 5.2). When averaged across all factors, plant community variables (flower community + productivity) and local landscape characteristics had approximately equal effects (6.2 +/- 1.39 and 5.8 +/- 1.07 respectively; mean rank +/- standard error). We find similar results using direct effects instead of total effect (see Appendix 3).

5.4. Discussion

5.4.1. Local landscape vs. plant community

Both the plant community and local landscape characteristics influenced bee visitation and visitor diversity. Previous work has found that both the plant

community (Potts *et al.* 2003b) and landscape configuration (Steffan-Dewenter *et al.* 2002; Brosi *et al.* 2008) can affect bee abundances, but few studies have assessed their relative importance (Grundel *et al.* 2010) and none have accounted for both the direct and indirect effects of fine-scale landscape characteristics on habitat use. Our results suggest that the plant community and landscape characteristics have approximately equal total effect on bee habitat use as aspects of both have relatively strong effects (Table 2) and they are of approximately equal mean rank.

Landscape effects on bee abundances are thought to be primarily indirect and to act through changes in resource availability (Roulston & Goodell 2011). While the local landscape certainly affected bee responses indirectly through the flower community and the availability of nesting sites, we show that local landscape characteristics can have direct effects on bee abundance and diversity within flower patches equivalent to or greater than the qualities of the patch itself. This is consistent with previous work showing that fine scale experimental manipulation of local landscape matrices can change bee foraging (Goverde *et al.* 2002; Diekötter *et al.* 2007) and suggests that both direct and indirect effects of landscape can be important in determining bee habitat use.

5.4.2. Plant community and landscape effects

The most important habitat characteristic determining use by bees is flower abundance (Table 2) which is consistent with previous results showing the importance of flower densities for bee abundance and diversity (Potts *et al.* 2003b; Hegland & Boeke 2006). However, the majority of studies also find

flower diversity to be a strong predictor of bee abundances (Potts *et al.* 2003b; Ghazoul 2006; Ebeling *et al.* 2008; Grundel *et al.* 2010). We found that diversity was relatively unimportant in determining bee habitat use at the site and that when it did have an effect, it was opposite of expected. However, the bivariate relationship between flower richness and bee response was positive for both bee diversity and abundance (see Appendix 3). This suggests that, at fine scales, the superficially positive effect of flower richness is an artifact related to other factors correlated with flower richness. This highlights the need to consider all potential relationships when assessing the factors important for bee habitat use, but does not mean that diversity among patches is unimportant at the site. We found that total bee visitation and diversity was related to two different flower community types, suggesting that among patch diversity may be important.

Irrespective of the flower community, bees also foraged in habitat patches closer to the forest edge. Edges are commonly home to an abundance and diversity of organisms (Chacoff & Aizen 2006), as they contain species from both environments and often have a favorable microclimate, with greater light and faster nutrient cycling relative to forests and increased relative humidity and soil moisture relative to open habitats (Gelhausen et al. 2000, Harper *et al.* 2005). However, in many sites containing both forest stands and grassland patches, forests are poorer foraging habitat for bees (Grundel *et al.* 2010) and would be unlikely to host a distinct bee assemblage. This suggests that the direct effect of distance to forest edge is more strongly related to microclimate at this site.

In part, the indirect effects of distance to forest edge are related to having access to both forest and grassland habitats. Landscape effects on bees are thought to be primarily related to resource availability (Roulston & Goodell 2011) and we found that many bees preferentially foraged in plots that were surrounded by low forest cover at the 10m scale and high forest cover at the 20m scale. Forest stands may be poor foraging habitats, but they contain an abundance of dead wood which serves as nesting substrate for many bee species (Roulston & Goodell 2011). However, this does not include bumblebees, whoch may explain the difference in responses among bee groups (see section 5.4.3). Thus our results are consistent with bees preferentially using habitat where flowers are available, but not too far from nesting sites. However, this is not the only indirect effect of edge proximity.

The beneficial microclimatic conditions near forest edges can also increase primary productivity (Harper *et al.* 2005). Although the effect of productivity was inconsistent among bee responses, the direct and indirect effects were always positive and the total effect was important overall. The direct effects are likely related to increased resource availability and the resultant increases in floral rewards (Cartar 2004; Baude *et al.* 2011), although increased resources do not always increase floral rewards (Burkle & Irwin 2010). However, more productive sites do produce more flowers (Burkle & Irwin 2010), which largely drove the indirect effects of productivity (Table 1). Productive sites also had more attractive flowering species, but the effect of flower community composition was relatively weak.

5.4.3. Bumblebees vs. other bees

Although all bees preferentially used plots with attractive flowers, bumblebees and other bees differed in their habitat use. Beyond flower abundance, non-bumblebee habitat use was primarily driven by forest cover and the need for both nesting and foraging resources. Most non-bumblebees were smaller species and smaller bees have reduced foraging distances (Greenleaf *et al.* 2007). This shorter foraging range would require both floral and nesting resources within close proximity. Conversely, bumblebees have larger foraging distances (Greenleaf *et al.* 2007) and do not treat forests as barriers (Kreyer *et al.* 2004); therefore showing little association with habitat composition at the fine scales used in this study. However, bumblebees did preferentially forage near edges, whereas other bees did not. Given that the foraging ranges of bumblebees can be multiple kilometers (Greenleaf et al. 2009), they may be able to choose edge habitats, regardless of nest proximity. In contrast smaller bees are restricted to a smaller range, which may or may not include edge habitats. Edge habitats have favorable microclimatic conditions (e.g. less wind, higher humidity, and increased water availability) (Gelhausen 2000, Harper *et al.* 2005). Such microclimatic conditions are more beneficial for plant growth (Harper 2005), which may increase reward availability (Cartar 2004; Baude *et al.* 2011). Alternatively, foraging in less windy areas may reduce energy expended during foraging (Brantjes 1981) or decrease disruptions to established foraging routines (Comba 1999).

Bumblebees also differed from other bees in the aspect of the plant community that influenced their habitat use; bumblebees preferentially used tall herbaceous flowers, while other bees preferred productive plots. Bumblebee preferences for certain flowering species are well documented (Rasheed & Harder 1997; Cahill *et al.* 2008). Many other bee species also have flower preferences (Eickwort & Ginsberg 1980); however, the diversity of species in our other bee category would have obscured any species-specific preferences. We did see a preference within non-bumblebees for productive plots, potentially related to increased reward availability (Cartar 2004; Baude *et al.* 2011).

5.4.4. Conservation implications

The integration of behavioral ecology into conservation planning shows potential to increase the efficacy of conservation strategies (Caro 2007; Berger-Tal *et al.* 2011). Adjusting management activities has been suggested as a means to maximize bee populations in other systems (Sjödin *et al.* 2008); however, few studies have attempted to integrate bee behavior into management plans. Our results suggest that most bees prefer to use habitats with high flower densities, but that flower densities alone are unlikely to conserve many bee populations. Local landscape characteristics are as important as flower availability for bee habitat use and therefore high quality bee habitat in the Aspen Parkland must include both foraging patches and forested areas within close proximity. These foraging habitats should occupy productive microsites when possible, as productive sites attract more non-bumblebees and produce more flowering stems of attractive

species. We expect that similar results would be found in other savannah habitats, although further research is necessary to identify generalities among sites.

Given the threatened nature of temperate grasslands and savannahs (Hoekstra *et al.* 2005), identifying general strategies to conserve biodiversity within these regions should be of utmost importance. However, the primary goal within many grazed grasslands is not bee conservation; it is to maximize forage (Fuller 1987; McCartney 1993). In savannahs, woody plants are an integral part of the landscape and are important for maintaining biodiversity; however, woody plant encroachment can threaten forage production (McCartney 1993; Brown & Archer 1999; Roques *et al.* 2001). Complete removal of woody plants is likely to reduce many bee populations and some forested patches must be retained. Further, woody plants are often controlled through grazing and fire management (McCartney 1993; Brown & Archer 1999; Roques *et al.* 2001). Both grazing and fire have large effects on bee abundances, but the magnitude of these effects are determined by their frequency and intensity (Potts *et al.* 2003a; Potts *et al.* 2003b; Sjödin *et al.* 2008). Thus, the management of woody plants can have multiple effects on bee populations. If we are to conserve bees within these habitats, management plans need to be developed that reduce impacts on bee populations, while maintaining forage production for grazing.

5.5. References

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Table 5.1 Standardized indirect effects of productivity and distance to trees on each bee response. Paths follow those shown in Fig. 1. Indirect effects of tree distance on bee responses through productivity are calculated using the total effect of productivity on bee responses (Table 2).

	Standardized indirect effects			
Path	**All bees**	**Bee richness**	**Bumblebees**	**Other bees**
Productivity->Flower abundance	0.219	0.186	0.137	0.216
Productivity->Flower axis 1	0.061	0.050	0.069	0.010
Productivity->Flower richness	-0.059	0.009	-0.034	-0.033
Tree distance->Forest cover 10	0.194	0.224	0.144	0.260
Tree distance->Forest cover 20	-0.211	-0.163	0.085	-0.339
Tree distance->Productivity	-0.170	-0.201	-0.080	-0.194

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Table 5.2 Total standardized effect of each potential factor on each bee response. Totals were calculated as the sum of direct and indirect effects. Factors were ranked for each bee response separately and the top three responses are shown in bold. Mean rank was calculated as the average rank across each of the four responses and bee responses are shown in descending order of mean rank.

	Total standardized effect (Rank)				
Factor	**All bees**	**Bee richness**	**Bumblebees**	**Other bees**	**Mean Rank**
Flower abundance	**0.630 (1)**	**0.534 (1)**	**0.395 (2)**	**0.620 (1)**	1.25
Tree distance	**-0.382 (2)**	-0.234 (4)	**-0.404 (1)**	0.151 (5)	3
Productivity	**0.343 (3)**	**0.404 (2)**	0.161 (6)	**0.390 (3)**	3.5
Forest cover 10m	-0.234 (5)	**-0.27 (3)**	-0.174 (5)	-0.313 (4)	4.25
Forest cover 20m	0.253 (4)	0.195 (5)	-0.102 (9)	**0.406 (2)**	5
Flower axis 1	0.205 (6)	0.169 (6)	**0.232 (3)**	0.032 (9)	6
Flower axis 2	0.133 (8)	0.148 (7)	0.069 (10)	0.117 (6)	7.75
Flower richness	-0.203 (7)	0.030 (10)	-0.118 (8)	-0.115 (7)	8
Prop. shrub 10m	0.117 (9)	0.117 (8)	0.122 (7)	0.072 (8)	8
Prop. shrub 20m	-0.063 (10)	-0.092 (9)	-0.185 (4)	0.028 (11)	8.5
Flower axis 3	0.028 (11)	0.019 (11)	0.061 (11)	-0.029 (10)	10.75

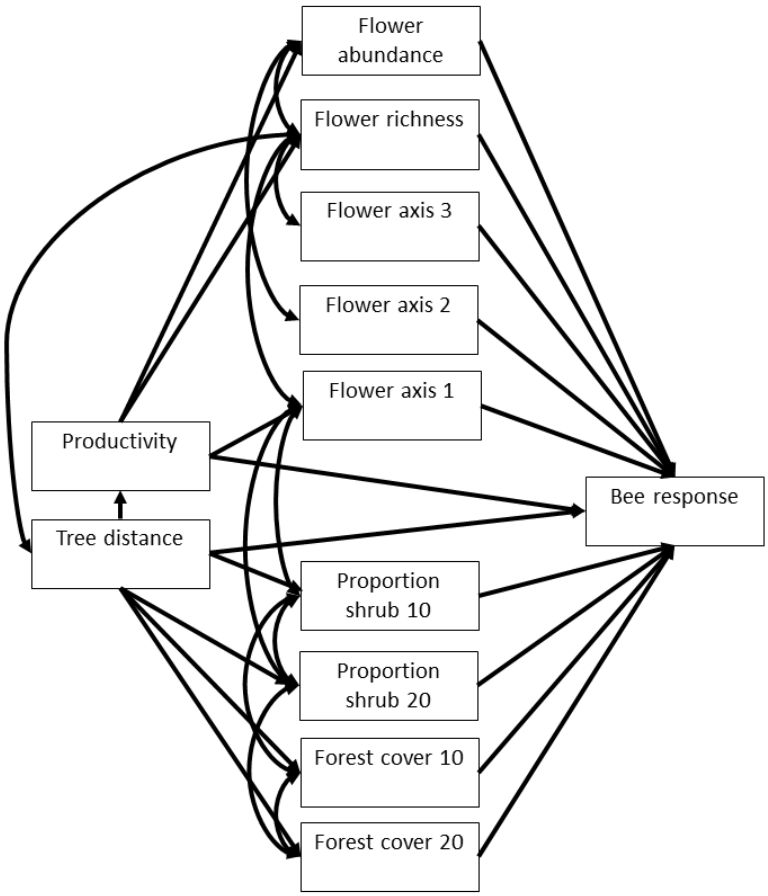

Figure 5.1 Overall structural equation model structure used to test direct and indirect effects of the local habitat matrix on bee responses. For a description of each path, see text on structural equation modeling.

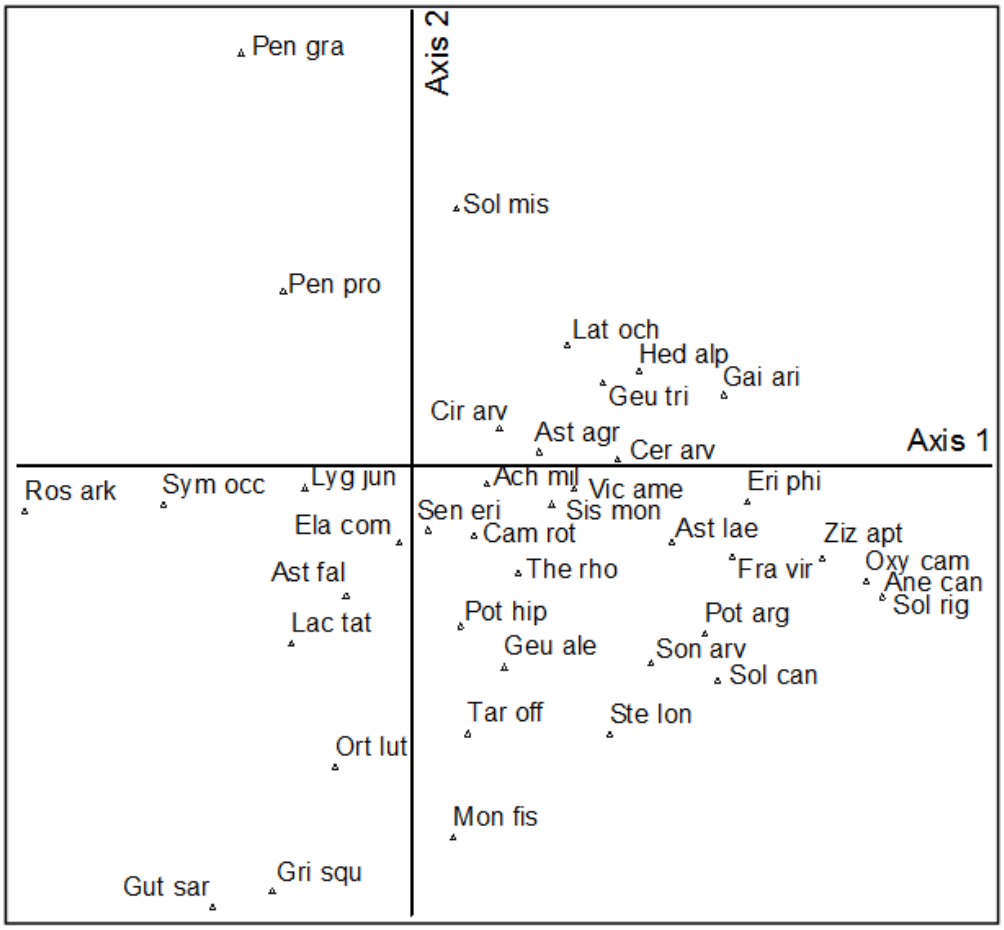

Figure 5.2: Results from non-metric multidimensional scaling ordination. Axis one explains 17% of the flower community, axis two 27% and axis three 23% (not shown). Axis one represents a gradient of productive plant community types near the forest edge ranging from shrubby (left) to herbaceous (right). Axis two represents a gradient of open grassland community types ranging from low productivity (bottom) to high (top) productivity.

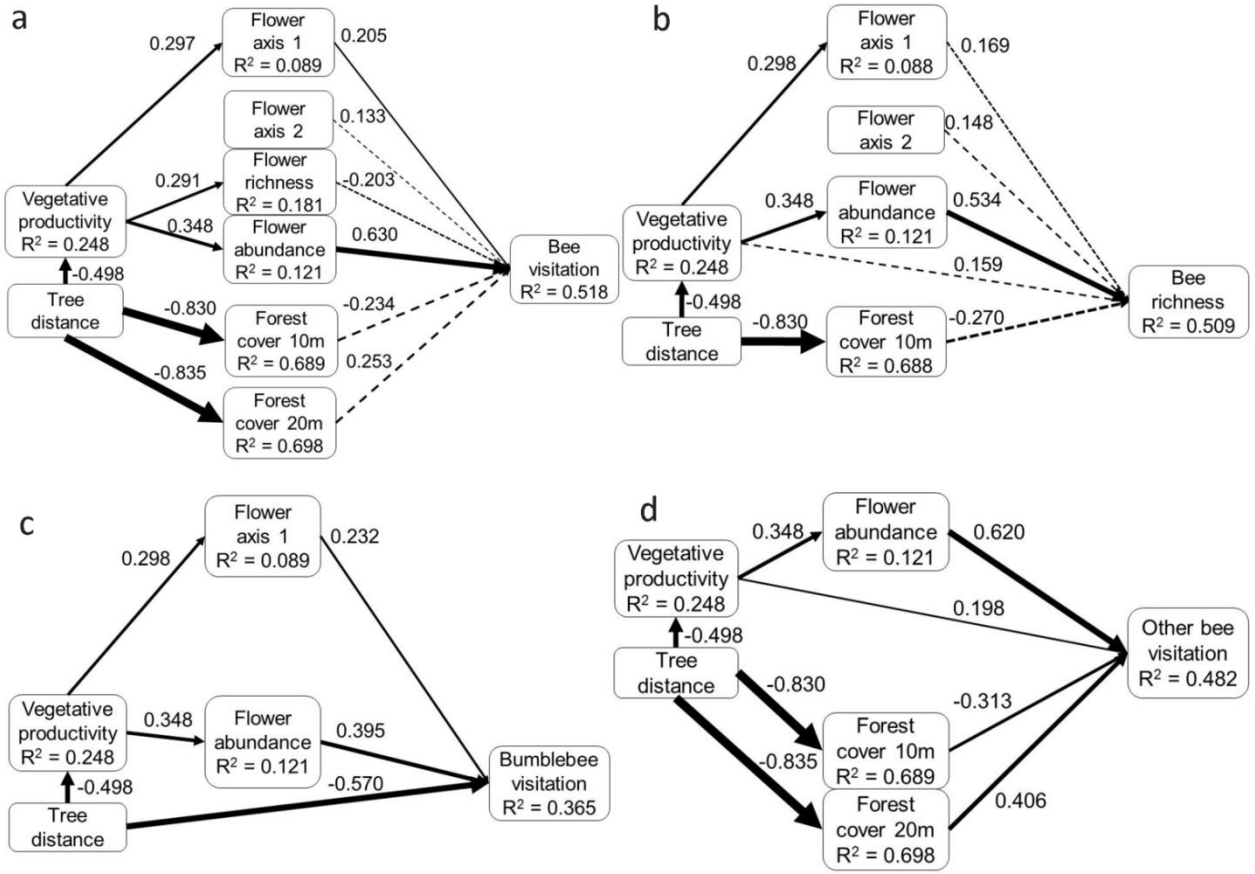

Figure. 5.3 Structural equation models showing the direct and indirect effects of the local habitat matrix on (a) total bee visitation, (b) bee visitor richness, (c) bumblebee visitation and (d) non-bumblebee visitation. Solid arrows show paths significant at $p < 0.05$ while dashed arrows show paths significant at $p < 0.10$. The width of each arrow is proportional to the size of the standardized effect, which is stated above the arrow. Non- significant paths, error terms and correlations among error terms are not shown. The total model can be seen in Fig. 1. Total variation explained for each endogenous variable is shown as R^2 within the box.

6. Flowering and visitation are more sensitive than vegetative growth to manipulation of mycorrhizae, nutrients and litter.

6.1. Introduction

Plant community structure can be affected by many environmental drivers. For example, in grassland plant communities elevated nitrogen usually reduces community diversity (Tilman 1987; Clark *et al.* 2007), while mycorrhizae can have variable effects, depending on the dominance structure of the community (Grime *et al.* 1987; Hartnett & Wilson 1999). Such studies have greatly improved our understanding of plant communities, yet they typically focus on the end result, the relative vegetative abundance of species within the community (vegetative community structure). However, plant communities are assembled in a continuous process where individual plants grow and die, and new individuals are recruited (Grubb 1977; Figure 1A). By only measuring changes in vegetative community structure, we implicitly assume that all processes involved in community assembly respond in the same fashion. However, sexual reproduction can become decoupled from vegetative growth (Neytcheva & Aarssen 2008). For individual plants, sexual reproduction is not always closely correlated with size because resources are allocated to fill the plant's needs (Perrin & Sibly 1993). As such, perennial plants often increase allocation to vegetative growth rather than sexual reproduction to maximize resource acquisition when resource stressed (Karlsson & Mendez 2005). This suggests that vegetative growth should be decoupled from sexual reproduction under a variety of situations. Such changes

in sexual reproduction have a large potential to impact both coexistence and community assembly (Sargent & Ackerly 2008).

Just as community assembly involves both vegetative and sexual processes (Figure 1B), sexual reproduction can be divided into the two aspects of the regeneration niche, seed production and recruitment (Grubb 1977). However, both theoretical (Jackson *et al.* 2009) and empirical (Tilman 1993; Stampfli & Zeiter 2004) studies related to the regeneration niche tend to focus on recruitment, although seed production is explicitly included in the original theory (Grubb 1977). Despite widespread belief that pollination can have important consequences for community assembly and the maintenance of diversity (Knight *et al.* 2005; Sargent & Ackerly 2008; Burkle *et al.* 2013) and experimental evidence that pollinators can alter patterns of plant species coexistence (Runquist & Stanton 2013), few studies have examined how the environment affects pollination at the community level (e.g. Burkle & Irwin 2010).

For many species, pollination is sensitive to changes in the environment. Flower production (Gange & Smith 2005; Cahill *et al.* 2008; Burkle & Irwin 2010), flowering phenology (Cleland *et al.* 2006), and pollinator attraction (Gange & Smith 2005; Cahill *et al.* 2008; Burkle & Irwin 2010; Baude *et al.* 2011) can all vary among species and with the environment, which ultimately could lead to local species extirpation (Willis *et al.* 2008; Burkle *et al.* 2013). As such, it seems vital that we understand how pollination changes within plant communities to evaluate potential impacts on diversity. Here, we consider how several key environmental factors (mycorrhizae, nutrients, and plant litter) affect the

relationship between vegetative growth, flowering, and floral visitation across a plant community.

Each of the three manipulated environmental drivers can be important for plant diversity and pollination, and could potentially have interactive effects. Mycorrhizal associations can affect plant diversity (Hartnett & Wilson 1999) and have strong, indirect effects on pollination (Gange & Smith 2005; Cahill *et al.* 2008; Becklin *et al.* 2011). Similarly, nutrient availability often alters plant diversity (Dickson & Foster 2011) and pollination (Burkle & Irwin 2010), as does litter accumulation (Facelli & Pickett 1991; Baude *et al.* 2011). Each of these factors affects the plants ability to gather certain resources. Mycorrhizal symbioses involve the exchange of photosynthetically derived carbon from the plant for soil nutrients from the fungi (Smith *et al.* 1997). As a result, adding nutrients can alter mycorrhizal effects on plant growth (Johnson 1993), flowering (Aguilar-Chama & Guevara 2012), and community structure (van der Heijden *et al.* 2008). Similarly, removing litter can affect nutrient, water, and light availability (Facelli & Pickett 1991), thereby affecting both the consequences of mycorrhizal symbioses (Johnson 2010; Aguilar-Chama & Guevara 2012) and resource interactions (Chapin *et al.* 1986) for individuals, populations, and communities. Thus we expect that each of these factors should affect vegetative abundance, flowering and flower visitation across the plant community, but we also expect that their effects should be interactive. However, given the prevalence of resource-induced changes in allocation (Karlsson & Mendez 2005), we

hypothesize that flowering and floral visitation should be more sensitive to the manipulations than vegetative abundance.

Using a multi-year experiment manipulating mycorrhizal fungi, soil nutrients and plant litter at a native grassland site, we identified the potential decoupling of relative abundance, flowering, and flower visitation for the 18 most common insect pollinated species. From these data, we tested whether species varied in their responses to the treatments, both in terms of the relationships abundance, flowering, and visitation, and in terms of phenology. Further, we tested whether these differences led to a decoupling of the relationship between abundance based estimates of diversity and the diversity of species flowering and being visited by pollinators. Finally, we used measurements of the abiotic and biotic environment, along with estimates of changes in phenology to identify the mechanisms by which our manipulations may interact to affect plant diversity.

6.2. Methods

6.2.1. Site description and study design

The study occurred in an unbroken and unseeded section of native prairie at the University of Alberta research ranch at Kinsella, Alberta, Canada (53^{o}05'N, 111^{o}33'W). The field site is a savannah type habitat with mixed grass prairie interspersed with stands of aspen (*Populus tremuloides* Michx.*)*. Though the site has historically been grazed by cattle, grazing had been halted three years prior to the onset of the experiment and did not occur for the duration of the study. Productivity at the site is limited by water and nitrogen availability (Lamb *et al.* 2007) and is dominated by graminoid species (Cahill *et al.* 2008). Diversity is

controlled by nitrogen and water availability as well as litter accumulation (Lamb 2008) and over 80% of species are forbs (Cahill *et al.* 2008). Thus, biomass is predominantly graminoid, and diversity is predominantly forb.

We established 20 blocks consisting of paired 5×7m plots in May 2008 distributed across 7 ha of grassland at the site. Beginning in May 2008, we suppressed mycorrhizae in one plot per block by applying the fungicide Rovral® FLO (Bayer Crop Science) biweekly at a rate of 82.3 $mg \cdot m^{-2}$ active ingredient (iprodione) in 7.5L water; control plots received only water. To minimize edge effects, for each plot, the outer 0.5m was designated as a buffer where no measurements were taken. Rovral® has been used successfully to reduce mycorrhizal colonization of plant roots and has limited documented non-target effects (Gange *et al.* 1990; Ganade & Brown 1997). Our fungicide application reduced hyphal colonization by mycorrhizal fungi to 70% of control and vesicle counts to 56% of control (see Appendix 4 for more details). We recognize that fungicide application can affect the plant community through mechanisms beyond mycorrhizal suppression (Allison *et al.* 2007). However, the methods for manipulating mycorrhizae in the field are limited and all have their limitations (Klironomos *et al.* 2011). As such, fungicide application was deemed the best method to assess the effect of mycorrhizae on a natural community.

In May 2009, each of the 5×7m plots was divided into four 2.5×3.5m sub-plots and we began a yearly treatment protocol where we applied a factorial combination of litter removal and fertilizer addition to these sub-plots for a total of eight treatment combinations. We removed litter from half of the plots by

raking and raked the control half to simulate the same level of mechanical disturbance, but replaced the litter. Half of the plots also received slow release fertilizer in May (14-14-14 NPK Osmocote® Classic, Scotts Professional) at a rate of 5.22g N/m^2. Boundaries between sub-plots were maintained by cutting roots to 10cm depth using an edging shovel every six weeks from May through September each year for 3 years.

6.2.2. Abiotic and biotic environmental effects

To identify potential mechanisms by which the treatments affected plant diversity, we measured plant available nitrogen, soil moisture, soil temperature, light availability within 9 blocks (72 plots) and the live biomass, relative graminoid abundance, and litter mass in all 20 blocks (160 plots). Nitrogen was measured using resin bags, soil moisture and temperature using ECH_2O® EC-TM sensors (Decagon Devices), and light availability using an AccuPAR light meter (Decagon Devices). Biomass measurements were taken in $0.1m^2$ quadrats, separated into graminoids, forbs and litter, dried and weighed. See Appendix A for detailed methods.

6.2.3. Focal plant vegetative abundance and diversity

Percent cover of all vascular plants was visually estimated in early June, mid-July and late August of 2010 in $0.25m^2$ permanent quadrats established within each of the sub-plots. We averaged these estimates to determine the relative abundance of the most common insect-pollinated plant species. Plant species were classified as insect-pollinated if we observed insects visiting the flowers of that species. In total, 18 insect-pollinated plant species were found in

the permanent quadrats and were used for subsequent analyses (Table 6.1). From these species-specific abundance estimates, we also determined total focal species cover, richness, and evenness.

6.2.4. Flower abundance and diversity

Flower abundance and diversity were assessed using biweekly flowering stem counts over the $6m^2$ usable area of each sub-plot, with all flowering stems counted and identified to species. For each sub-plot, we determined the abundance of individual flowering species as the maximum count obtained for that species across counts within that sub-plot. These maximum abundances were then used to create estimates of the flower community, characterized as total flower abundance, flower species richness, and flower species evenness within that sub-plot.

6.2.5. Flowering phenology

For each sub-plot, we determined the first and last day that a flower species was found in that sub-plot. From these values we derived two values, the first date an open flower was recorded and the length of the flowering period for each species, which were then used to determine treatment effects on flowering phenology.

6.2.6. Flower visitation

Flower visitation was monitored in each sub-plot by direct observation of insect visits to flowers every two weeks from June 1 to mid-September 2010. All observations occurred on mostly sunny days with little wind between 0900h and 1900h; however, the timing of observations was adjusted over the summer to

avoid cool mornings and evenings in June and September and to avoid hot periods near solar noon in July and August. Each sub-plot was observed every two weeks for three intervals of four minutes (12 total minutes per 2-week period), with each plot observed once in the morning, early afternoon and late afternoon every two weeks. In each observation interval, we recorded the number of visits to each flower species to determine the visitation rate to individual species. To avoid potential direct effects of fungicide application on flower visitors, we applied fungicide only after all observations were completed for each two week interval, with a minimum of five days between fungicide application and insect observation. From the individual species visitation data, we calculated the number of flowering species visited and the evenness of these visits across flowering species for each sub-plot.

6.2.7. Statistical analysis

Our statistical analyses were designed to answer questions related to both the whole community and individual species; as such we used two related, yet different statistical frameworks. To determine if the treatments affected the abiotic and biotic environment and if communities were affected we used very similar analyses. For analyses of the environment, we included treatments factorially as fixed effects and block as a random effect. We also included a block-by-fungicide interaction as a random effect to account for the split-plot design. We used this same model structure to test whether the treatments affected vegetative abundance, richness, and evenness across the community. However, when measuring changes in flowering within the community, we were primarily

interested in how the treatments caused the diversity of species flowering to deviate from the diversity estimates based on vegetative abundances. Therefore we included total vegetative cover, species richness, and species evenness as continuous covariates for our models of flower density, flowering species richness and flowering species evenness. Similarly, we were primarily interested in the decoupling of flowering and flower visitation, so we included total flower density, flowering richness and flowering evenness as covariates when analyzing treatment effects on number of species visited and the evenness of visits across species. To normalize the residuals, we used separate transformations on each response variable (See Appendix 4 for details). For our model with vegetative evenness as the response variable, the block by fungicide interaction resulted in a non-positive Hessian matrix when using variance components as the covariance structure, so compound symmetry was used instead. When flower species visited was the response variable, block explained no variation regardless of the covariance structure specified and was removed from the model.

To determine if the treatments had variable effects on flowering phenology among species, we used two models, one for square root transformed first date of flower and one for length of flowering period. For both models, all treatment effects and flower identity were included factorially as fixed effects with the same random structure as the environmental models. We used an identical model to test if the treatments affected the relative abundance of individual plant species. We also tested whether the treatments decoupled flowering from vegetative growth and pollinator attraction from flowering. This

required some modification of the data to enable easier comparison among species; we relativized each of the measurements for the individual species by the mean for that measurement for that species. To test for decoupling of flowering from abundance among individual species, we used the same basic model structure as for phenology, but with relative flowering as the response variable and including relative abundance as a continuous covariate in a fully factorial fashion. Similarly, for the decoupling of visitation from flowering, we included relative flowering as a factorial covariate with relative visitation as the response variable. To present the results for these tests graphically, we calculated slopes for each species within each treatment combination, but excluded several species as they were too infrequent to calculate reliable slope estimates. All analyses were conducted in SPSS (v. 20).

6.3. Results

6.3.1. Abiotic and biotic environmental effects

Of the three treatments, only mycorrhizal suppression had no effect on the aspects of the abiotic environment and biotic environments that we measured. Fertilizer addition increased plant available nitrogen ($F_{1,52}$ = 101.50, P < 0.001), live biomass ($F_{1,122}$ = 58.40, P < 0.001) and the proportional abundance of graminoids ($F_{1,122}$ = 11.47, P = 0.001). This increase in biomass was likely why we found reductions in soil moisture ($F_{1,48}$ = 9.48, P = 0.003), light penetration through the canopy ($F_{1,48}$ = 53.05, P < 0.001), and soil temperature ($F_{1,48}$ = 19.91, P < 0.001). Contrary to our expectations, litter removal had no effect on soil nitrogen ($F_{1,48}$ = 0.28, P =0.597) or water ($F_{1,48}$ = 0.32, P = 0.576), but did reduce

litter biomass ($F_{1,114}$ = 656.16, P < 0.001), which increased light penetration ($F_{1,48}$ = 34.20, P < 0.001) and soil temperature ($F_{1,48}$ = 17.31, P < 0.001). However, fertilizer addition and litter removal had interactive effects on light penetration, where the effect of litter removal was reduced in fertilized plots relative to control plots ($F_{1,48}$ = 8.19, P = 0.006). We also found an unexpected three way interaction between mycorrhizae, fertilizer, and litter on soil temperature ($F_{1,48}$ = 4.67, P = 0.036), where mycorrhizal suppression decreased soil temperature, but only in two scenarios: 1) without fertilizer and with litter intact and 2) with fertilizer and litter removed. However, the mechanism behind this change is unclear. Full statistical and graphical results can be found in Appendix 4.

6.3.2. Flowering phenology

Mycorrhizal suppression had no effects on phenology, but both fertilizer addition and litter removal had some effect. Litter removal was the only factor to have consistent effects across species and caused flowering to start approximately 2 days earlier on average (Figure 2A; $F_{1,1266}$ = 6.57, P = 0.011). However, this had no effect on the mean length of the flowering period, but did cause both increases and decreases for some species ($F_{17,1261}$ = 1.82, P = 0.021) as is seen in the highly variable responses (Figure 2B). Fertilizer addition caused some species to increase, but others to decrease relative to controls in both the onset of flowering (Figure 2A; $F_{17,1259}$ = 1.65, P = 0.046) and the length of the flowering period (Figure 2B; $F_{17,1262}$ = 1.80, P = 0.024). Detailed species-specific responses and statistical results can be found in Appendix 4.

6.3.3. Decoupling of species-specific flowering and abundance

Although species varied in their abundance, we found no significant changes among species in abundance in response to the treatments (see Appendix 4). Across species, abundance was a strong indicator of flower densities ($F_{1,1273}$ = 52.38, $P < 0.001$). Under control conditions flower-abundance slopes were usually positive (mean 0.23 +/– 0.47 SD), but ranged from positive to negative (–0.96 to 1.04). However, species differed in their relative allocations to flowering (species * abundance interaction; $F_{17,1284} = 5.80$, $P < 0.001$), which is consistent with variability among species in reproductive strategies (Grubb 1977). Few treatments had relatively consistent effects across species (see Appendix 4). Litter removal increased the degree of flowering relative to abundance (Figure 3A; litter * abundance interaction; $F_{1,1283} = 4.94$, $P = 0.026$), but there was also a significant four way interaction between mycorrhizal suppression, fertilizer, litter removal, and abundance ($F_{1,1279} = 9.42$, $P = 0.002$). Litter removal only increased visitation in isolation or when both mycorrhizae were suppressed and fertilizer added, but not in combination with only one of the other treatments (Figure 3A). Consistent with strong environmental niche differences among species (Silvertown 2004), most factors had variable effects among species on the flowering-abundance relationship, as seen in the great deal of variability in the species-specific slopes (Figure 3A). Further, each factor exhibited a significant treatment * species * abundance interaction and almost all the interactions among treatments were significant in that context. In the interest of space, we do not present the full results here, but they are available in Appendix 4.

6.3.4. Decoupling of species-specific visitation and flowering

Flower densities were a strong indicator of visitation frequencies for all species ($F_{1,1278}$ = 40.52, P < 0.001) and visit-flower slopes under control conditions were also mostly positive (0.95 +/– 0.74), but did exhibit a wide range of values (–0.04 to 2.42). The importance of flower densities for determining visitation is consistent with a number of previous findings (e.g. Hegland & Boeke 2006). None of the treatments had a consistent effect on this relationship (see Appendix 4) and the mean change in the visit-flower slope relative to control was always near zero (Figure 3B). However, the visit-flower relationship did vary among plant species as a function of each factor independently and with each of the two way interactions among factors (see Appendix 4). This variability among species is easily visible in how the slope of the visit-flower relationship varies from control for many of the species (Figure 3B).

6.3.5. Community vegetative abundance and diversity

In total, the 18 focal insect-pollinated plant species considered here accounted for 33.8% (+/-standard deviation of 14.5%; range 0.7% – 61.1%) of total plant cover within the experiment. On average each permanent quadrat contained 6.1 (+/- 2.2; range 1 – 12) focal species which were fairly even in relative abundance on average (0.79 +/- 0.16. range 0.00 – 1.00). However, we did not find any significant treatment effects on either vegetative richness or evenness of the focal species (See Appendix 4 for detailed statistical results), despite large changes in the abiotic and biotic environment.

6.3.6. Community flower density and diversity

Across all plots, we counted 23,500 flowering stems, with an average of 149.7 (+/- 80.2; range 12 – 424) stems per plot. These flowers belonged to an average of 8.9 (+/- 2.8; range 1 –14) focal species and were relatively evenly distributed among species (0.72 +/- 0.14; range 0.00 – 0.95). Generally, there were more flowers when focal species cover was higher ($F_{1,147}$ = 7.95, P = 0.005) and when there were more focal species ($F_{1,147}$ = 4.91, P = 0.028). Only litter removal had a significant effect on flowering stems (Figure 4A; $F_{1,112}$ = 5.73, P = 0.018) which is consistent with the effect seen across species.

As would be expected, the diversity of the focal species that flowered was dependent on their vegetative abundance and diversity. Flowering species richness was positively correlated with total focal species cover ($F_{1,147}$ = 12.09, P = 0.001), and with richness ($F_{1,149}$ = 11.16, P = 0.001) and evenness estimates ($F_{1,145}$ = 5.73, P = 0.018) based on vegetative cover, but flowering species evenness was only positively correlated with focal species total cover ($F_{1,152}$ = 5.53, P = 0.020). However, in contrast to the highly variable effects of the treatments on flowering among species, we found far fewer effects on the diversity of species flowering. Both flowering species richness ($F_{1,114}$ = 5.27, P = 0.023) and evenness ($F_{1,119}$ = 8.76, P = 0.004) were affected by the interactive effects of mycorrhizal suppression and fertilizer. This suggests that relative nutrient acquisition among species is important for determining which species flower, and that mycorrhizae are important determinants of the balance of resource competition. When applied independently, mycorrhizal suppression and

fertilizer addition reduced the number and evenness of flowering species, but when both treatments were applied, the number and evenness of flowering species was no different than the control (Figure 4B,C). Litter removal had no effect on flower richness, but decreased flower evenness (Figure 4C; $F_{1,120} = 6.04$, $P = 0.015$). Full statistical results can be found in Appendix 4.

6.3.7. Flower visitation

We observed a total of 7450 visits over the study; however, these visits were highly variable among plots (mean 47.4 +/- 43.2) as were the number of flower species visited (mean 4.0 +/- 2.1) and visit evenness (mean 0.63 +/- 0.27). The number of flowering species visited was strongly related to the total number of flowers ($F_{1,140} = 11.74$, $P = 0.001$), the number of flowering species ($F_{1,115} = 65.08$, $P < 0.001$), and how evenly distributed flowers were among species ($F_{1,157} = 9.84$, $P = 0.002$), suggesting an important role of the floral display in attracting floral visitors. Unlike the number of flowering species visited, the evenness of visits among them was unaffected by flowering within the community (see Appendix 4).

Despite the widespread differences among species in how the treatments affected the relationship between flowering and visitation, effects on the diversity of flowering species visited were more limited. Independent of the floral display, mycorrhizal suppression did reduce the number of species visited (Figure 4A; $F_{1,40} = 6.78$, $P = 0.013$). However, for the evenness of visits, the effect of mycorrhizal suppression was dependent on the fertilizer treatment ($F_{1,118} = 6.44$, $P = 0.012$). In contrast to their effects on flower diversity, but still consistent with

mycorrhizae primarily playing a role in nutrient acquisition, both mycorrhizal suppression and fertilizer addition increased visit evenness when applied independently, but had no effect when both treatments were applied (Figure 4B). Further, fertilizer altered the effect of litter removal ($F_{1,116} = 6.20$, $P = 0.014$). Litter removal also increased visit evenness, but the effects were non-additive when fertilizer was applied, resulting in similar visit evenness regardless of litter status in fertilized plots (Figure 4B).

6.4. Discussion

As predicted, we observed decoupled responses to the manipulations between abundance and flowering and between flowering and visitation. These effects were widespread across members of the community and caused a decoupling of the responses in diversity of species present, flowering, and being visited. Many studies have shown that environmental factors can influence relative reproductive allocation (Bazzaz *et al.* 2000; Karlsson & Mendez 2005), yet few have experimentally assessed effects on flowering and pollination under field conditions (e.g. Becklin *et al.* 2011) and fewer still have manipulated the whole plant community (Cahill *et al.* 2008; Burkle & Irwin 2010). While previous studies have shown differences among species in how flowering and floral visitation change following some manipulation of the environment (Cahill *et al.* 2008; Burkle & Irwin 2010), ours is the first to quantify the potential impacts of environmental manipulations on plant diversity. Our findings suggest that flowering and the attraction of floral visitors are more sensitive than abundance to the environmental factors manipulated and that they are decoupled

from changes in abundance. Such changes in flowering and potentially in pollination could have important impacts on plant diversity (Knight *et al.* 2005; Sargent & Ackerly 2008).

6.4.1. Variation among species and effects on diversity

Species are expected to vary in how they respond to different environmental factors (Silvertown 2004) and in how they regenerate (Grubb 1977), with such differences driving patterns of coexistence. These two aspects of the niche are not independent and environmental requirements for growth may not be the same for regeneration (Holt 2009). However, studies of the regeneration niche typically focus on differences in environmental conditions that are suitable for adult growth versus those suitable for germination and seedling survival (Jackson *et al.* 2009). Our data suggest that species vary greatly in how they flower and attract visitors under a variety of environmental conditions. Further, there is no reason to believe that these conditions necessarily correspond to those that enable germination and survival. The regeneration niche was initially intended to encompass all aspects of regeneration from flowering to seedling survival and growth (Figure 1B; Grubb 1977). Both flowering and visitation were highly sensitive to environmental changes and if no seeds are produced, then germination conditions cannot matter. We suggest that flowering and pollination must be considered in any study of the regeneration niche and omitting these factors may miss an important coexistence mechanism.

Almost all treatment combinations caused decoupling among abundance, flowering, and visitation for some species, but not all caused the decoupling of

diversity. However, our study is the first to show that differences in how species respond to variation in their environment can cause changes in the diversity of species flowering and attracting visitors. Many authors have suggested a potential link between changes in pollination and diversity (Knight *et al.* 2005; Sargent & Ackerly 2008), but this would require changes in relative pollen limitation among species. Although we have no estimates of pollination efficacy and seed production, it seems unlikely that declines in flowering will be compensated for by more efficient seed production of the remaining flowers. Further, visitation networks and pollination networks can be highly congruent (Alarcón 2010); although our visitation results may not be a precise estimate of pollination, they should show the general trend. In addition to the decoupling of growth and reproduction, we found changes in flowering phenology among species. Changes in flowering phenology can have important consequences for plant-pollinator interactions by causing phenological mismatches among plants and pollinators (Memmott *et al.* 2007). Such mismatches can have large consequences on pollination efficacy (Rafferty & Ives 2012) and ultimately alter community structure through species extinction (Burkle *et al.* 2013).

6.4.2. Decoupling flowering from abundance

Although mycorrhizae (Grime *et al.* 1987; van der Heijden *et al.* 1998; Hartnett & Wilson 1999), nutrient availability (Tilman 1987; Turkington *et al.* 2002), and litter mass (Facelli & Pickett 1991; Xiong & Nilsson 1999) can have strong effects on plant diversity, we found no evidence of this treatment, despite changes in the availability of most essential resources. We were particularly

surprised that we found no effect of fertilizer on focal species diversity, given the increases in both biomass and graminoid dominance. Such changes suggest future losses among the focal species if these treatments are continued, but mortality of the focal species may be lagged due to storage effects (Chesson 2000).

There are many potential mechanisms behind the decoupling of flowering from abundance. Storage effects could explain this if resources become stored rather than allocated to reproduction. Further, stress can reduce reproductive allocation (Karlsson & Mendez 2005) as the limited resource pool becomes allocated towards resource acquisition and reproduction waits until conditions improve (Perrin & Sibly 1993). Of course the conditions that are stressful vary among plant species (Chapin *et al.* 1986; Grime 2001) and thus we found that different conditions caused decoupling for different species.

Mycorrhizae, fertilizer, and litter all decoupled flowering species diversity from abundance-based diversity in some way. Mycorrhizal suppression decreased flowering diversity, consistent with previous results showing that mycorrhizae increase flowering for the majority of species (Gange & Smith 2005; Cahill *et al.* 2008). However, mycorrhizae had limited effects when fertilizer was applied, consistent with theory suggesting that resource availability determines mycorrhizal effects (Johnson 2010). In isolation, fertilizer decreased flowering diversity, likely through increased competition with the dominant graminoids for a reduced light and soil moisture pool. However, our results do suggest that light availability was relatively unimportant for flowering species diversity, as we saw

no interaction with litter removal which increased light availability. Litter removal did reduce the evenness of flowers among species, predominantly through variable increases in flower production among species, with some species increasing more than other. The precise mechanism for this increase is unknown, but is likely related to warming and increased light availability as both can affect flowering to a certain extent (Dyer & Rice 1999; Cleland *et al.* 2006).

6.4.3. Linking mycorrhizae, nutrients, and litter to flowering and visitation

Many factors influence the attractiveness of flowers to pollinators. These can include aspects of the flower community, such as flower abundance and diversity, but other factors such as floral rewards can also have important effects (Potts *et al.* 2003). Mycorrhizae (Gange & Smith 2005; Cahill *et al.* 2008), nutrient availability (Cleland *et al.* 2006; Burkle & Irwin 2010), and litter mass (Baude *et al.* 2011) can have strong, yet variable, effects on flower visitation independent of any change in flower production. Further, each of the treatments can affect floral traits and the production of floral rewards (Gange & Smith 2005; Burkle & Irwin 2010; Baude *et al.* 2011; Becklin *et al.* 2011). Each of these factors decoupled visitation from the floral display to some extent. While we have no direct evidence for any change in traits or rewards, they likely played a role. Further, both fertilizer and litter altered phenology, which could drive changes in visitation (Memmott *et al.* 2007; Rafferty & Ives 2012). However, the effects of litter and fertilizer on phenology were not interactive, while their effects on visitation were. This suggests that the story is more complex than a shift in

phenology and that additional experiments are necessary to identify the precise mechanism.

6.4.4. Synthesis

Although the vegetative abundance of the focal species did not change over the duration of the study, these effects could take years to manifest (Tilman 1997). However, we did find strong evidence that flowering and floral visitation were more sensitive than vegetative growth to each of our manipulations. Changes in flowering and floral visitation can lead to changes pollination (Alarcón 2010), which can alter patterns of pollen limitation and potentially plant community structure (Knight *et al.* 2005; Sargent & Ackerly 2008; Runquist & Stanton 2013). The decoupling of vegetative and reproductive responses demonstrates that monitoring changes in biomass alone may be insufficient to predict long term community dynamics in response to environmental variability and global change.

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Table 6.1 Flowering species observed in the experiment and their mean cover, number of flowering stems, and floral visits.

Species	Mean cover	Mean flowering stems	Mean visits
Achillea millefolium L.	5.1	14.8	4.1
Astragalus agrestis G. Don	1.5	7.2	1.4
Astragalus flexuosus (Hook.) Douglas ex G. Don	<0.1	2.2	0.1
Campanula rotundifolia L.	0.9	6.2	1.5
Cerastium arvense L.	0.5	1.6	4.7
Descurainia sophia (L.) Webb. ex Prantl	<0.1	0.7	0.3
Drymocallis arguta (Pursh) Rydb.	0.2	0.5	0.7
Erigeron glabellus Nutt.	1.1	19	1.8
Galium boreale L.	7.9	16.2	0.5
Geum triflorum Pursh	1.7	6.2	0.6
Hieracium umbellatum L.	0.1	0.8	0.3
Orthocarpus luteus Nutt.	0.2	16	2.8
Oxytropis campestris (L.) DC.	0.3	4.1	0.5
Rosa arkansana Porter	5.5	2.3	1.1
Solidago missouriensis Nutt.	4	5.4	0.8
Symphyotrichum falcatum (Lindl.) G.L. Nesom	3.2	36.7	12.9
Symphyotrichum laeve (L.) Á. Löve & D. Löve	0.8	6.4	12.4
Vicia americana Muhl. ex Willd.	0.8	0.9	<0.1

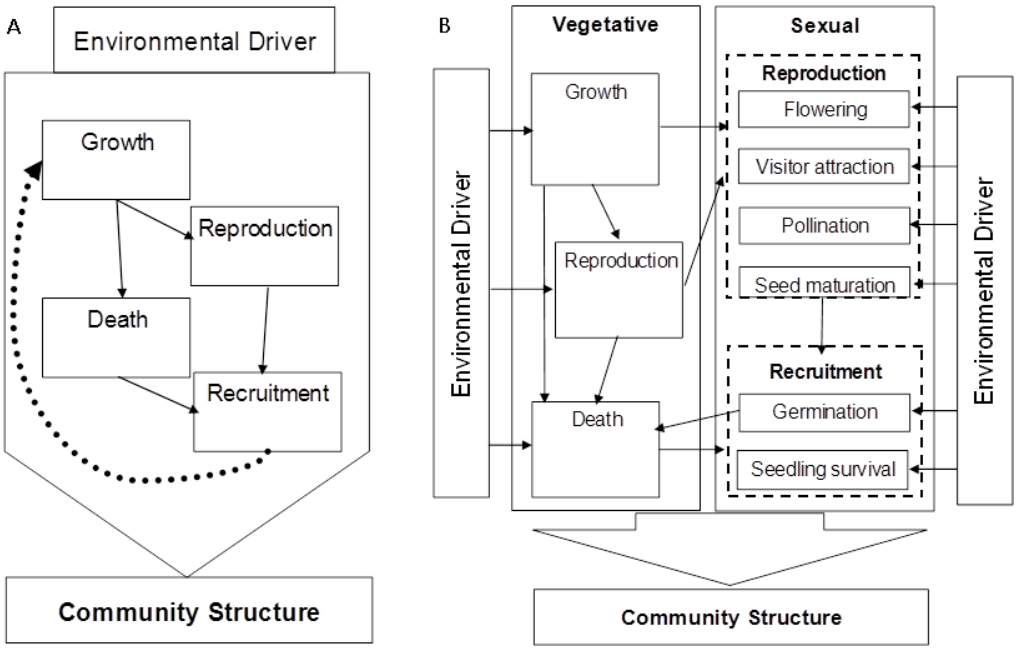

Figure 6.1 Hypothesized pathways by which communities respond to changes in the environment. Panel A shows a simplified version in which all processes are assumed to respond in the same fashion and is characteristic of the way that experiments measure changes in the community. Panel B shows a more holistic perspective of how communities change. Each process can potentially respond independently to changes in the environment, but is also dependent on a number of other factors.

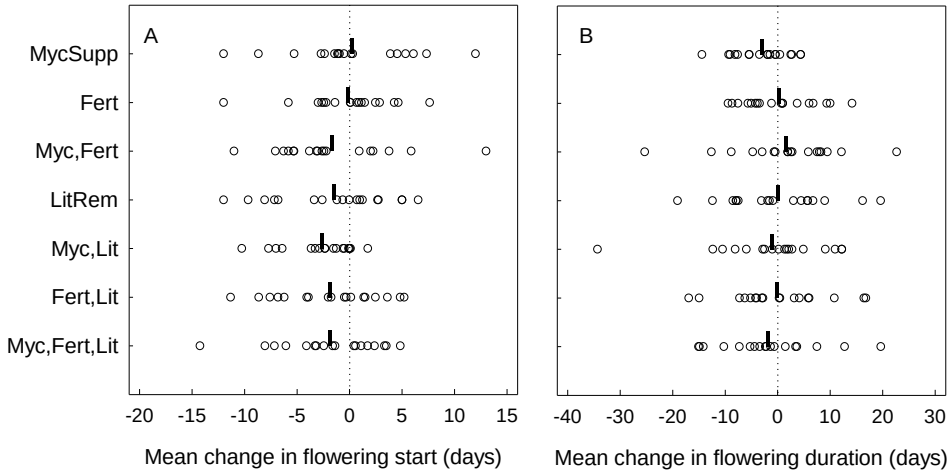

Figure 6.2: Changes in (A) the onset of flowering and (B) the length of the flowering period following mycorrhizal suppression, fertilizer addition, and litter removal. Open circles represent the average change in phenology for a single species in their response to a given treatment combination relative to control. The mean change in phenology across species is shown as a vertical line for each treatment.

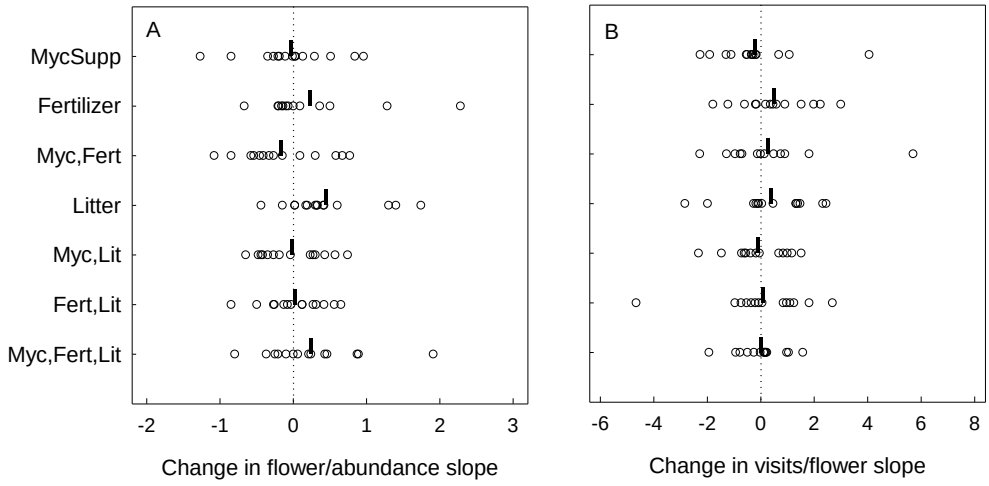

Figure 6.3 Changes in (A) the relationship between flowering and abundance and (B) the relationship between visitation and flowering following mycorrhizal suppression, fertilizer addition, and litter removal. The relationships were calculated as the slope of a linear regression between the two variables for each species under each treatment combination. Species for which we could not calculate a slope under control conditions were not included. Open circles represent the slope for a single species within a given treatment combination relative to the slope in control plots. The mean change in slope across species is shown as a vertical line for each treatment.

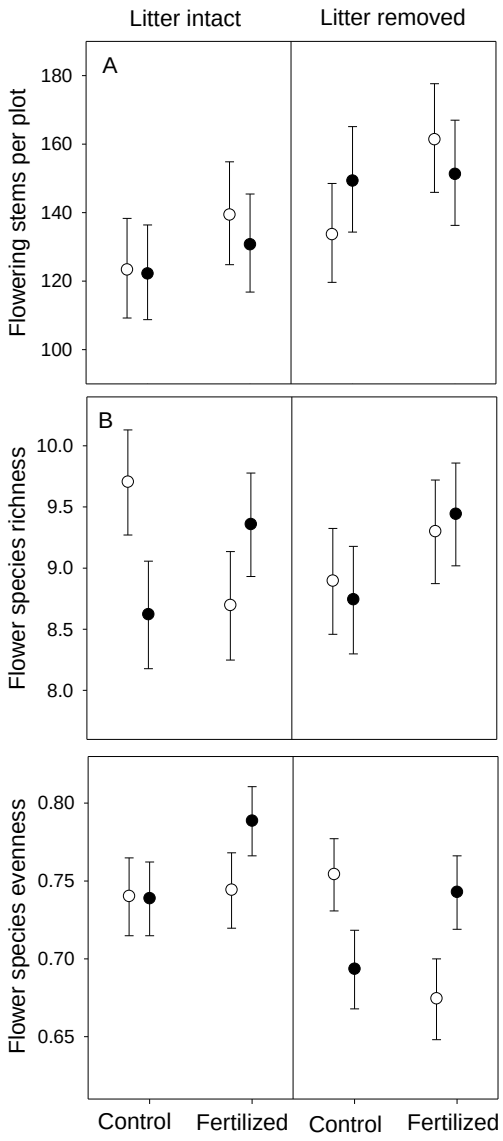

Figure 6.4 The effects of litter removal, mycorrhizal suppression and nutrient addition on (A) flower species richness and (B) flower species evenness. Open circles represent areas with mycorrhizae intact and filled circles mycorrhizal suppression. Error bars represent one standard error. Values shown are model estimated means after accounting for vegetative abundance and diversity of insect pollinated plants within the plant community. For model results see Table 3.

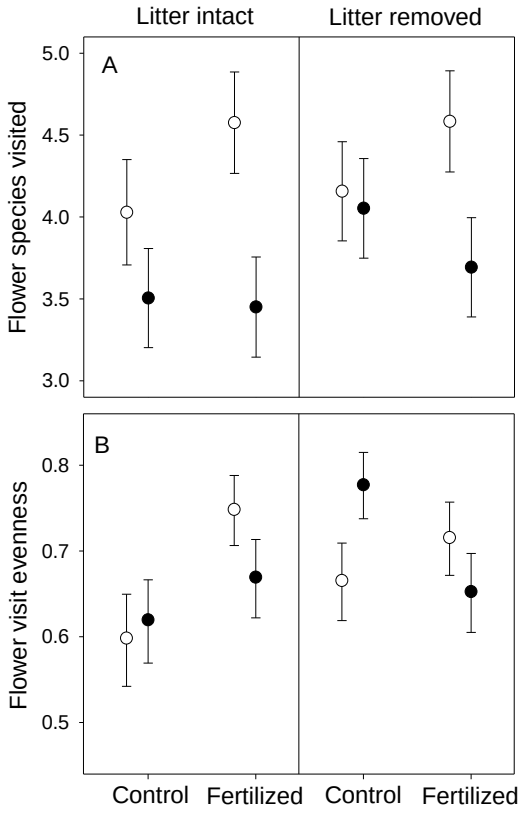

Figure 6.5 The effects of litter removal, mycorrhizal suppression and nutrient addition on (A) the number of flower species visited and (B) the evenness of visits among flower species. Open circles represent areas with mycorrhizae intact and filled circles mycorrhizal suppression. Error bars represent one standard error. Values shown are estimated marginal means after accounting for flower abundance and diversity.

7. General discussion

Community assembly is not a simple process. Any factor that can affect the recruitment of individuals or their coexistence once established has the potential to alter the outcome of community assembly (e.g. mycorrhizae (Bever 2003), herbivory (Carson & Root 2000), resource availability (Suding *et al.* 2005)). As such, understanding community assembly in its entirety may be near to impossible. The goal of this thesis was to understand some of this complexity for a single grassland system. Specifically, I was interested in how evolutionary history and environmental variation affected plant community assembly through plant species interactions with the environment, with each other, and with pollinators.

7.1. Evolutionary history

In niche-based models of community assembly, there is little doubt that evolutionary history is important; the characteristics of each species that define its niche are a product of that history. The controversy lies in how we use evolutionary history to help us understand community assembly and dynamics. Two uses of evolutionary history related to communities have been proposed. First, we can use the phylogenetic relationships among species to predict how species will respond to environmental change, with the expectation that closely related species should respond similarly (Mouquet *et al.* 2012). Second, the field of community phylogenetics contends that we can use the phylogenetic relationships among species to infer the processes that were important in community assembly (Webb *et al.* 2002). If species in a particular community are

more related than expected under a null model, then environmental conditions may have acted to select species with similar characteristics; in contrast, if species are less similar than expected, competition among similar species may have caused the exclusion of related species. Inherent in both of these proposed usages, is the assumption that related species are ecologically similar (Webb *et al.* 2002; Cavender-Bares *et al.* 2009). Essentially, this assumption requires that niches be broadly conserved (Wiens *et al.* 2010).

In Chapter 2, I found that plant population responses to many important ecological drivers were not phylogenetically conserved, despite morphological similarity among species at the site (Kembel & Cahill 2011). Further, even responses to very similar factors varied in whether they were conserved (e.g. responses to low intensity clipping (simulated herbivory) were conserved, but not high intensity clipping). This suggests a highly contextual nature to niche conservatism, which is consistent with the mixed results reported by others (Prinzing 2001; Cavender-Bares *et al.* 2004; Niinemets & Valladares 2006; Silvertown *et al.* 2006; Thuiller *et al.* 2011; Reinhart *et al.* 2012). Interestingly, I did find that responses to belowground factors as a group appeared to be phylogenetically conserved, but the same was not true for aboveground factors, which suggests that there is something unique about the belowground environment.

The extension to the assumption that related species are ecologically similar is that, due to their similarity, closely related species will compete more strongly (Darwin 1859). This 'competition-relatedness' hypothesis (Cahill *et al.* 2008b),

has rarely been tested and the results from these limited tests have been mixed (Cahill *et al.* 2008b; Burns & Strauss 2011; Violle *et al.* 2011; Best *et al.* 2013). In Chapter 3, I tested whether the mean relatedness of an individual to its neighbors influenced the strength of competition and found no relationship. While this is not a test of related species having similar competitive abilities, it does tell us that relatedness is not important for determining the outcome of competition within communities where the nature of competition is similar to the study system where this work was conducted. If related species are not similar, then the utility of phylogenetic relationships for predicting population responses to changes in the environment is questionable. Further, the contextuality of niche conservatism and the lack of a relationship between competition and mean relatedness raise questions about the generality of community phylogenetics theory.

No single process drives the assembly of ecological communities; community assembly is the result of many simultaneously processes (e.g. competition, facilitation, predation, disease, water limitation, temperature stress) acting to filter potential colonists from the community (Soliveres et al. 2012, Spasojevic and Suding 2012, Heard and Sax 2013, Schöb et al 2013). Thus, current attempts to attribute community assembly to a single process based on the degree of relatedness among community members seem overly simple. While there has been some effort to partition variation in phylogenetic community structure among multiple drivers (Soliveres et. al 2012), much more work is needed in this area before the field of community phylogenetics can advance.

Understanding how each of these drivers affects phylogenetic community structure requires understanding which traits are conserved and how each of these traits are related to the different environmental drivers. For example, if maximum relative growth rate (RGR) is a conserved trait and species with a high RGR are better competitors for light than slower growing species, under existing theory we would expect competition to exclude slow growing species and the community to become phylogenetically clustered (Mayfield and Levine 2010). However, if species with high RGR are more susceptible to herbivores, coexistence is then possible (Heard and Sax 2013), and the opposing effects of herbivory and competition are likely to lead to random phylogenetic structure. Disentangling these relationships is time consuming and requires careful measurement of phylogenetic community structure and the relevant traits along gradients of herbivory and competition, or the manipulation of competition and herbivory to look at changes in community trait values and phylogenetic community structure. This example illustrates the complexities involved if only two factors are considered, and studies will become more complicated, time-consuming, and labor intensive as more factors are considered. However, despite the labor required for such studies, they are necessary for us to understand the role of evolutionary history in plant community assembly.

7.2. Interactions among plants

Interactions among plants, especially competition, are expected to be important in determining the structure of communities (Tilman 1988; Grime 2001). However, there is debate over when competition is expected to be

important for community assembly (Grace 1991; Craine 2005; Brooker & Kikividze 2008). In Chapter 3, I explored whether competition was an important assembly mechanism by testing whether competition actually led to the predicted pattern of phylogenetic overdispersion within communities (Webb *et al.* 2002). As expected, given the limited niche conservatism and the limited influence of relatedness on competition, I found that the intensity of competition was unrelated to the degree of phylogenetic dispersion. I did find some evidence that overdispersion occurred at multiple spatial scales, but never consistently. However, phylogenetic dispersion did increase on average when light was limited. Competition for light is much more likely to cause competitive exclusion than competition for soil resources (Schwinning & Weiner 1998; Lamb *et al.* 2009), which is necessary for overdispersion to occur (Mayfield & Levine 2010). Thus, competition may only cause competitive exclusion when competition is for light, limiting potential effects on phylogenetic community structure (and community assembly) to such situations.

In Chapter 4, I more thoroughly investigated the role of environmental heterogeneity in determining the strength of competition by manipulating competition for 22 species across a range of productivities. Counter to theoretical predictions of increasing (Grime 2001) or invariant (Tilman 1988) relationships between competition and productivity, I found that competition generally declined with productivity. Given that light limitation is more likely to cause competitive exclusion than competition for soil resources (Chapter 3), it seems surprising that I found lower competition at high productivity, where light

is more likely to be limiting (Hautier *et al.* 2009). However, light availability is often heterogeneous in moderate to high productivity herbaceous communities (Abrams 1995) and thus competitive exclusion may also have been heterogeneous and not easily observed at these productivities. If competition does increase at productivities greater than those used in the study, this, at a minimum, suggests a nonlinear relationship between competition and productivity (e.g. Rees 2013). Such context dependency in competition needs to be better understood for us to advance our understanding of competition's role in community assembly. A more detailed study of the nature of resource supply and demand may help us reach this goal (Taylor *et al.* 1990; Davis *et al.* 1998; Suding *et al.* 2004). Further, competition-productivity relationships vary widely among species and species-specific models may need to be developed to help us better understand the role of competition in structuring plant communities (Holmgren & Scheffer 2010).

7.3. The role of pollinators

Plant communities are dynamic, with the relative abundance of species changing due to temporally heterogeneous growth rates, mortality, and recruitment (Chesson 2000). Species vary in how they recruit new individuals; this variation has been termed the "regeneration niche" and is an important mechanism of coexistence (Grubb 1977). Many authors have speculated on the role of pollination in species coexistence (Knight *et al.* 2005; Sargent & Ackerly 2008) and recent empirical results suggest that changes in pollination over long periods of time can cause dramatic changes in local communities through extinction (Willis *et al.* 2008; Burkle *et al.* 2013). Despite its inclusion in the

original regeneration niche theory (Grubb 1977), the role of pollination in recruitment is often neglected in experimental studies of coexistence. Further, pollination is rarely even measured at the community level (e.g. Cahill *et al.* 2008a; Burkle & Irwin 2010).

Pollination is likely overlooked in coexistence studies due to the difficulty and time-intensive nature quantifying any aspect of pollination relative to the time it takes to measure abundance. As the reproductive output of a plant is often correlated with size (Bonser & Aarssen 2009), the latter may seem a useful approximation for the former, but this is not always true (Neytcheva & Aarssen 2008). Plants often reallocate resources away from reproduction when stressed (Karlsson & Mendez 2005) and many plants are pollen limited (Larsen *et al.* 2005; Knight *et al.* 2006). Such decoupling of sexual reproduction from abundance must be accounted for. Plant communities are often seed limited (Clark *et al.* 2007a; Aicher *et al.* 2011), with changes in plant communities resulting from altered seed availability taking years to manifest (Tilman 1993). I argue that without a good understanding of pollination at the community level, our understanding of community assembly remains incomplete.

In Chapters 5 and 6, I examined how plant interactions with pollinators are affected by variability in the environment. In my survey of flower patch preference by bees (Chapter 5), I found that both the local environment and the position of that patch within the environment affected flower densities and diversity. In turn, landscape position, local conditions, and flower density all had strong effects on bee patch use. This spatial heterogeneity in bee patch use

suggests a high potential for spatial patterns of pollen limitation, dependent on all three factors that influenced bee patch use. After experimentally manipulating the local environment in Chapter 6, I found similar results. Both flowering and floral visitation were dependent on local environmental conditions, but the specific environmental effects varied among species. This resulted in a decoupling of diversity between flowering and abundance, and between visitation and flowering. This decoupling suggests a large potential for environmental change to affect plant community composition and diversity through altered flowering and pollination, and that these effects may not be predicted by measuring changes in abundance. However, the studies conducted in Chapters 5 and 6 are but a first step to understanding how pollination influences plant community structure, and future work should focus on integrating flower production, pollination, seed production, and recruitment success. Until we know if changes in flower production and altered visitation patterns lead to measurable changes in seed production and ultimately recruitment of new individuals, we are left with only hypotheses about the potential effects of pollination on community structure. Such an understanding requires both manipulative experiments and long-term measurements focusing on each of these processes. Given the effort required for such studies, these questions will likely be open for years to come.

7.4. Conclusions

Many processes have the potential to drive patterns of community assembly (Weiher *et al.* 2011; HilleRisLambers *et al.* 2012). Throughout this thesis I have addressed just a small fraction of these, and although the effect size

varied, nearly every factor I examined showed some potential to alter community assembly. Evolutionary history can influence how plants respond to some factors, but not all. Competition can structure communities, but likely only when light is limiting. Pollinators can change community trajectories, but again only under some conditions and the effects are likely lagged. From the combined inference of these studies, the lasting impression that I am left with is the high degree of contextuality in the relative importance of different assembly mechanisms. As many authors have noted before, the world is a heterogeneous place (Chesson 2000; Kelly & Bowler 2005; Hillebrand & Matthiessen 2009; Wisz *et al.* 2013), but it is this heterogeneity that drives biodiversity. If communities are largely structured by niche differences (Chesson 2000; HilleRisLambers *et al.* 2012), as I am inclined to believe, then each of the many dimensions of the niche (Clark *et al.* 2007b) may require a separate assembly rule (Weiher & Keddy 1999). If community assembly is context dependent, then assembly rules should be as well.

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8. Appendix 1

8.1. Phylogeny construction

We sampled 146 species across 35 families found at the study site (Table S1). Ninety-six specimens were collected directly from the ranch, while 48 additional taxa were sampled from herbarium specimens collected in Alberta. All specimens are housed in the University of Alberta Vascular Plant Herbarium (ALTA). We were unable to generate sequences from *Monarda fistulosa* L. (Lamiaceae) and *Lithospermum incisium* Lehm. (Boraginaceae). Instead, GenBank sequences were used for these species in analyses. GenBank sequences of *Amborella trichopoda* Baill. and *Nymphaea alba* var. *rubra* Lönnr. were used as outgroups.

Total genomic DNA was extracted from fresh, frozen, or herbarium material using either a modified CTAB method (McNickle et al. 2008), or DNeasy Plant Mini Kits (Qiagen Inc. Mississauga, ON). Standard PCR amplification and cycle-sequencing protocols were used (Hall *et al.* 2002; Hall *et al.* 2004). 1400bp of the partial coding sequence for the large subunit of the ribulose-biphosphate carboxylase gene (*rbcL*) was amplified using the forward and reverse primers from Duvall and Morton (Duvall & Morton 1996), referred to by us as 5'poa and 3'poa. All PCR products were cleaned using QiaQuick PCR Purification Kits (Qiagen Inc.). Five primers were used then for cycle sequencing: 5'poa, 3'poa, and 523F, 674R, 1020F (Conti *et al.* 1997). Sequences were obtained with an ABI-3730 DNA Analyzer (Applied Biosystems, Foster City, California, USA) after being cleaned with Performa DTR V3 96-well Short

Plate Kit (Edge BioSystems, Gaithersburg, MD). Sequence reads were edited in Sequencher v. 4.10.1 (Gene Codes Corporation, Ann Arbor, MI) then codon aligned. The highly conserved nature of this gene resulted in no indels in our alignment.

Phylogenetic relationships were inferred using Bayesian inference (BI) and maximum likelihood (ML). The optimum model of molecular evolution was GTR + I + Γ, which was determined using the Akaike Information Criterion (AIC) as implemented in jModeltest v. 0.1.1 (Posada 2008). Bayesian inference was executed in MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003), searching two simultaneous runs in tree space. Default priors and search parameters were used, except the temperature variable was reduced to 0.1 and the number of chains was increased to 8, in order to improve mixing. Runs were stopped after 2 million generations when there was sufficient indication for convergence (0.017 split-range frequencies, 1.00 PSRF (Potential Scale Reduction Factor) for all variables). Tracer v. 1.5 (Rambaut & Drummond) was used to assess convergence as well, indicating above adequate ESS (Effective Sample Size) for all variables except tree length and –lnL, which were below 200 (considered low by the software). Maximum likelihood analyses were conducted using GARli v0.95 (Zwickl 2006) starting from random trees, using 10,000 generations per search and estimating model parameters. Values for ML bootstrap were determined by conducting 100 replicates of the ML search except improvement of tree topology was limited to 5000 iterations. The majority rule tree excluding burn-in is shown in Figure S1 with posterior probability (PP) and ML bootstrap values provided.

8.2. Phylogenetic signal

To quantify phylogenetic signal, we first pruned the larger site-specific phylogenetic tree using the prune function of Picante in R (Kembel *et al.* 2010), such that only the species for which we had response data remained. These trees were then used to investigate the phylogenetic signal in species responses to individual treatments and more broadly for aboveground, belowground, resource, and non-resource treatment categories. When sequence data was missing for a species for which we had response data, we either substituted that species with a congener (*Hesperostipa curtiseta* - *Hesperostipa comata*) or removed that species from the analysis if no congener was present (*Gentiana amarella*). Similarly, if our response data sets contained estimates for congeners that could not be identified to species in that data set (*Astragalus* spp., *Arabis* spp.), we assumed that the species was the most common of the congeners and used the phylogenetic relationships of that species in our analysis.

Phylogenetic signal was determined by decomposing variation in responses across the nodes of the sub-tree using the methods of Pavoine et al. (Pavoine *et al.* 2010) using the ade4 package in R. This method can be combined with abundance data for the species to test for the correlations between certain traits or phylogenetic position and the abundance of species in the field. For the current analyses, we chose to limit ourselves to tests of phylogenetic signal (Pavoine *et al.*, 2010), and thus treated all species as equal in abundance. The decomposition of trait variation also allows for the calculation of response diversity using multiple responses (Pavoine *et al.*, 2010). However, we chose to

use estimated mean factor responses instead of using multiple responses within a single analysis as species were not included in any analyses unless we had at least three response values for that analysis and missing values would have reduced the number of species we could include in the factor categories to the same number of species as the factor within that category with the least species.

While, the randomization tests are generally powerful in their ability to detect phylogenetic signal when present, they do decline in power when the number of species used is small. Increasing the number of responses used may have compensated for the power lost due to reduced number of species included (Pavoine *et al.*, 2010); however, the removal of the less common species would have reduced the overall phylogenetic diversity within the analysis as the site is dominated in both species richness and abundance by Asteraceae and Poaceae (Cahill, 2003).

8.3. References:

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Table 8.1 Accession table for phylogenetic analysis.

Family	**Taxon**	**Herbarium voucher**	**GenBank #**
Amaryllidaceae	*Allium textile* A. Nelson & J.F. Macbr.	ALTA Acc. No. 119854	**JX848396**
Amborellaceae	*Amborella trichopoda* Baill.	See GenBank	L12628
Apiaceae	*Zizia aptera* (A. Gray) Fernald	G.G. McNickle 40, 20 May 2006	**JX848397**
Asparagaceae	*Maianthemum stellatum* (L.) Link	G.G. McNickle	**JX848398**

		s.n., 24 May 2006	
Asteraceae	*Achillea millefolium* L.	J.M. Taggart *s.n.*, 15 Jun 2009	**JX848399**
Asteraceae	*Agoseris glauca* (Pursh) Raf. var. *dasycephela* (Torr. & A. Gray) Jeps.	ALTA Acc. No. 118276	**JX848400**
Asteraceae	*Antennaria neglecta* Greene	J.M. Taggart *s.n.*, 15 Jun 2009	**JX848401**
Asteraceae	*Antennaria parvifolia* Nutt.	G.G. McNickle 29, 22 Jun 2005	**JX848402**
Asteraceae	*Artemisia campestris* L.	ALTA Acc. No. 121968	**JX848403**
Asteraceae	*Artemisia frigida* Willd.	G.G. McNickle *s.n.*, 31 Jul 2006	**JX848404**
Asteraceae	*Artemisia ludoviciana* Nutt.	G.G. McNickle *s.n.*, 24 Aug 2006	**JX848405**
Asteraceae	*Cirsium arvense* (L.) Scop.	G.G. McNickle 62, 13 Jul 2006	**JX848406**
Asteraceae	*Cirsium undulatum* (Nutt.) Spreng.	B.C. Alexander 98, 31 Jul 2010	**JX848407**
Asteraceae	*Coreopsis tinctoria* (Nutt.)	ALTA Acc. No. 105800	**JX848408**
Asteraceae	*Crepis tectorum* L.	G.G. McNickle 84, 11 Jun 2006	**JX848409**
Asteraceae	*Erigeron caespitosus* Nutt.	G.G. McNickle 77, 29 Jun 2006	**JX848410**
Asteraceae	*Erigeron glabellus* Nutt.	G.G. McNickle 26, 26 Jul 2005	**JX848411**
Asteraceae	*Erigeron philadelphicus* L.	J.M. Taggart *s.n.*, 15 Jun 2009	**JX848412**
Asteraceae	*Gaillardia aristata* Pursh	G.G. McNickle 56, 29 Jun 2006	**JX848413**
Asteraceae	*Grindelia squarrosa* (Pursh) Dunal	B.C. Alexander 91, 31 Jul 2010	**JX848414**
Asteraceae	*Gutierrezia sarothrae* (Pursh) Britton & Rusby	G.G. McNickle 72, 13 Jul 2006	**JX848415**
Asteraceae	*Helianthus pauciflorus* Nutt. subsp. *subrhomboideus* (Rydb.) O. Spring & E.E. Schill. [Basionym: *Helianthus subrhomboideus* Rydb.]	ALTA Acc. No. 125536	**JX848416**
Asteraceae	*Heterotheca villosa* (Pursh) Shinners var. *villosa*	G.G. McNickle 70, 13 Jul 2006	**JX848417**
Asteraceae	*Hieracium umbellatum* L.	ALTA Acc. No. 120163	**JX848418**
Asteraceae	*Lygodesmia juncea* (Pursh) D. Don	G.G. McNickle	**JX848419**

	ex Hook.	80, 13 Jul 2006	
Asteraceae	*Mulgedium pulchellum* (Pursh) G. Don [Homotypic syn.: *Lactuca tatarica* var. *pulchella* (Pursh) Breitung]	G.G. McNickle 24, 26 Jul 2005	**JX848420**
Asteraceae	*Packera cana* (Hook.) W.A. Weber & Á. Löve [Basionym: *Senecio canus* Hook.]	G.G. McNickle 86, 11 Jun 2006	**JX848421**
Asteraceae	*Pyrrocoma lanceolata* (Hook.) Greene [Homotypic syn.: *Haplopappus lanceolatus* (Hook.) Torr. & A. Gray]	ALTA Acc. No. 123530	**JX848422**
Asteraceae	*Senecio eremophilus* Richardson	G.G. McNickle *s.n.*, 31 Jul 2006	**JX848423**
Asteraceae	*Solidago canadensis* L.	ALTA Acc. No. 120212	**JX848424**
Asteraceae	*Solidago missouriensis* Nutt.	G.G. McNickle 17, 26 Jul 2005	**JX848425**
Asteraceae	*Solidago rigida* L. subsp. *humilis* (Porter) S.B. Heard & Semple	G.G. McNickle *s.n.*, 31 Jul 2006	**JX848426**
Asteraceae	*Sonchus arvensis* L.	B.C. Alexander 141, 15 Aug 2010	**JX848427**
Asteraceae	*Symphyotrichum ericoides* (L.) G.L. Nesom [Basionym: *Aster ericoides* L.]	ALTA Acc. No. 114827	**JX848428**
Asteraceae	*Symphyotrichum falcatum* (Lindl.) G.L. Nesom [Basionym: *Aster falcatus* Lindl.]	B.C. Alexander 94, 31 Jul 2010	**JX848429**
Asteraceae	*Symphyotrichum laeve* (L.) Á. Löve & D. Löve [Basionym: *Aster laevis* L.]	G.G. McNickle *s.n.*, 31 Jul 2006	**JX848430**
Asteraceae	*Tanacetum vulgare* L.	ALTA Acc. No. 113964	**JX848431**
Asteraceae	*Taraxacum officinale* F.H. Wigg.	G.G. McNickle 7, 8 Jun 2005	**JX848432**
Asteraceae	*Tragopogon dubius* Scop.	G.G. McNickle 48, 11 Jun 2006	**JX848433**
Asteraceae	*Xanthisma spinulosum* (Pursh) D.R. Morgan & R.L. Hartm. [Homotypic syn.: *Haplopappus spinulosus* (Pursh) DC.]	G.G. McNickle *s.n.*, 18 Aug 2005	**JX848434**
Boraginaceae	*Lithospermum incisum* Lehm.	See GenBank	EU599857
Brassicaceae	*Arabis hirsuta* (L.) Scop.	ALTA Acc. No. 121949	**JX848435**
Brassicaceae	*Arabis x divaricarpa* A. Nelson	G.G. McNickle	**JX848436**

	(pro sp.)	44, 20 May 2006	
Brassicaceae	*Capsella bursa-pastoris* (L.) Medik.	B.C. Alexander 85, 31 Jul 2010	**JX848437**
Brassicaceae	*Descurainia pinnata* (Waltor) Britton	G.G. McNickle *s.n.*, 8 Jun 2005	**JX848438**
Brassicaceae	*Descurainia sophia* (L.) Webb. ex Prantl	ALTA Acc. No. 115932	**JX848439**
Brassicaceae	*Draba nemorosa* L.	G.G. McNickle *s.n.*, 21 May 2006	**JX848440**
Brassicaceae	*Erysimum inconspicuum* (S. Watson) MacMill.	G.G. McNickle 82, 11 Jun 2006	**JX848441**
Brassicaceae	*Lepidium densiflorum* Schrad.	G.G. McNickle 49, 11 Jun 2006	**JX848442**
Brassicaceae	*Physaria arenosa* (Richardson) O'Kane & Al-Shehbaz [Homotypic syn.: *Lesquerella arenosa* (Richardson) Rydb.]	G.G. McNickle *s.n.*, 24 May 2006	**JX848443**
Campanulaceae	*Campanula rotundifolia* L.	ALTA Acc. No. 121437	**JX848444**
Caprifoliaceae	*Symphoricarpos occidentalis* Hook.	G.G. McNickle 23, 26 Jul 2006	**JX848445**
Caryophyllaceae	*Cerastium arvense* L.	G.G. McNickle 4, 8 Jun 2005	**JX848446**
Caryophyllaceae	*Silene drummondii* Hook.	ALTA Acc. No. 119292	**JX848447**
Caryophyllaceae	*Stellaria longifolia* Muhl. Ex Willd.	ALTA Acc. No. 117877	**JX848448**
Caryophyllaceae	*Stellaria longipes* Goldie	ALTA Acc. No. 118509	**JX848449**
Chenopodiaceae	*Axyris amaranthoides* L.	G.G. McNickle *s.n.*, 31 Jul 2006	**JX848450**
Chenopodiaceae	*Chenopodium album* L.	ALTA Acc. No. 124917	**JX848451**
Chenopodiaceae	*Monolepis nuttalliana* (Schult.) Greene	ALTA Acc. No. 115960	**JX848452**
Crassulaceae	*Sedum lanceolatum* Torr.	ALTA Acc. No. 124426	**JX848453**
Cyperaceae	*Carex siccata* Dewey	G.G. McNickle *s.n.*, 23 May 2006	**JX848454**
Cyperaceae	*Carex stenophylla* Wahlb.	G.G. McNickle *s.n.*, 22 May 2006	**JX848455**
Elaeagnaceae	*Elaeagnus commutata* Bernh. ex Rydb.	G.G. McNickle 42, 20 May 2006	**JX848456**
Fabaceae	*Astragalus agrestis* G. Don	G.G. McNickle *s.n.*, 22 May 2006	**JX848457**
Fabaceae	*Astragalus drummondii* Douglas	G.G. McNickle	**JX848458**

		33, 20 May 2006	
Fabaceae	*Astragalus flexuosus* (Hook.) Douglas ex G. Don	G.G. McNickle *s.n.*, 11 Jun 2006	**JX848459**
Fabaceae	*Astragalus laxmannii* Jacq. var. *robustior* (Hook.) Barneby & S.L. Welsh	G.G. McNickle 74, 13 Jul 2006	**JX848460**
Fabaceae	*Hedysarum alpinum* L.	G.G. McNickle 45, 11 Jun 2006	**JX848461**
Fabaceae	*Lathyrus ochroleucus* Hook.	G.G. McNickle *s.n.*, 24 May 2006	**JX848462**
Fabaceae	*Melilotus officinalis* (L.) Lam.	G.G. McNickle 60, 29 Jun 2006	**JX848463**
Fabaceae	*Oxytropis campestris* (L.) DC.	G.G. McNickle 32, 26 Jul 2005	**JX848464**
Fabaceae	*Pediomelum argophyllum* (Pursh) J.W. Grimes [Basionym: *Psoralea argophylla* Pursh]	G.G. McNickle 66, 13 Jul 2006	**JX848465**
Fabaceae	*Pediomelum esculentum* (Pursh) Rydb. [Basionym: *Psoralea esculenta* Pursh]	G.G. McNickle 46, 11 Jun 2006	**JX848466**
Fabaceae	*Petalostemon purpureus* (Vent.) Rydb. [Basionym: *Dalea purpurea* Vent.]	G.G. McNickle *s.n.*, 13 Jul 2006	**JX848467**
Fabaceae	*Thermopsis rhombifolia* (Nutt. ex Pursh) Richardson	G.G. McNickle *s.n.*, 22 May 2006	**JX848468**
Fabaceae	*Vicia americana* Muhl. ex Willd.	G.G. McNickle *s.n.*, 11 Jun 2006	**JX848469**
Fabaceae	*Vicia venosa* (Willd. ex Link) Maxim. [Heterotypic syn.: *Lathyrus venosus* Muhl. ex Willd.]	ALTA Acc. No. 121462	**JX848470**
Grossulariaceae	*Ribes americanum* Mill.	ALTA Acc. No. 117783	**JX848471**
Iridaceae	*Sisyrinchium montanum* Greene	G.G. McNickle 25, 29 Jun 2006	**JX848472**
Juncaginaceae	*Triglochin maritima* L.	ALTA Acc. No. 118269	**JX848473**
Lamiaceae	*Monarda fistulosa* L.	See GenBank	Z37419
Lamiaceae	*Stachys pilosa* Nutt.	ALTA Acc. No. 124964	**JX848474**
Liliaceae	*Lilium philadelphicum* L.	ALTA Acc. No. 121854	**JX848475**
Linaceae	*Linum lewisii* Pursh	G.G. McNickle *s.n.*, 31 Jul 2006	**JX848476**

Malvaceae	*Sphaeralcea coccinea* (Nutt.) Rydb.	G.G. McNickle 79, 29 Jun 2006	**JX848477**
Nymphaeaceae	*Nymphaea alba* L. var. *rubra* Lönnr.	See GenBank	GQ358627
Onagraceae	*Gaura coccinea* Pursh [Heterotypic syn.: *Oenothera suffrutescens* (Ser.) W.L. Wagner & Hoch]	G.G. McNickle 81, 29 Jun 2006	**JX848478**
Orobanchaceae	*Orthocarpus luteus* Nutt.	G.G. McNickle 67, 26 Jul 2006	**JX848479**
Plantaginaceae	*Linaria vulgaris* Mill.	G.G. McNickle *s.n.*, 31 Jul 2006	**JX848480**
Plantaginaceae	*Penstemon gracilis* Nutt.	G.G. McNickle *s.n.*, 11 Jun 2006	**JX848481**
Plantaginaceae	*Penstemon procerus* Douglas ex Graham	G.G. McNickle 28, 29 Jun 2006	**JX848482**
Poaceae	*Agropyron cristatum* (L.) Gaertn.	ALTA Acc. No. 095997	**JX848483**
Poaceae	*Agropyron cristatum* (L.) Gaertn. subsp. *pectinatum* (M. Bieb) Tzvelev [Heterotypic syn.: *Agropyron pectiniforme* Roem. & Schult.]	ALTA Acc. No. 114738	**JX848484**
Poaceae	*Agropyron dasystachyum* (Hook.) Scribn.	ALTA Acc. No. 118161	**JX848485**
Poaceae	*Agrostis scabra* Willd.	G.G. McNickle 22, 26 Jul 2005	**JX848486**
Poaceae	*Anthoxanthum nitens* (Weber) Y. Schouten & Veldkamp [Heterotypic syn.: *Hierochloe odorata* (L.) P. Beauv.]	G.G. McNickle 52, 23 May 2006	**JX848487**
Poaceae	*Avenula hookeri* (Scribn.) Holub [Homotypic syn.: *Helictochloa hookeri* (Scribn.) Zomero Zarco]	ALTA Acc. No. 118225	**JX848488**
Poaceae	*Bouteloua gracilis* (Kunth) Lag. ex Griffiths	G.G. McNickle 13, 26 Jul 2005	**JX848489**
Poaceae	*Bromus ciliatus* L.	ALTA Acc. No. 120064	**JX848490**
Poaceae	*Bromus inermis* Leyss.	ALTA Acc. No. 121745	**JX848491**
Poaceae	*Bromus porteri* (J.M. Coult.) Nash	G.G. McNickle 65, 13 Jul 2006	**JX848492**
Poaceae	*Calamovilfa longifolia* (Hook.) Hack. ex Scribn. & Southw.	G.G. McNickle *s.n.*, 24 Aug 2006	**JX848493**
Poaceae	*Dactylis glomerata* L.	G.G. McNickle *s.n.*, 24 Aug 2006	**JX848494**

Poaceae	*Deschampsia cespitosa* (L.) P. Beauv.	ALTA Acc. No. 112673	**JX848495**
Poaceae	*Elymus glaucus* Buckley	G.G. McNickle 31, 26 Jul 2006	**JX848496**
Poaceae	*Elymus trachycaulus* (Link) Gould ex Shinners	G.G. McNickle 30, 26 Jul 2006	**JX848497**
Poaceae	*Festuca hallii* (Vasey) Piper	J.M. Taggart *s.n.*, 15 Jun 2009	**JX848498**
Poaceae	*Hesperostipa comata* (Trin. & Rupr.) Barkworth [Basionym: *Stipa comata* Trin. & Rupr.]	ALTA Acc. No. 112468	**JX848499**
Poaceae	*Hordeum jubatum* L.	G.G. McNickle 64, 13 Jul 2006	**JX848500**
Poaceae	*Koeleria macrantha* (Ledeb.) Schult.	J.M. Taggart *s.n.*, 15 Jun 2009	**JX848501**
Poaceae	*Muhlenbergia cuspidata* (Torr. ex Hook.) Rydb.	G.G. McNickle *s.n.*, 24 Aug 2006	**JX848502**
Poaceae	*Muhlenbergia richardsonis* (Trin.) Rydb.	ALTA Acc. No. 105977	**JX848503**
Poaceae	*Nassella viridula* (Trin.) Barkworth [Basionym: *Stipa viridula* Trin.]	ALTA Acc. No. 106022	**JX848504**
Poaceae	*Pascopyrum smithii* (Rydb.) Barkworth & D.R. Dewey	G.G. McNickle *s.n.*, 24 Aug 2006	**JX848505**
Poaceae	*Poa pratensis* L.	ALTA Acc. No. 120115	**JX848506**
Poaceae	*Poa sandbergii* Vasey	ALTA Acc. No. 121885	**JX848507**
Polemoniaceae	*Phlox hoodii* Richardson	G.G. McNickle 38, 20 May 2006	**JX848508**
Polygonaceae	*Fallopia convolvulus* (L.) Á. Löve [Basionym: *Polygonum convolvulus* L.]	ALTA Acc. No. 119281	**JX848509**
Polygonaceae	*Rumex crispus* L.	ALTA Acc. No. 120206	**JX848510**
Polygonaceae	*Rumex salicifolius* Weinm. var. *mexicanus* (Meisn.) C.L. Hitchc. [Basionym: *Rumex mexicanus* Meisn.)	G.G. McNickle 76, 29 Jun 2006	**JX848511**
Polygonaceae	*Rumex triangulivalvis* (Danser) Rech. f.	ALTA Acc. No. 119019	**JX848512**
Primulaceae	*Androsace septentrionalis* L.	G.G. McNickle *s.n.*, May 2006	**JX848513**
Ranunculaceae	*Anemone canadensis* L.	ALTA Acc. No. 125491	**JX848514**

Ranunculaceae	*Anemone cylindrica* A. Gray	ALTA Acc. No. 115908	**JX848515**
Ranunculaceae	*Anemone patens* L. [Homotypic syn.: *Pulsatilla patens* (L.) Mill.]	G.G. McNickle 36, 20 May 2006	**JX848516**
Ranunculaceae	*Ranunculus pedatifidus* Sm. var. *affinis* (R. Br.) L.D. Benson	G.G. McNickle *s.n.*, 21 May 2006	**JX848517**
Ranunculaceae	*Ranunculus rhomboideus* Goldie	G.G. McNickle *s.n.*, 21 May 2006	**JX848518**
Ranunculaceae	*Thalictrum venulosum* Trel.	G.G. McNickle 47, 11 Jun 2006	**JX848519**
Rosaceae	*Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem.	ALTA Acc. No. 115905	**JX848520**
Rosaceae	*Argentina anserina* (L.) Rydb. [Basionym: *Potentilla anserina* L.]	G.G. McNickle 53, 11 Jun 2006	**JX848521**
Rosaceae	*Drymocallis arguta* (Pursh) Rydb. [Basionym: *Potentilla arguta* Pursh]	G.G. McNickle *s.n.*, 24 Aug 2006	**JX848522**
Rosaceae	*Fragaria virginiana* Duchesne	G.G. McNickle *s.n.*, May 2006	**JX848523**
Rosaceae	*Geum aleppicum* Jacq.	ALTA Acc. No. 124935	**JX848524**
Rosaceae	*Geum triflorum* Pursh	G.G. McNickle 6, 8 Jun 2005	**JX848525**
Rosaceae	*Potentilla bipinnatifida* Douglas	G.G. McNickle 55, 29, Jun 2006	**JX848526**
Rosaceae	*Potentilla concinna* Richardson	G.G. McNickle 41, 20 May 2006	**JX848527**
Rosaceae	*Potentilla gracilis* Douglas ex Hook.	B.C. Alexander 71, 1 Jul 2010	**JX848528**
Rosaceae	*Potentilla hippiana* Lehm.	G.G. McNickle 59, 29 Jun 2006	**JX848529**
Rosaceae	*Potentilla norvegica* L.	ALTA Acc. No. 121490	**JX848530**
Rosaceae	*Potentilla pensylvanica* L.	ALTA Acc. No. 115097	**JX848531**
Rosaceae	*Rosa arkansana* Porter	G.G. McNickle *s.n.*, 29 Jun 2006	**JX848532**
Rosaceae	*Rubus idaeus* L.	ALTA Acc. No. 120202	**JX848533**
Rubiaceae	*Galium boreale* L.	G.G. McNickle 27, 26 Jul 2005	**JX848534**
Salicaceae	*Populus tremuloides* Michx.	ALTA Acc. No. 117777	**JX848535**
Santalaceae	*Comandra umbellata* (L.) Nutt.	G.G. McNickle 35, 20 May 2006	**JX848536**

Saxifragaceae	*Heuchera richardsonii* R. Br.	ALTA Acc. No. 109724	**JX848537**
Violaceae	*Viola adunca* Sm.	G.G. McNickle 9, 8 Jun 2005	**JX848538**
Violaceae	*Viola pedatifida* G. Don	G.G. McNickle 39, 20 May 2006	**JX848539**

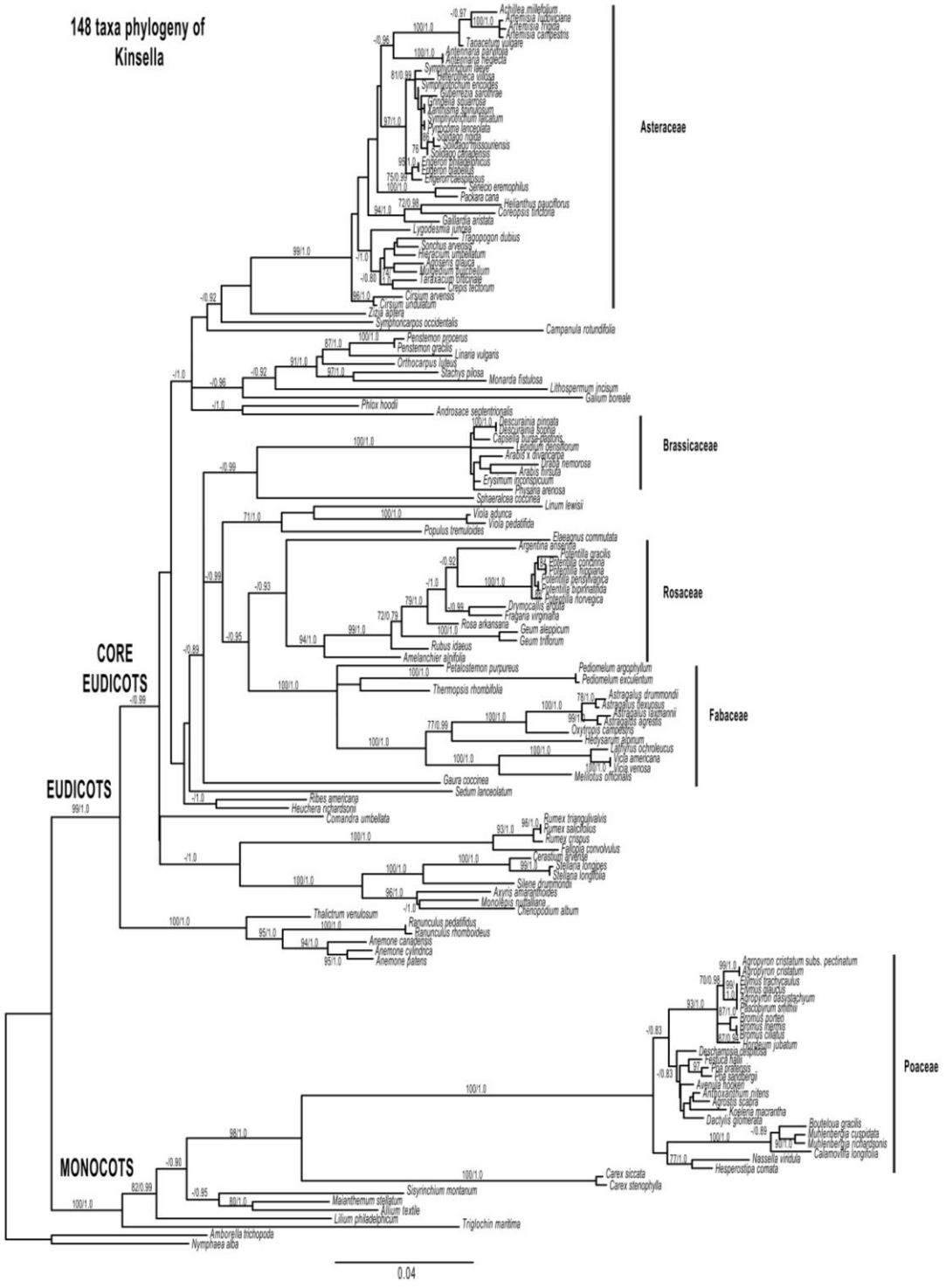
148 taxa phylogeny of Kinsella
Asteraceae
Brassicaceae
Rosaceae
Fabaceae
Poaceae
CORE EUDICOTS
EUDICOTS
MONOCOTS
Achillea millefolium
Artemisia ludoviciana
Artemisia tridata
Artemisia campestris
Tanacetum vulgare
Antennaria parvifolia
Antennaria neglecta
Symphyotrichum laeve
Heterotheca villosa
Symphyotrichum ericoides
Eutrochium serotinae
Grindelia squarrosa
Xanthisma texanum
Symphyotrichum falcatum
Pyrrocoma lanceolata
Solidago nana
Solidago missouriensis
Solidago canadensis
Erigeron philadelphicus
Erigeron bloomeri
Erigeron caespitosus
Senecio eremophilus
Packera cana
Helianthus pauciflorus
Coreopsis tinctoria
Gaillardia aristata
Lygodesmia juncea
Tragopogon dubius
Sonchus arvensis
Hieracium umbellatum
Agoseris glauca
Wolffiella lingulata
Taraxacum officinale
Crepis tectorum
Cirsium arvense
Cirsium undulatum
Zizia aptera
Symphoricarpos occidentalis
Campanula rotundifolia
Penstemon procerus
Penstemon gracilis
Linaria vulgaris
Orthocarpus luteus
Stachys pilosa
Monarda fistulosa
Lithospermum incisum
Galium boreale
Phlox hoodii
Androsace septentrionalis
Descurainia pinnata
Descurainia sophia
Capsella bursa-pastoris
Lepidium densiflorum
Arabis x divaricarpa
Arabis hirsuta
Erysimum inconspicuum
Physaria arenosa
Sphaeralcea coccinea
Linum lewisii
Viola adunca
Viola pedatifida
Populus tremuloides
Elaeagnus commutata
Argentina anserina
Potentilla gracilis
Potentilla concinna
Potentilla hippiana
Potentilla pensylvanica
Potentilla pulcherrima
Fragaria norvegica
Drymocallis arguta
Fragaria virginiana
Rosa arkansana
Geum aleppicum
Geum triflorum
Rubus idaeus
Amelanchier alnifolia
Petalostemon purpureus
Pediomelum argophyllum
Pediomelum esculentum
Thermopsis rhombifolia
Astragalus drummondii
Astragalus flexuosus
Astragalus tetrapterus
Astragalus agrestis
Oxytropis campestris
Hedysarum alpinum
Lathyrus ochroleucus
Vicia americana
Vicia venosa
Melilotus officinalis
Gaura coccinea
Sedum lanceolatum
Ribes americana
Heuchera richardsonii
Comandra umbellata
Rumex triangulivalvis
Rumex salicifolius
Rumex crispus
Fallopia convolvulus
Cerastium brachypodum
Stellaria longipes
Stellaria longifolia
Silene drummondii
Axyris amaranthoides
Monolepis nuttalliana
Chenopodium album
Thalictrum venulosum
Ranunculus pedatifidus
Ranunculus rhomboideus
Anemone canadensis
Anemone cylindrica
Anemone patens
Agropyron cristatum subs. pectinatum
Agropyron cristatum
Elymus trachycaulus
Elymus glaucus
Agropyron dasystachyum
Decaryum smithii
Bromus porteri
Bromus hermis
Bromus ciliatus
Hordeum jubatum
Deschampsia cespitosa
Festuca hallii
Poa pratensis
Poa sandbergii
Avenula hookeri
Anthoxanthum nitens
Agrostis scabra
Koeleria macrantha
Dactylis glomerata
Bouteloua gracilis
Muhlenbergia cuspidata
Muhlenbergia richardsonis
Calamovilfa longifolia
Nassella viridula
Hesperostipa comata
Carex siccata
Carex stenophylla
Sisyrinchium montanum
Maianthemum stellatum
Allium textile
Lilium philadelphicum
Triglochin maritima
Amborella trichopoda
Nymphaea alba
0.04

Figure 8.1 Bayesian consensus tree of Kinsella community based on 148 rbcL sequences. Maximum likelihood bootstrap values / Bayesian posterior probabilities are indicated above branches. Branch lengths are proportional to the number of changes and represent the average branch length across the post burn-in trees.

9. Appendix 2

9.1. Spatial survey environmental measures

Plant available nitrate, ammonium, and phosphate were extracted from the soil using ion exchange resin bags following standard methods (Roberston *et al.* 1999) and sent to the University of Alberta Biogeochemical Analytical Laboratory where concentrations were determined on a Lachat QuikChem 8500 Flow Injection Analysis automated ion analyzer. Soil texture and pH were determined from soil collected from each plot. Soil texture was determined by the Natural Resources Analytical Laboratory at the University of Alberta using the hydrometer method, while pH was measured using standard methods (Roberston *et al.* 1999) where soil samples were suspended in solution and pH determined using an Orion 2-Star Benchtop pH meter (Thermo-Scientific Inc.). Slope and soil moisture were determined at the site; slope was measured for each plot using a Suunto clinometer and soil moisture by using a ML2x ThetaProbe soil moisture sensor coupled to a HH2 moisture meter (Delta-T Devices).

9.2. Principal components analysis

We used principal components analysis (PCA) to extract components (PCs) from the environmental data for both the competition experiment (see Lamb & Cahill 2008 for methods details) and the spatial survey using identical methods. PCs were extracted from the covariance matrix if their eigenvalues were equal to or greater than the mean eigenvalue (SPSS v. 20.0).

For the competition data, two PCs were extracted. PC1 explained 30% of the variation in the environmental data and was positively associated with light

interception by the canopy and negatively correlated with high incident radiation (as expressed by topographic position). PC 2 explained 26% of the variation and was largely correlated with nitrogen availability (Table S1).

For the spatial survey, three PCs were extracted. PC1 explained 26% or the variation and was positively associated with sand and to some extent nitrate, but negatively associated with clay and silt. PC2 explained 19% of the variation and was positively associated with clay, pH, and soil moisture, but negatively associated with silt. PC3 only explained 7% of the variation was positively associated with silt and negatively associated with clay (Table S2).

9.3. References

Lamb E.G. & Cahill J.F. (2008). When competition does not matter: Grassland diversity and community composition. *Am. Nat.*, 171, 777-787.

Roberston P.G., Coleman D.C. & Bledsoe C. (1999). *Standard Soil Methods for Long-Term Ecological Research*. Oxford University Press, Cary, NC, USA.

Webb C.O., Ackerly D.D., McPeek M.A. & Donoghue M.J. (2002). Phylogenies and community ecology. *Annu. Rev. Ecol. Syst.*, 33, 475-505.

Table 9.1 Factor loadings for the extracted principal components from the environmental data in the competition experiment.

	Component 1	Component 2
Soil moisture	-0.092	0.200
Light interception	0.998	-0.058
Topographic position	-0.412	-0.081
Total soil nitrogen	0.155	0.988

Table 9.2 Factor loadings for the extracted principal components from the environmental data in the spatial survey.

	Component 1	Component 2	Component 3
Slope	0.507	0.656	0.556
Clay	-0.446	0.817	-0.365
Silt	-0.822	-0.403	0.401
Sand	0.965	-0.256	-0.060
Nitrate	0.340	0.212	0.147
Ammonium	0.008	-0.066	0.004
Phosphate	-0.148	-0.109	0.015
pH	0.294	0.399	0.079
Soil moisture	0.097	0.434	-0.011

Table 9.3 Results from mixed model analyses of the effect of relatedness and nitrogen treatment on competitive intensity.

Relatedness metric	Factor	d.f.	F	P
Mean phylogenetic distance to target (MPD.t)	MPD.ph	1,67	0.051	0.823
	Nitrogen (N)	1,186	0.687	0.408
	MPD.ph x N	1,186	0.678	0.411
Abundance weighted mean phylogenetic distance to target (MPD.t.a)	MPD.ph.a	1,142	0.011	0.915
	Nitrogen (N)	1,185	2.672	0.104
	MPD.ph.a x N	1,185	2.775	0.097

Table 9.4 Results from mixed model analysis of the effects of competitive intensity and nitrogen treatment on phylogenetic community structure.

Response variable*	Factor	d.f.	F	P
NRI	Comp. intensity (CI)	1,188	0.015	0.902
	Nitrogen (N)	1,181	0.108	0.742
	CI x N	1,184	2.476	0.117
NRI.a	Comp. intensity (CI)	1,188	0.852	0.357
	Nitrogen (N)	1,178	0.032	0.858
	CI x N	1,182	2.015	0.157
NTI	Comp. intensity (CI)	1,188	0.411	0.522
	Nitrogen (N)	1,188	0.414	0.521
	CI x N	1,188	0.166	0.684
NTI.a	Comp. intensity (CI)	1,188	0.423	0.516
	Nitrogen (N)	1,178	0.305	0.581
	CI x N	1,182	0.649	0.422

* NRI denotes the net relatedness index and NTI the nearest taxon index (Webb *et al.* 2002), while the suffix .a denotes abundance weighted statistics.

Table 9.5 Results of regression analyses determining the effect of environmental filters on phylogenetic community structure.

Study	Response variable*	Principal component†	F	d.f.	P‡
Competition experiment	NRI	**1 (Light)**	**7.048**	**1,189**	**0.009**
	(R^2 = 0.038)	2 (Soil resources)	0.439	1,189	0.508
	NTI	1 (Light)	0.201	1,189	0.655
	(R^2 = 0.001)	2 (Soil resources)	0.082	1,189	0.776
	NRI.a	**1 (Light)**	**9.764**	**1,189**	**0.002**
	(R^2 = 0.055)	2 (Soil resources)	1.267	1,189	0.262
	NTI.a	1 (Light)	2.018	1,189	0.157
	(R^2 = 0.011)	2 (Soil resources)	0.111	1,189	0.740
Spatial survey	NRI	1 (Sand/Silt)	2.363	1,92	0.128
	(R^2 = 0.066)	**2 (Clay/Water/pH)**	**4.167**	**1,92**	**0.044**
		3 (Silt/Clay)	0.005	1,92	0.941
	NTI	1 (Sand/Silt)	0.832	1,92	0.364
	(R^2 = 0.060)	**2 (Clay/Water/pH)**	**4.577**	**1,92**	**0.035**
		3 (Silt/Clay)	0.502	1,92	0.480

* NRI denotes the net relatedness index and NTI the nearest taxon index (Webb *et al.* 2002), while the suffix .a denotes abundance weighted statistics. † Details of the principal components analysis can be found in the supplementary materials. ‡Values in bold are significant at $\alpha = 0.05$.

10. Appendix 3

Table 10.1 Abundances and visitation rates for flower species visited by bees across all plots. Visits per stem is abbreviated as VPS.

Species	Stems	Bee visits	Bumblebees	Other bees	Bee VPS	Bumblebee VPS	Other bee VPS
Solidago missouriensis Nutt.	390	114	72	42	0.3	0.2	0.1
Symphyotrichum laeve (L.) Á. Löve & D. Löve	171	96	75	21	0.6	0.4	0.1
Solidago canadensis L.	54	86	57	29	1.6	1.1	0.5
Symphyotrichum falcatum (Lindl.) G.L. Nesom	394	71	13	58	0.2	0	0.1
Achillea millefolium L.	207	46	7	39	0.2	0	0.2
Symphoricarpos occidentalis Hook.	64	25	12	13	0.4	0.2	0.2
Senecio eremophilus Richardson	13	24	3	21	1.8	0.2	1.6
Penstemon procerus Douglas ex Graham	16	17	12	5	1.1	0.8	0.3
Grindelia squarrosa (Pursh) Dunal	155	16	0	16	0.1	0	0.1
Cirsium arvense (L.) Scop.	15	16	7	9	1.1	0.5	0.6
Zizia aptera (A. Gray) Fernald	5	16	0	16	2.7	0	2.7
Elaeagnus commutata Bernh. ex Rydb.	39	15	8	7	0.4	0.2	0.2
Oxytropis campestris (L.) DC.	49	13	0	13	0.3	0	0.3
Campanula rotundifolia L.	42	11	2	9	0.3	0	0.2
Mulgedium pulchellum (Pursh) G. Don	15	10	1	9	0.7	0.1	0.6
Drymocallis arguta (Pursh) Rydb.	41	9	0	9	0.2	0	0.2
Erigeron glabellus Nutt.	73	9	0	9	0.1	0	0.1
Monarda fistulosa L.	11	8	8	0	0.7	0.7	0
Hedysarum alpinum L.	6	6	0	6	1	0	1
Geum triflorum Pursh	78	4	2	2	0.1	0	

Species	Stems	Bee visits	Bumblebees	Others	Bee VPS	Bumblebee VPS	Other bee VPS
Solidago rigida var. *humilis* (Porter) Heard & Semple	3	3	2	1	1	0.7	0.3
Cerastium arvense L.	40	1	0	1	<0.1	0	<0.1
Gutierrezia sarothrae (Pursh) Britton & Rusby	61	0					
Astragalus agrestis Douglas ex G. Don	26	0					
Vicia americana Muhl. ex Willd.	18	0					
Sisyrinchium montanum Greene	10	0					
Thermopsis rhombifolia (Nutt. ex Pursh) Richardson	8	0					
Fragaria virginiana Duchesne	7	0					
Stellaria longipes Goldie	6	0					
Hieracium umbellatum L.	5	0					
Gaillardia aristata Pursh	4	0					
Penstemon gracilis Nutt.	4	0					
Rosa arkansana Porter	4	0					
Potentilla hippiana Lehm.	3	0					
Taraxacum officinale F.H. Wgg.	2	0					
Anemone cylindrica A. Gray	1	0					
Geum aleppicum Jacq.	1	0					
Lathyrus ochroleucus Hook.	1	0					
Lygodesmia juncea (Pursh) D. Don ex Hook.	1	0					
Orthocarpus luteus Nutt.	1	0					

Table 10.2 Bee species found in the study.

Family	Genus	Estimated spp #	Known species
Andrenidae	Andrena	5	5 unknown
Apidae	Anthophora	2	*A. virgata*, 1 unknown
	Bombus	8	*B. borealis*, *B. melanopygus, B. morrisoni, B. rufocinctus, B. sylvicola, B. ternarius, B. terricola, B. vagans*
Colletidae	Colletes	2	2 unknown
	Hylaeus	2	*H. annulatus*, 1 unknown
Halictidae	Augochloropsis	1	*A. metallica*
	Agapostemnon	1	1 unknown
	Halictus	4	*H. rubicundus*, 3 unknown
	Lasioglossum	6	*L. laevissimum, L. succinipenne, L. leucocomum*, 3 unknown
Megachilidae	Megachile	4	*M. fortis*, *M. relativa*, 2 unknown
	Heriades	2	*H. carinata*, 1 unknown
	Hoplitis	4	4 unknown
	Osmia	3	*O. bucephala*, 2 unknown

Table 10.2 Unstandardized and standardized path coefficients and covariances from the full model.

Path type	Path	Unstandardized	S.E.	P	Standardized
Regression	Productivity<-Tree proximity	-9.398	1.773	<0.001	-0.498
Regression	Proportion shrub 10m<-Tree proximity	-0.026	0.026	0.319	-0.106
Regression	Proportion shrub 20m<-Tree proximity	-0.038	0.024	0.111	-0.158
Regression	Forest cover 10m<-Tree proximity	-1.471	0.107	<0.001	-0.83
Regression	Forest cover 20m<-Tree proximity	-2.007	0.143	<0.001	-0.835
Regression	Floral richness<-Productivity	0.007	0.002	0.003	0.291
Regression	Floral abundance<-Productivity	0.022	0.006	<0.001	0.348
Regression	Flower axis 1<-Productivity	0.009	0.003	0.002	0.298
Regression	Total bee visitation<-Tree proximity	-0.225	0.207	0.276	-0.192
Regression	Total bee visitation<-Forest cover 10m	-0.155	0.091	0.089	-0.234
Regression	Total bee visitation<-Forest cover 20m	0.123	0.07	0.076	0.253
Regression	Total bee visitation<-Proportion shrub 10m	0.556	0.479	0.246	0.117
Regression	Total bee visitation<-Proportion shrub 20m	-0.305	0.545	0.575	-0.063
Regression	Total bee visitation<-Productivity	0.008	0.006	0.2	0.122
Regression	Total bee visitation<-Floral richness	-0.51	0.275	0.064	-0.203
Regression	Total bee visitation<-Floral abundance	0.627	0.096	<0.001	0.63
Regression	Total bee visitation<-Flower axis 1	0.445	0.197	0.024	0.205
Regression	Total bee visitation<-Flower axis 2	0.291	0.17	0.086	0.133
Regression	Total bee visitation<-Flower axis 3	0.064	0.179	0.718	0.028
Regression	Bee richness<-Tree proximity	-0.069	0.128	0.588	-0.096
Regression	Bee richness<-Forest cover 10m	-0.109	0.056	0.052	-0.27
Regression	Bee richness<-Forest cover 20m	0.058	0.043	0.174	0.195
Regression	Bee richness<-Proportion shrub 10m	0.34	0.296	0.251	0.117

Path type	Path	Unstandardized	S.E.	P	Standardized
Regression	Bee richness<-Proportion shrub 20m	-0.272	0.336	0.419	-0.092
Regression	Bee richness<-Productivity	0.006	0.004	0.099	0.159
Regression	Bee richness<-Floral richness	0.046	0.17	0.786	0.03
Regression	Bee richness<-Floral abundance	0.326	0.059	<0.001	0.534
Regression	Bee richness<-Flower axis 1	0.224	0.122	0.066	0.169
Regression	Bee richness<-Flower axis 2	0.198	0.105	0.059	0.148
Regression	Bee richness<-Flower axis 3	0.027	0.11	0.805	0.019
Regression	Bumblebee visitation<-Tree proximity	-0.544	0.193	0.005	-0.57
Regression	Bumblebee visitation<-Forest cover 10m	-0.094	0.085	0.271	-0.174
Regression	Bumblebee visitation<-Forest cover 20m	-0.04	0.065	0.533	-0.102
Regression	Bumblebee visitation<-Proportion shrub 10m	0.472	0.447	0.292	0.122
Regression	Bumblebee visitation<-Proportion shrub 20m	-0.73	0.508	0.151	-0.185
Regression	Bumblebee visitation<-Productivity	-0.001	0.006	0.916	-0.011
Regression	Bumblebee visitation<-Floral richness	-0.242	0.257	0.347	-0.118
Regression	Bumblebee visitation<-Floral abundance	0.32	0.09	<0.001	0.395
Regression	Bumblebee visitation<-Flower axis 1	0.409	0.184	0.026	0.232
Regression	Bumblebee visitation<-Flower axis 2	0.122	0.158	0.441	0.069
Regression	Bumblebee visitation<-Flower axis 3	0.115	0.167	0.492	0.061
Regression	Other bee visitation<-Tree proximity	0.138	0.186	0.459	0.135
Regression	Other bee visitation<-Forest cover 10m	-0.18	0.082	0.028	-0.313
Regression	Other bee visitation<-Forest cover 20m	0.172	0.063	0.006	0.406
Regression	Other bee visitation<-Proportion shrub 10m	0.296	0.432	0.493	0.072
Regression	Other bee visitation<-Proportion shrub 20m	0.116	0.49	0.813	0.028
Regression	Other bee visitation<-Productivity	0.011	0.005	0.046	0.198
Regression	Other bee visitation<-Floral richness	-0.251	0.248	0.311	-0.115

Path type	Path	Unstandardized	S.E.	P	Standardized
Regression	Other bee visitation<-Floral abundance	0.536	0.087	<0.001	0.62
Regression	Other bee visitation<-Flower axis 1	0.06	0.178	0.734	0.032
Regression	Other bee visitation<-Flower axis 2	0.222	0.153	0.146	0.117
Regression	Other bee visitation<-Flower axis 3	-0.057	0.161	0.722	-0.029
Covariance	Flower abundance<->Flower richness	0.255	0.059	<0.001	0.457
Covariance	Forest cover 10<->Forest cover 20	0.289	0.169	0.087	0.186
Covariance	Proportion shrub 10<->Proportion shrub 20	0.045	0.009	<0.001	0.639
Covariance	Forest cover 20<->Proportion shrub 20	0.055	0.029	0.056	0.145
Covariance	Flower richness<->Tree distance	-0.184	0.056	<0.001	-0.366
Covariance	Flower richness<->Flower axis 1	0.046	0.02	0.025	0.175
Covariance	Flower richness<->Flower axis 3	0.048	0.022	0.033	0.186
Covariance	Flower abundance<->Flower axis 2	-0.145	0.068	0.032	-0.205
Covariance	Forest cover 10<->Proportion shrub 10	-0.02	0.024	0.407	-0.068
Covariance	Proportion shrub 10<->Flower axis 1	-0.023	0.016	0.161	-0.15
Covariance	Proportion shrub 20<->Flower axis 1	-0.061	0.017	<0.001	-0.414

Table 10.3 Rank of standardized direct effects of each factor on each bee response. Factors significant at $P < 0.05$ are denoted by ** and between $0.05 < P < 0.10$ by *. Ranks are directly adjacent to the standardized scores.

Factor class	Factor	All bees	Rank	Bee richness	Rank	Bumblebees	Rank	Other bees	Rank	Mean Rank
Plant community	Flower axis 1	**0.205	4	*0.169	4	**0.232	3	0.032	9	5
	Flower axis 2	*0.133	7	*0.148	6	0.069	9	0.117	6	7
	Flower axis 3	0.028	11	0.019	11	0.061	10	-0.029	10	10.5
	Flower abundance	**0.630	1	**0.534	1	**0.395	2	**0.620	1	1.25
	Flower richness	*-0.203	5	0.03	10	-0.118	7	-0.115	7	7.25
	Vegetative productivity	0.122	8	*0.159	5	-0.011	11	**0.198	4	7
Local habitat	Tree distance	-0.192	6	-0.096	8	**-0.570	1	0.135	5	5
	Forest cover 10m	*-0.234	3	*-0.270	2	-0.174	5	**-0.313	3	3.25
	Forest cover 20m	*0.253	2	0.195	3	-0.102	8	**0.406	2	3.75
	Proportion shrub 10m	0.117	9	0.117	7	0.122	6	0.072	8	7.5
	Proportion shrub 20m	-0.063	10	-0.092	9	-0.185	4	0.028	11	8.5

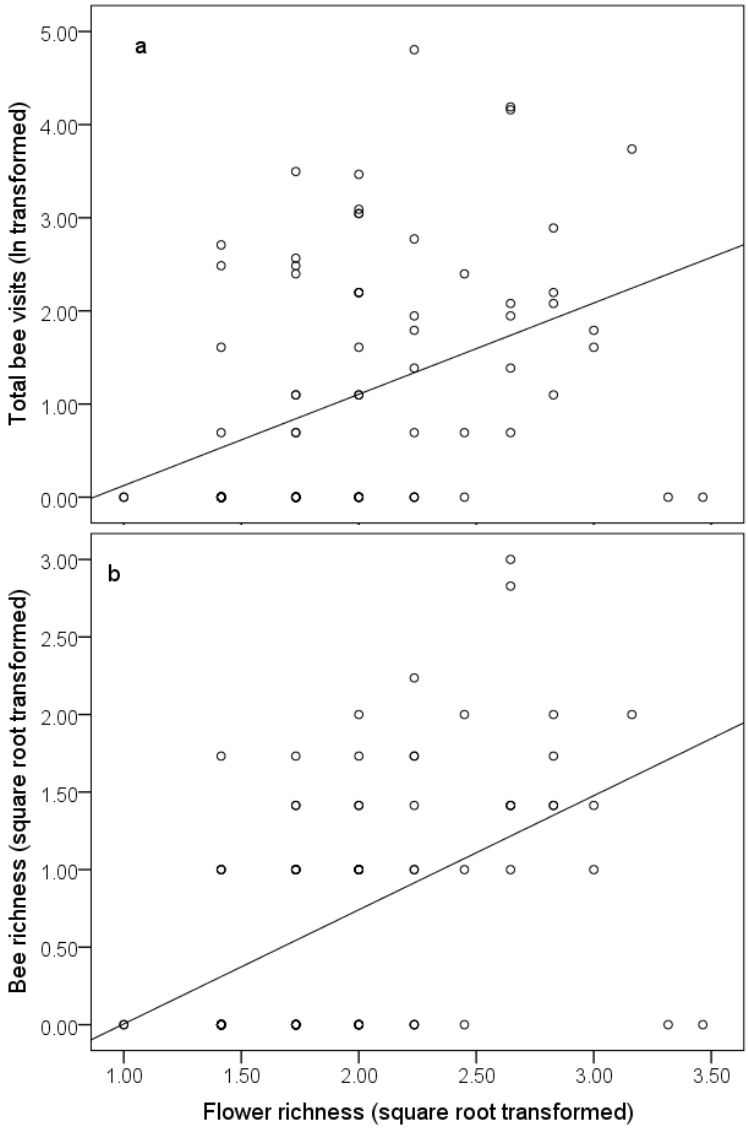

Fig. 10.1 Bivariate relationships between flower species richness and (a) total bee visitation and between flower richness and (b) bee richness. Lines denote the line of best fit as determined by linear regression. Both regressions for bee visits ($F_{1,84} = 16.59$, $P < 0.001$, $R^2 = 0.165$) and bee richness ($F_{1,84} = 27.71$, $P < 0.001$, $R^2 = 0.248$) were highly significant.

11. Appendix 4

11.1. Mycorrhizal inoculum potential

Fungicide effects on mycorrhizal inoculum potential were quantified using trap plants in the field and greenhouse. In the field, seedlings of four insect-pollinated plant species (*Campanula rotundifolia, Potentilla arguta, Gaillardia aristata* and *Heterotheca villosa*) were transplanted into twelve plots in June 2011, half of which had received fungicide application. Plants were allowed to grow for 5 weeks, harvested and returned to the lab. In the greenhouse assessment, soil was collected from sixteen plots in July 2011, half of which received the fungicide treatment. For each plot, we planted *Sorghum bicolor* seeds into a 50ml centrifuge tube filled with soil. Plants were allowed to grow for two weeks and then harvested. For both the field and greenhouse assessment, plant roots were washed free of soil, cleared and stained using Trypan blue under ambient room conditions (Pitet et al. 2009).

Fungal colonization was determined for AMF hyphae, non-AMF hyphae, arbuscles and vesicles at 400× magnification using a modification of the line intersect method (McGonigle et al. 1990). Vesicles are not considered further as they were too rare. Hyphae were classified as AMF if they lacked septae and non-AMF if they had septae. For field samples, we quantified fungal colonization as the number of fungal structures per intersection and for greenhouse samples fungal colonization was recorded as intersection occupancy for each structure type. To standardize across species and quantification methods, colonization was generalized to maximum colonization for each type of fungal structure within each plant species. This data was used to determine fungicide effects on fungal colonization using a mixed model in SPSS (v. 20) specifying fungicide treatment as a fixed effect with both quantification method and plant species nested within quantification method as random effects.

Fungicide application reduced colonization of trap plants by AMF hyphae to 70% of control ($F_{1,54.6} = 6.15$, $P = 0.016$) and arbuscles to 56% of control ($F_{1,59} = 4.73$, $P = 0.034$). We found no differences in non-AMF hyphae between fungicide treated soils and control ($F_{1,55.3} = 0.06$, $P = 0.807$). This suggests that fungicide application primarily reduced mycorrhizae within plant roots. Therefore we refer to fungicide effects as mycorrhizal suppression, although we cannot eliminate the possibility that fungicide application affected fungi outside of the plant roots.

11.2. Abiotic and biotic environmental effects

Treatment effects on abiotic environmental conditions were measured for nine blocks (72 plots). Treatment effects on were assessed using plant root simulator (PRS^{TM}) probes (Western Ag Innovations), which are strips of cation or anion exchange membrane in a plastic casing. Probes were buried to 10cm depth on July 1, 2010 and recovered after 70 days; however, probes were not recovered from three plots. Recovered probes were washed in deionized water and sent to Western Ag for analysis. Nitrogen availability values were ln transformed prior to analysis.

Within the same blocks, we measured soil moisture and temperature using ECH$_2$O$^{®}$ EC-TM soil moisture sensors buried to a depth of 10cm (Decagon Devices). Sensors were coupled to Em50$^{®}$ data loggers (Decagon Devices) and measurements were recorded every two hours from May 28 to September 28, 2010. From these data we calculated mean soil moisture and temperature. We also measured light penetration to the soil surface near peak biomass in late July 2010 using an AccuPAR light meter (Decagon Devices). All light measurements were taken within 2 hours of solar noon when the sky was clear.

We measured the effects of the treatments on the biotic environment as primary productivity in all 160 sub-plots. Productivity was estimated as standing live biomass during peak biomass in late July 2010. Biomass was clipped in a 10×100 cm strip and sorted into live

and dead material. The live material was further sorted into graminoids, forbs and woody components. Samples were dried at 65^{o}C for 72h and weighed. From these weights, we calculated total litter biomass, total live biomass and the proportion of biomass belonging to graminoids with each variable transformed prior to analysis to normalize residuals.

Treatment effects on abiotic and biotic environmental conditions were analyzed using the same mixed model structure in SPSS (v. 20). All treatments were included factorially as fixed effects with block included as a random effect. An interaction between fungicide and block was also included as a random effect in each of the models to account for the split plot design. In the models for nitrogen, live biomass and proportion graminoid biomass, the interaction between fungicide and block was found to be redundant and resulted in a non-positive definite Hessian matrix. To correct this, we changed the covariance structure from variance components to compound symmetry.

11.3. References

McGonigle, T. P., M. H. Miller, D. G. Evans, G. L. Fairchild, and J. A. Swan. 1990. A new method which gives an objective-measure of colonization of roots by vesicular arbuscular mycorrhizal fungi. New Phytologist **115**:495 - 501.

Pitet, M., A. Camprubi, C. Calvet, and V. Estaun. 2009. A modified staining technique for arbuscular mycorrhiza compatible with molecular probes. Mycorrhiza **19**:125-131.

Table 11.1 Treatment effects on the abiotic environment. Significant effects (P<0.05) are in bold.

		Moisture		Temperature		Light		Nitrogen		
	d.f.	F	P	F	P	F	P	d.f.	F	P
Fungicide	1,8	0.48	0.509	0.46	0.519	0.37	0.552	1,50.8	0.87	0.354
Fertilized	1,48	**9.48**	**0.003**	**19.91**	**<0.001**	**53.05**	**<0.001**	**1,51.7**	**101.50**	**<0.001**
Litter removal	1,48	0.32	0.576	**17.31**	**<0.001**	**34.20**	**<0.001**	1,51.7	0.28	0.597
Fung*Fert	1,48	0.29	0.593	0.02	0.885	1.07	0.307	1,51.7	1.63	0.208
Fung*Lit	1,48	3.13	0.083	0.29	0.592	0.38	0.542	1,51.7	0.05	0.825
Fert*Lit	1,48	0.63	0.430	2.13	0.151	**8.19**	**0.006**	1,51.7	1.05	0.311
Fung*Fert*Lit	1,48	0.19	0.663	**4.67**	**0.036**	0.01	0.989	1,51.7	0.50	0.485

Table 11.2 Treatment effects on community biomass**.** Terms in brackets refer to the transformation used for that variable and significant effects are in bold.

	(ln) Live biomass			Litter biomass$^{(1/3)}$			Proportion graminoid$^{(2)}$		
	d.f.	*F*	*P*	*d.f.*	*F*	*P*	*d.f.*	*F*	*P*
Fungicide	1,80.3	0.02	0.898	1,19	0.84	0.371	1,87.3	0.03	0.857
Fertilized	**1,121.9**	**58.4**	**<0.001**	1,114	0.62	0.432	**1,122.2**	**11.47**	**0.001**
Litter removal	1,121.9	0.43	0.512	**1,114**	**656.16**	**<0.001**	1,122.2	2.84	0.095
Fung*Fert	1,121.9	0.09	0.764	1,114	0.04	0.851	1,122.2	0.25	0.621
Fung*Lit	1,121.9	1.20	0.275	1,114	3.52	0.063	1,122.2	3.00	0.086
Fert*Lit	1,121.9	0.27	0.608	1,114	3.14	0.079	1,122.2	0.69	0.409
Fung*Fert*Lit	1,121.9	0.54	0.465	1,114	1.34	0.249	1,122.2	0.13	0.719

Table 11.3 Mixed model results testing whether species flowering and visitation responses become decoupled from abundance as a function of mycorrhizal suppression, fertilizer addition, and litter removal.

Response	Factor	d.f.	F	P
Abundance	Mycorrhizae	1,19	0.356	0.558
Abundance	Fertilizer	1,2698	0.48	0.488
Abundance	Litter	1,2698	3.802	0.051
Abundance	**Species**	**17,2698**	**6.201**	**0**
Abundance	Myc * Fert	1,2698	0.066	0.797
Abundance	Myc* Litter	1,2698	0.78	0.377
Abundance	Myc * Species	17,2698	0.908	0.564
Abundance	Fert* Litter	1,2698	0.027	0.87
Abundance	Fert* Species	17,2698	0.882	0.596
Abundance	Litter * Species	17,2698	1.177	0.275
Abundance	Myc * Fert * Litter	1,2698	2.006	0.157
Abundance	Myc * Fert * Species	17,2698	0.494	0.957
Abundance	Myc * Litter * Species	17,2698	0.354	0.993
Abundance	Fert * Litter * Species	17,2698	0.4	0.986
Abundance	Myc * Fert * Litter * Species	17,2698	0.663	0.841
Flowering	Mycorrhizae	1,71	0.01	0.946
Flowering	Fertilizer	1,1277	1.30	0.254
Flowering	Litter	1,1268	1.91	0.167
Flowering	**Species**	**17,1283**	**2.51**	**0.001**
Flowering	**Cover**	**1,1273**	**52.38**	**<0.001**
Flowering	Myc * Fert	1,1277	0.63	0.426
Flowering	Myc* Litter	1,1272	0.48	0.490
Flowering	**Myc * Species**	**17,1280**	**1.78**	**0.026**
Flowering	Myc * Cover	1,1285	1.37	0.242
Flowering	Fert* Litter	1,1274	0.60	0.438
Flowering	Fert* Species	17,1275	1.12	0.330
Flowering	Fert * Cover	1,1280	0.06	0.809
Flowering	Litter * Species	17,1273	0.74	0.768
Flowering	**Litter * Cover**	**1,1283**	**4.94**	**0.026**
Flowering	**Species * Cover**	**17,1284**	**5.80**	**<0.001**
Flowering	Myc * Fert * Litter	1,1273	1.42	0.234
Flowering	Myc * Fert * Species	17,1275	0.38	0.989
Flowering	Myc * Fert * Cover	1,1284	0.43	0.512
Flowering	Myc * Litter * Species	17,1273	0.76	0.737
Flowering	Myc * Litter * Cover	1,1280	0.51	0.473
Flowering	**Myc * Species * Cover**	**16,1275**	**2.40**	**0.002**

Response	Factor	d.f.	F	P
Flowering	Fert * Litter * Species	17,1273	0.67	0.836
Flowering	Fert * Litter * Cover	1,1274	1.33	0.250
Flowering	**Fert * Species * Cover**	**16,1280**	**2.66**	**<0.001**
Flowering	**Litter * Species * Cover**	**16,1281**	**1.67**	**0.046**
Flowering	Myc * Fert * Litter * Species	17,1273	1.50	0.088
Flowering	**Myc * Fert * Litter* Cover**	**1,1279**	**9.42**	**0.002**
Flowering	Myc * Fert * Species * Cover	15,1279	1.14	0.317
Flowering	**Myc * Litter * Species * Cover**	**15,1280**	**1.80**	**0.030**
Flowering	**Fert * Litter * Species * Cover**	**15,1279**	**2.05**	**0.010**
Flowering	**Myc * Fert * Litter * Species * Cover**	**12,1279**	**1.98**	**0.023**
Visitation	Mycorrhizae	1,249	2.57	0.110
Visitation	Fertilizer	1,1278	0.54	0.464
Visitation	Litter	1,1271	0.63	0.428
Visitation	Species	17,1273	1.04	0.414
Visitation	**Flower**	**1,1278**	**40.52**	**<0.001**
Visitation	Myc * Fert	1,1269	0.19	0.660
Visitation	Myc* Litter	1,1258	0.04	0.835
Visitation	Myc * Species	17,1272	0.97	0.493
Visitation	Myc * Flower	1,1278	3.02	0.083
Visitation	Fert* Litter	1,1268	0.25	0.619
Visitation	Fert* Species	17,1262	0.64	0.858
Visitation	Fert * Flower	1,1278	0.25	0.620
Visitation	Litter * Species	17,1262	0.94	0.524
Visitation	Litter * Flower	1,1272	0.29	0.593
Visitation	Species * Flower	17,1275	1.11	0.336
Visitation	Myc * Fert * Litter	1,1267	1.80	0.180
Visitation	Myc * Fert * Species	16,1261	1.33	0.170
Visitation	Myc * Fert * Flower	1,1272	1.87	0.172
Visitation	Myc * Litter * Species	17,1262	0.62	0.876
Visitation	Myc * Litter * Flower	1,1261	0.00	0.987
Visitation	**Myc * Species * Flower**	**17,1275**	**4.06**	**<0.001**
Visitation	Fert * Litter * Species	17,1261	0.84	0.651
Visitation	Fert * Litter * Flower	1,1270	0.00	0.978
Visitation	**Fert * Species * Flower**	**17,1266**	**1.93**	**0.013**
Visitation	**Litter * Species * Flower**	**17,1266**	**2.93**	**<0.001**
Visitation	Myc * Fert * Litter * Species	16,1260	0.60	0.885
Visitation	Myc * Fert * Litter* Flower	1,1265	2.04	0.153
Visitation	**Myc * Fert * Species * Flower**	**16,1265**	**3.71**	**<0.001**
Visitation	**Myc * Litter * Species * Flower**	**17,1265**	**1.81**	**0.022**
Visitation	**Fert * Litter * Species ***	**17,1266**	**3.00**	**<0.001**

Response	Factor	d.f.	F	P
	Flower			
Visitation	Myc * Fert * Litter * Species * Flower	16,1265	1.35	0.158

Table 11.4 Species specific regression results within each treatment. Species are abbreviated as *Achillea millefolium* (Achmil), *Astragalus agrestis* (Astagr), *Aster falcatus* (Astfal), *Astragalus flexuosus* (Astfle), *Aster laevis* (Astlae), *Campanula rotundifolia* (Camrot), *Cerastium arvense* (Cerarv), *Descurainia sophia* (Dessop), *Erigeron glabellus* (Erigla), *Galium boreale* (Galbor), *Geum triflorum* (Geutri), *Hieracium umbellatum* (Hieumb), *Orthocarpus luteus* (Ortlut), *Oxytropis campestris* (Oxycam), *Potentilla arguta* (Potarg), *Rosa arkansana* (Rosark), *Solidago missouriensis* (Solmis), *Vicea americana* (Vicame).

Response variable	Treatment	Species	d.f.	Slope	SE	P
Visitation	Control	Achmil	1,15	0.585	0.423	0.187
Visitation	Control	Astagr	1,11	1.932	0.478	0.002
Visitation	Control	Astfal	1,14	0.617	0.417	0.161
Visitation	Control	Astlae	1,7	1.635	0.250	0.000
Visitation	Control	Camrot	1,12	0.198	0.573	0.736
Visitation	Control	Cerarv	1,9	0.596	0.247	0.039
Visitation	Control	Erigla	1,14	0.784	0.323	0.029
Visitation	Control	Galbor	1,12	0.642	0.234	0.018
Visitation	Control	Geutri	1,8	0.293	0.490	0.566
Visitation	Control	Ortlut	1,9	1.348	0.157	0.000
Visitation	Control	Oxycam	1,11	2.416	1.233	0.076
Visitation	Control	Potarg	1,3	1.910	0.415	0.019
Visitation	Control	Rosark	1,12	1.137	0.340	0.006
Visitation	Control	Solmis	1,14	-0.044	0.063	0.496
Visitation	Control	Vicame	1,7	0.211	2.431	0.933
Visitation	Litter	Achmil	1,14	0.467	0.149	0.007
Visitation	Litter	Astagr	1,14	-0.063	0.386	0.872
Visitation	Litter	Astfal	1,13	1.082	0.321	0.005
Visitation	Litter	Astlae	1,8	1.549	0.213	0.000
Visitation	Litter	Camrot	1,13	1.670	0.631	0.020
Visitation	Litter	Cerarv	1,9	1.908	0.578	0.009
Visitation	Litter	Erigla	1,15	0.513	0.336	0.147
Visitation	Litter	Galbor	1,13	1.961	0.565	0.004
Visitation	Litter	Geutri	1,11	2.613	0.360	0.000
Visitation	Litter	Ortlut	1,9	2.728	0.257	0.000
Visitation	Litter	Oxycam	1,5	-0.417	0.484	0.428

Response variable	Treatment	Species	d.f.	Slope	SE	P
Visitation	Litter	Potarg	1,2	1.727	0.851	0.180
Visitation	Litter	Rosark	1,14	3.581	0.244	0.000
Visitation	Litter	Solmis	1,13	-0.002	0.046	0.973
Visitation	Fertilizer	Achmil	1,16	2.091	0.692	0.008
Visitation	Fertilizer	Astagr	1,13	0.140	0.180	0.451
Visitation	Fertilizer	Astfal	1,13	1.202	0.393	0.009
Visitation	Fertilizer	Astlae	1,9	1.025	0.120	0.000
Visitation	Fertilizer	Camrot	1,14	2.434	0.293	0.000
Visitation	Fertilizer	Cerarv	1,13	0.435	0.139	0.008
Visitation	Fertilizer	Erigla	1,15	0.580	0.152	0.002
Visitation	Fertilizer	Galbor	1,12	2.625	1.268	0.061
Visitation	Fertilizer	Geutri	1,10	1.193	0.200	0.000
Visitation	Fertilizer	Ortlut	1,9	0.124	0.296	0.685
Visitation	Fertilizer	Potarg	1,2	2.366	1.465	0.248
Visitation	Fertilizer	Rosark	1,12	1.316	0.194	0.000
Visitation	Fertilizer	Solmis	1,14	0.320	0.182	0.100
Visitation	Fertilizer	Vicame	1,5	3.196	1.782	0.133
Visitation	Fert, Litter	Achmil	1,17	1.674	0.260	0.000
Visitation	Fert, Litter	Astagr	1,14	1.189	0.248	0.000
Visitation	Fert, Litter	Astfal	1,15	0.674	0.306	0.044
Visitation	Fert, Litter	Astlae	1,9	1.274	0.189	0.000
Visitation	Fert, Litter	Camrot	1,14	2.006	0.318	0.000
Visitation	Fert, Litter	Cerarv	1,14	1.556	0.199	0.000
Visitation	Fert, Litter	Erigla	1,14	0.246	0.099	0.026
Visitation	Fert, Litter	Galbor	1,12	1.875	0.417	0.001
Visitation	Fert, Litter	Geutri	1,12	0.078	0.197	0.700
Visitation	Fert, Litter	Ortlut	1,14	0.378	0.216	0.102
Visitation	Fert, Litter	Oxycam	1,8	-2.240	1.780	0.244
Visitation	Fert, Litter	Rosark	1,15	1.060	0.481	0.044
Visitation	Fert, Litter	Solmis	1,14	0.795	0.371	0.050
Visitation	Fert, Litter	Vicame	1,5	2.895	0.222	0.000
Visitation	Myc. Supp.	Achmil	1,14	0.231	0.193	0.250
Visitation	Myc. Supp.	Astagr	1,8	1.652	0.288	0.000
Visitation	Myc. Supp.	Astfal	1,14	1.690	0.185	0.000
Visitation	Myc. Supp.	Astlae	1,10	1.095	0.290	0.004
Visitation	Myc. Supp.	Camrot	1,12	0.866	0.139	0.000
Visitation	Myc. Supp.	Cerarv	1,3	0.426	0.060	0.006
Visitation	Myc. Supp.	Erigla	1,14	0.446	0.394	0.277
Visitation	Myc. Supp.	Galbor	1,13	0.127	0.855	0.884
Visitation	Myc. Supp.	Geutri	1,8	0.091	0.158	0.581
Visitation	Myc. Supp.	Ortlut	1,9	0.052	0.056	0.384

Response variable	Treatment	Species	d.f.	Slope	SE	P
Visitation	Myc. Supp.	Oxycam	1,6	0.151	0.305	0.639
Visitation	Myc. Supp.	Potarg	1,4	0.000	0.120	1.000
Visitation	Myc. Supp.	Rosark	1,14	0.029	0.083	0.730
Visitation	Myc. Supp.	Solmis	1,14	4.013	0.797	0.000
Visitation	Myc, Litter	Achmil	1,16	-0.041	0.116	0.731
Visitation	Myc, Litter	Astagr	1,12	0.463	0.379	0.245
Visitation	Myc, Litter	Astfal	1,14	1.461	0.253	0.000
Visitation	Myc, Litter	Astlae	1,5	0.912	0.473	0.112
Visitation	Myc, Litter	Camrot	1,12	1.187	0.453	0.022
Visitation	Myc, Litter	Cerarv	1,4	1.269	0.308	0.015
Visitation	Myc, Litter	Erigla	1,14	0.727	0.289	0.025
Visitation	Myc, Litter	Galbor	1,14	0.085	0.435	0.848
Visitation	Myc, Litter	Geutri	1,8	0.108	0.047	0.050
Visitation	Myc, Litter	Ortlut	1,10	2.856	0.330	0.000
Visitation	Myc, Litter	Oxycam	1,8	0.086	0.666	0.901
Visitation	Myc, Litter	Rosark	1,13	0.763	0.341	0.043
Visitation	Myc, Litter	Solmis	1,13	1.123	0.477	0.035
Visitation	Myc, Fert	Achmil	1,15	0.573	0.235	0.028
Visitation	Myc, Fert	Astagr	1,9	1.229	0.100	0.000
Visitation	Myc, Fert	Astfal	1,12	1.524	0.404	0.003
Visitation	Myc, Fert	Astlae	1,9	0.665	0.343	0.084
Visitation	Myc, Fert	Camrot	1,11	0.334	0.261	0.228
Visitation	Myc, Fert	Cerarv	1,8	2.410	0.475	0.001
Visitation	Myc, Fert	Erigla	1,12	1.265	0.129	0.000
Visitation	Myc, Fert	Galbor	1,11	0.513	0.827	0.548
Visitation	Myc, Fert	Geutri	1,10	5.986	0.670	0.000
Visitation	Myc, Fert	Ortlut	1,8	0.067	0.095	0.499
Visitation	Myc, Fert	Oxycam	1,6	0.137	0.220	0.555
Visitation	Myc, Fert	Rosark	1,14	0.372	0.584	0.534
Visitation	Myc, Fert	Solmis	1,13	0.705	0.905	0.450
Visitation	Myc, Fert, Lit	Achmil	1,17	0.557	0.257	0.045
Visitation	Myc, Fert, Lit	Astagr	1,10	1.666	0.780	0.058
Visitation	Myc, Fert, Lit	Astfal	1,12	0.117	0.100	0.263
Visitation	Myc, Fert, Lit	Astlae	1,5	0.690	0.219	0.025
Visitation	Myc, Fert, Lit	Camrot	1,13	1.167	0.234	0.000
Visitation	Myc, Fert, Lit	Cerarv	1,10	0.787	0.070	0.000
Visitation	Myc, Fert, Lit	Erigla	1,14	0.938	0.247	0.002
Visitation	Myc, Fert, Lit	Galbor	1,12	0.748	0.568	0.212
Visitation	Myc, Fert, Lit	Geutri	1,7	0.428	0.272	0.160
Visitation	Myc, Fert, Lit	Ortlut	1,10	2.386	0.490	0.001
Visitation	Myc, Fert, Lit	Oxycam	1,5	0.485	0.202	0.061

Response variable	Treatment	Species	d.f.	Slope	SE	P
Visitation	Myc, Fert, Lit	Rosark	1,11	1.371	0.328	0.002
Visitation	Myc, Fert, Lit	Solmis	1,16	1.534	1.037	0.159
Visitation	Myc, Fert, Lit	Vicame	1,2	-0.570	4.558	0.912
Flowering	Control	Achmil	1,15	0.279	0.260	0.300
Flowering	Control	Astagr	1,11	0.079	0.175	0.662
Flowering	Control	Astfal	1,14	0.452	0.105	0.001
Flowering	Control	Astlae	1,7	0.523	0.100	0.001
Flowering	Control	Camrot	1,12	-0.021	0.215	0.925
Flowering	Control	Cerarv	1,9	-0.366	0.171	0.060
Flowering	Control	Erigla	1,14	0.218	0.077	0.014
Flowering	Control	Galbor	1,12	0.312	0.130	0.034
Flowering	Control	Geutri	1,8	0.179	0.117	0.165
Flowering	Control	Ortlut	1,9	0.567	0.414	0.205
Flowering	Control	Oxycam	1,11	0.262	0.054	0.000
Flowering	Control	Potarg	1,3	-0.964	0.952	0.386
Flowering	Control	Rosark	1,12	0.737	0.391	0.084
Flowering	Control	Solmis	1,14	1.036	0.424	0.028
Flowering	Control	Vicame	1,7	0.132	0.078	0.134
Flowering	Litter removal	Achmil	1,14	1.683	0.456	0.002
Flowering	Litter removal	Astagr	1,14	0.684	0.278	0.028
Flowering	Litter removal	Astfal	1,13	0.472	0.101	0.000
Flowering	Litter removal	Astlae	1,8	0.371	0.061	0.000
Flowering	Litter removal	Camrot	1,13	0.385	0.168	0.039
Flowering	Litter removal	Cerarv	1,9	-0.051	0.191	0.794
Flowering	Litter removal	Erigla	1,15	0.644	0.247	0.020
Flowering	Litter removal	Galbor	1,13	0.331	0.277	0.253
Flowering	Litter removal	Geutri	1,11	0.513	0.319	0.137
Flowering	Litter removal	Ortlut	1,9	0.866	0.231	0.005
Flowering	Litter removal	Oxycam	1,5	-0.178	0.268	0.537
Flowering	Litter removal	Potarg	1,2	0.344	0.665	0.656
Flowering	Litter removal	Rosark	1,14	0.909	0.387	0.034
Flowering	Litter removal	Solmis	1,13	2.779	0.309	0.000
Flowering	Litter removal	Vicame	1,4	0.324	1.290	0.814
Flowering	Fertilizer	Achmil	1,16	0.070	0.169	0.683
Flowering	Fertilizer	Astagr	1,13	0.076	0.027	0.015
Flowering	Fertilizer	Astfal	1,13	0.354	0.122	0.012
Flowering	Fertilizer	Astlae	1,9	0.362	0.326	0.296
Flowering	Fertilizer	Camrot	1,14	1.257	0.250	0.000
Flowering	Fertilizer	Cerarv	1,13	0.133	0.405	0.748
Flowering	Fertilizer	Dessop	1,2	-0.547	0.596	0.456
Flowering	Fertilizer	Erigla	1,15	0.084	0.255	0.747

Response variable	Treatment	Species	d.f.	Slope	SE	P
Flowering	Fertilizer	Galbor	1,12	0.666	0.433	0.150
Flowering	Fertilizer	Geutri	1,10	2.459	0.521	0.001
Flowering	Fertilizer	Ortlut	1,9	-0.097	0.267	0.726
Flowering	Fertilizer	Rosark	1,12	0.538	0.426	0.231
Flowering	Fertilizer	Solmis	1,14	0.970	0.326	0.010
Flowering	Fertilizer	Vicame	1,5	0.215	0.138	0.180
Flowering	Fert, Litter	Achmil	1,17	0.699	0.261	0.016
Flowering	Fert, Litter	Astagr	1,14	-0.772	0.521	0.160
Flowering	Fert, Litter	Astfal	1,15	0.415	0.071	0.000
Flowering	Fert, Litter	Astfle	1,3	0.071	0.013	0.011
Flowering	Fert, Litter	Astlae	1,9	0.827	0.165	0.001
Flowering	Fert, Litter	Camrot	1,14	0.626	0.112	0.000
Flowering	Fert, Litter	Cerarv	1,14	-0.100	0.332	0.767
Flowering	Fert, Litter	Dessop	1,3	0.377	0.096	0.029
Flowering	Fert, Litter	Erigla	1,14	0.784	0.181	0.001
Flowering	Fert, Litter	Galbor	1,12	0.232	0.395	0.567
Flowering	Fert, Litter	Geutri	1,12	0.050	0.109	0.656
Flowering	Fert, Litter	Ortlut	1,14	0.299	0.074	0.001
Flowering	Fert, Litter	Oxycam	1,8	0.001	0.116	0.993
Flowering	Fert, Litter	Rosark	1,15	0.855	0.154	0.000
Flowering	Fert, Litter	Solmis	1,14	0.539	0.686	0.445
Flowering	Fert, Litter	Vicame	1,5	0.246	1.066	0.827
Flowering	Myc. Supp.	Achmil	1,14	0.298	0.130	0.037
Flowering	Myc. Supp.	Astagr	1,8	0.206	0.566	0.726
Flowering	Myc. Supp.	Astfal	1,14	0.741	0.230	0.006
Flowering	Myc. Supp.	Astlae	1,10	0.410	0.175	0.041
Flowering	Myc. Supp.	Camrot	1,12	0.495	0.416	0.257
Flowering	Myc. Supp.	Cerarv	1,3	0.585	0.260	0.109
Flowering	Myc. Supp.	Erigla	1,14	0.011	0.238	0.964
Flowering	Myc. Supp.	Galbor	1,13	0.341	0.285	0.253
Flowering	Myc. Supp.	Geutri	1,8	-0.087	0.149	0.577
Flowering	Myc. Supp.	Ortlut	1,9	0.564	0.322	0.114
Flowering	Myc. Supp.	Oxycam	1,6	-0.090	0.271	0.752
Flowering	Myc. Supp.	Potarg	1,4	-0.119	0.067	0.151
Flowering	Myc. Supp.	Rosark	1,14	-0.531	1.207	0.667
Flowering	Myc. Supp.	Solmis	1,14	0.193	0.093	0.058
Flowering	Myc. Supp.	Vicame	1,6	-0.056	0.358	0.880
Flowering	Myc, Litter	Achmil	1,16	1.016	0.588	0.103
Flowering	Myc, Litter	Astagr	1,12	-0.570	0.489	0.266
Flowering	Myc, Litter	Astfal	1,14	0.407	0.153	0.018
Flowering	Myc, Litter	Astfle	1,3	-0.180	0.268	0.549

Response variable	Treatment	Species	d.f.	Slope	SE	P
Flowering	Myc, Litter	Astlae	1,5	0.254	0.036	0.001
Flowering	Myc, Litter	Camrot	1,12	0.407	0.242	0.118
Flowering	Myc, Litter	Cerarv	1,4	0.201	0.090	0.089
Flowering	Myc, Litter	Erigla	1,14	0.520	0.198	0.020
Flowering	Myc, Litter	Galbor	1,14	0.540	0.216	0.026
Flowering	Myc, Litter	Geutri	1,8	0.451	0.281	0.147
Flowering	Myc, Litter	Ortlut	1,10	0.151	0.145	0.321
Flowering	Myc, Litter	Oxycam	1,8	0.067	0.072	0.379
Flowering	Myc, Litter	Rosark	1,13	0.386	0.312	0.238
Flowering	Myc, Litter	Solmis	1,13	0.555	0.308	0.094
Flowering	Myc, Litter	Vicame	1,5	-0.311	0.159	0.108
Flowering	Myc, Fert	Achmil	1,15	0.133	0.152	0.394
Flowering	Myc, Fert	Astagr	1,9	-0.774	0.563	0.203
Flowering	Myc, Fert	Astfal	1,12	0.543	0.142	0.002
Flowering	Myc, Fert	Astlae	1,9	0.059	0.163	0.726
Flowering	Myc, Fert	Camrot	1,11	0.748	0.304	0.031
Flowering	Myc, Fert	Cerarv	1,8	0.206	0.217	0.369
Flowering	Myc, Fert	Dessop	1,3	-0.119	0.061	0.148
Flowering	Myc, Fert	Erigla	1,12	0.889	0.203	0.001
Flowering	Myc, Fert	Galbor	1,11	-0.103	0.248	0.687
Flowering	Myc, Fert	Geutri	1,10	0.479	0.081	0.000
Flowering	Myc, Fert	Ortlut	1,8	0.035	0.382	0.930
Flowering	Myc, Fert	Oxycam	1,6	-0.073	0.220	0.751
Flowering	Myc, Fert	Rosark	1,14	-0.344	0.652	0.605
Flowering	Myc, Fert	Solmis	1,13	0.459	0.212	0.049
Flowering	Myc, Fert	Vicame	1,8	-0.135	0.081	0.135
Flowering	Myc, Fert, Lit	Achmil	1,17	1.165	0.323	0.002
Flowering	Myc, Fert, Lit	Astagr	1,10	-0.129	0.229	0.587
Flowering	Myc, Fert, Lit	Astfal	1,12	0.506	0.148	0.005
Flowering	Myc, Fert, Lit	Astlae	1,5	0.517	0.115	0.007
Flowering	Myc, Fert, Lit	Camrot	1,13	0.854	0.262	0.006
Flowering	Myc, Fert, Lit	Cerarv	1,10	1.536	0.253	0.000
Flowering	Myc, Fert, Lit	Dessop	1,3	0.048	0.153	0.777
Flowering	Myc, Fert, Lit	Erigla	1,14	0.679	0.126	0.000
Flowering	Myc, Fert, Lit	Galbor	1,12	0.523	0.433	0.250
Flowering	Myc, Fert, Lit	Geutri	1,7	0.609	0.187	0.014
Flowering	Myc, Fert, Lit	Hieumb	1,2	2.059	0.075	0.001
Flowering	Myc, Fert, Lit	Ortlut	1,10	0.322	0.106	0.013
Flowering	Myc, Fert, Lit	Oxycam	1,5	0.159	0.147	0.327
Flowering	Myc, Fert, Lit	Rosark	1,11	0.373	0.438	0.413
Flowering	Myc, Fert, Lit	Solmis	1,16	0.244	0.069	0.003

Response variable	Treatment	Species	d.f.	Slope	SE	P
Flowering	Myc, Fert, Lit	Vicame	1,2	0.371	1.726	0.850

Table 11.5 Treatment effects on plant community diversity. Numbers in brackets refer to the power transformation used for that variable.

	Total cover			Vegetative richness$^{(0.5)}$			Vegetative evenness$^{(3)}$		
Source	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Fungicide	1,19	0.35	0.558	1,20	1.12	0.303	1,93	1.97	0.164
Fertilized	1,111	0.17	0.677	1,116	0.31	0.579	1,115	0.01	0.911
Litter removal	1,112	1.84	0.177	1,117	1.66	0.201	1,116	0.06	0.802
Fung*Fert	1,111	1.07	0.303	1,116	2.85	0.094	1,115	0.81	0.371
Fung*Lit	1,112	0.54	0.463	1,117	1.38	0.242	1,116	0.85	0.357
Fert*Lit	1,111	1.57	0.213	1,116	0.42	0.519	1,115	0.20	0.659
Fung*Fert*Lit	1,111	0.68	0.411	1,116	0.24	0.626	1,115	1.19	0.278

Table 11.6 Treatment and vegetative community effects on flowering species diversity. Numbers in brackets refer to the power transformation used for that variable.

	Total flowers$^{(0.5)}$			Flower richness$^{(1.5)}$			Flower evenness$^{(2)}$		
Source	*df*	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Fungicide	1,19	<0.01	0.970	1,21	0.23	0.638	1,20	0.41	0.530
Fertilized	1,109	2.54	0.114	1,112	0.88	0.352	1,117	0.20	0.654
Litter removal	**1,112**	**5.73**	**0.018**	1,115	<0.01	0.989	**1,120**	**6.04**	**0.015**
Fung*Fert	1,112	0.79	0.376	**1,114**	**5.27**	**0.023**	**1,119**	**8.76**	**0.004**
Fung*Lit	1,109	0.15	0.695	1,113	0.23	0.635	1,118	0.43	0.513
Fert*Lit	1,111	0.01	0.929	1,113	2.42	0.123	1,118	2.10	0.150
Fung*Fert*Lit	1,110	0.29	0.590	1,112	2.68	0.104	1,118	1.76	0.187
Total cover	1,147	7.95	0.005	1,147	12.09	0.001	1,152	5.53	0.020
Veg. richness$^{(0.5)}$	1,147	4.91	0.028	1,149	11.16	0.001	1,153	0.96	0.330
Veg. evenness$^{(3)}$	1,146	2.05	0.154	1,145	5.73	0.018	1,151	1.61	0.206

Table 11.7 Treatment effects on flower phenology. Significant treatment effects are in bold. Numbers in brackets following response variables denote the power transformation used.

	Date of first flower$^{(0.5)}$			Length of flowering period		
Source	*df*	*F*	*P*	*df*	*F*	*P*
Fungicide	1,36	0.19	0.669	1,35	0.85	0.362
Fertilized	1,1265	0.04	0.840	1,1268	0.88	0.350
Litter removal	**1,1266**	**6.57**	**0.011**	1,1269	0.13	0.716
Fung*Fert	1,1266	0.20	0.654	1,1268	0.90	0.344
Fung*Lit	1,1266	0.14	0.707	1,1268	0.10	0.757
Fert*Lit	1,1267	1.42	0.233	1,1269	2.00	0.157
Fung*Fert*Lit	1,1264	2.12	0.145	1,1268	1.43	0.233
Flower	17,1272	413.58	<0.001	17,1274	25.79	<0.001
Fung*Flower	17,1267	0.82	0.665	17,1271	0.69	0.817
Fert*Flower	**17,1259**	**1.65**	**0.046**	**17,1262**	**1.80**	**0.024**
Lit*Flower	17,1259	0.95	0.518	**17,1261**	**1.82**	**0.021**
Fung*Fert*Flower	17,1259	0.65	0.853	17,1262	0.70	0.800
Fung*Lit*Flower	17,1258	1.12	0.324	17,1261	0.57	0.915
Fert*Lit*Flower	17,1258	0.45	0.973	17,1261	0.88	0.598
Fung*Fert*Lit*Flower	17,1258	0.72	0.789	17,1261	0.85	0.637

Table 11.8 Treatments and flower community effects on the diversity of flowering species visited by insects. Numbers in brackets refer to the power transformation used for that variable.

	Flower species visited			Flower visit evenness $^{(2)}$		
Source	*df*	*F*	*P*	*df*	*F*	*P*
Fungicide	**1,40.3**	**6.78**	**0.013**	1,20.1	<0.01	0.963
Fertilized	1,118.7	0.48	0.489	1,115.5	1.12	0.293
Litter removal	1,124.0	1.26	0.263	1,120.8	2.38	0.126
Fung*Fert	1,121.7	2.86	0.093	**1,118.3**	**6.44**	**0.012**
Fung*Lit	1,119.6	0.65	0.421	1,116.1	1.15	0.287
Fert*Lit	1,119.8	0.27	0.605	**1,116.2**	**6.20**	**0.014**
Fung*Fert*Lit	1,119.3	0.05	0.819	1,115.9	0.52	0.471
Total flowers$^{(0.5)}$	1,140.3	11.74	0.001	1,141.9	0.40	0.530
Flower richness$^{(1.5)}$	1,114.7	65.08	<0.001	1,89.8	0.97	0.327
Flower evenness$^{(2)}$	1,156.5	9.84	0.002	1,155.0	0.01	0.927

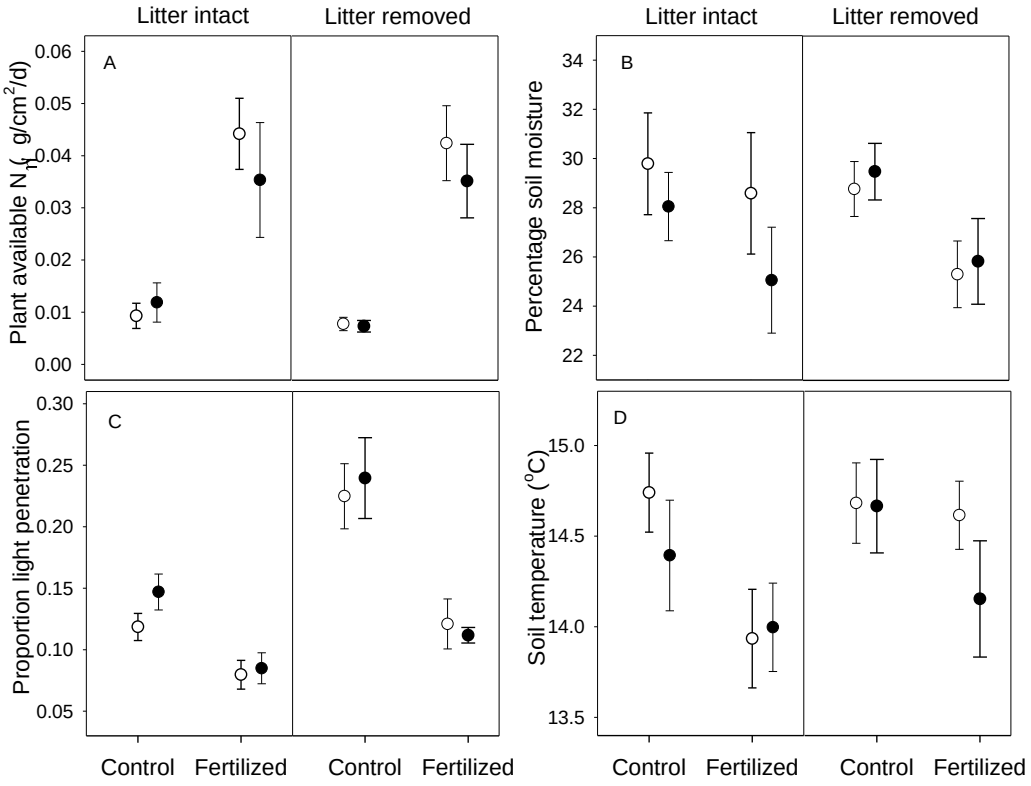

Figure 11.1 Changes in (A) plant available nitrogen, (B) soil moisture, (C) light penetration, and (D) soil temperature following mycorrhizal suppression, fertilizer addition, and litter removal. Open circles represent intact mycorrhizae conditions and filled circles suppressed mycorrhizae. Error bars represent one standard error.

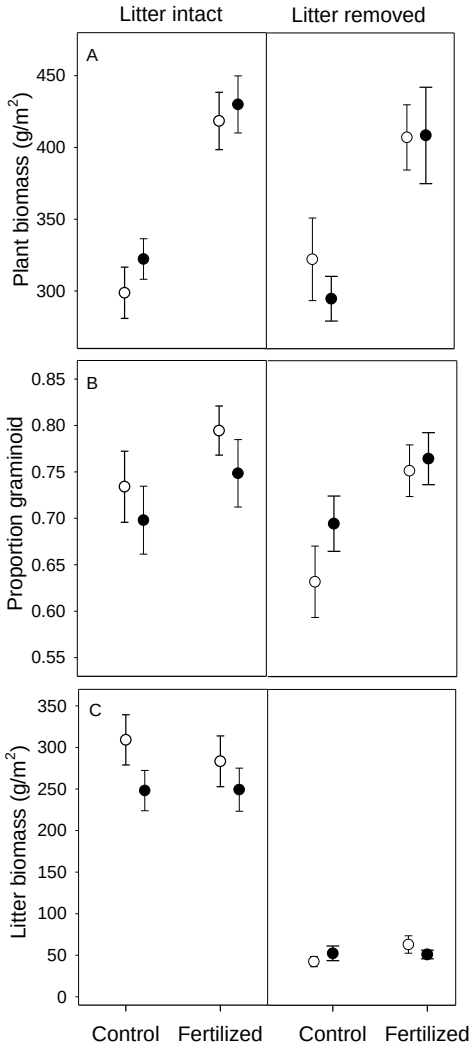

Figure 11.2 Changes in (A) plant biomass, (B) proportion graminoid abundance moisture, and (C) litter biomass following mycorrhizal suppression, fertilizer addition, and litter removal. Open circles represent intact mycorrhizae conditions and filled circles suppressed mycorrhizae. Error bars represent one standard error.