

THE INFLUENCE OF SOIL HETEROGENEITY ON PLANT SPECIES RICHNESS

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The following faculty members have examined the final copy of this thesis for form and content, and recommend that it be accepted in partial fulfillment of the requirement for the degree of Master of Science with a major in Biological Sciences.

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ABSTRACT

Environmental heterogeneity is often cited as one of the driving mechanisms behind community species composition and diversity. However, its contribution to species composition in plant communities remains unclear because few experiments demonstrate a causal link between environmental heterogeneity and plant diversity. This is particularly true for soil manipulations despite that fact that soil is expected to provide the key resources necessary for plant growth. Here, I utilize a unique manipulation of the soil profile to create communities with spatially heterogeneous and homogeneous soil arrangements and examine the influence of soil heterogeneity on community structure, through species composition and flowering patterns, during community assembly.

I employed an annual census of the assembling communities and recorded the identity and density of all species within the patches. After two years, I found that species richness was significantly higher in heterogeneous than in homogeneous plots. In the heterogeneous plots, thirteen species had higher greater establishment rates in a specific patch type representing one of the three soil strata. However, no species had greater association with the mixed stratum, comprising the homogeneous plots, than one of the heterogeneous strata. This pattern of species sorting between soil types suggests that the increased richness in heterogeneous plots is due to the increased variety of soil types comprising those communities. Alternatively, species richness in homogeneous plots, where species did not sort to distinct soils, was strongly associated with total plant density. This experiment is one of the first to provide clear, experimental evidence that fine-scale soil heterogeneity increases species richness through species sorting during community assembly.

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CHAPTER 1

MECHANISMS OF FINE-SCALE SPECIES DIVERSITY

Understanding the mechanisms behind fine-scale species diversity patterns is an enduring challenge in ecology. On one hand, the competitive exclusion principle predicts that only one species will persist over time if the competing species rely on a shared resource (Gause, 1932, Gause, 1934). On the other hand, communities that have similar environmental conditions can often have large differences in species richness suggesting that not only do species coexist, but that aspects of the environment lead to coexistence of large numbers of species. Hutchinson (1961) terms this apparent contradiction to Gause's principle the "paradox of the plankton" spotlighting communities of plankton, which are highly diverse despite having only a handful of limiting resources. The same question as to why communities have high species diversity is unresolved within plant communities, provoking continued examination of the mechanisms governing community diversity and composition.

Classic competition models support the competitive exclusion principle and predict that at equilibrium there will only be as many species coexisting as there are limiting resources for those species (Amarasekare, 2003, Armstrong *et al.*, 1980, MacArthur *et al.*, 1964, Tilman *et al.*, 1993, Volterra, 1928). However, these models assume simple environments that may not reflect natural conditions (Hutchinson, 1959, Tilman and Pacala, 1993). More complicated models of competition that relax one or more of these assumptions, such as allowing for more complicated trophic interactions (Coberly *et al.*, 2009, Levin *et al.*, 1977, Tilman, 1986), or allowing for heterogeneous

resource availability either spatially (Bolker *et al.*, 2003, Chesson, 2000a, Jeltsch *et al.*, 1998, Questad *et al.*, 2008, Reynolds *et al.*, 2009, Snyder *et al.*, 2003) or temporally (Chesson, 1994, Grover, 1991, Levins, 1979, Schmidt *et al.*, 2000), can generate stable coexistence or delay the onset of competitive exclusion depending upon the types of mechanisms introduced to the model.

Understanding the ramifications of these assumptions necessitates distinguishing between competition as the struggle to grow and reproduce under limited resource availability rather than simply a contest for resource accumulation (Chesson, 2000b). In this light, a species' R^* , the resources required to maintain a stable population size (Armstrong and McGehee, 1980, Tilman, 1980), becomes a measure of overall fitness and the mechanisms that alter a species' R^* are then categorized as either equalizing or stabilizing mechanisms (Reviewed in Wilson, 2011). Equalizing mechanisms mitigate the differences in species fitness effectively delaying the onset of competitive exclusion and include mechanisms like chance (Chave, 2004, Hubbell, 2005, Hubbell, 2001) and species aggregation (Stoll *et al.*, 2001). Stabilizing mechanisms increase the impact of intraspecific competition relative to interspecific competition leading to an increase-when-rare function central to many models of coexistence (Chesson, 2000b, Wilson, 2011). They can include several of the classical mechanisms for stable coexistence such as environmental heterogeneity and density or frequency dependent predation (Chesson, 2000b), which increase intraspecific competition relative to interspecific competition through species sorting to favored patch types.

Explanations for species diversity patterns and mechanisms of coexistence frequently reference environmental heterogeneity as potentially important since

environmental conditions are neither spatially nor temporally constant as mandated by competitive exclusion in the classical models (Chesson, 2000a). The environmental heterogeneity hypothesis is an intuitive expansion of the habitat heterogeneity hypothesis, used to explain consumer diversity through the spatial variation in habitat availability and quality, largely constructed from the accompanying vegetation (MacArthur and Levins, 1964, Pianka, 1966). Ricklefs (1977) laid the foundations for mainstream use of the environmental heterogeneity hypothesis in plant communities while suggesting mechanisms for the observed latitudinal gradient in species diversity. Ricklefs posits that “environmental heterogeneity provides the basis for resource partitioning and coexistence of competing species” and hypothesizes that species should have clear responses to experimental manipulation due to species sorting. By extension, heterogeneous environments are expected to have greater species richness owing to the greater variety in available alpha niche space (Costanza *et al.*, 2011, Houseman *et al.*, 2011, Melbourne *et al.*, 2007, Questad and Foster, 2008, Tamme *et al.*, 2010, Tews *et al.*, 2004a). Since then, the implications of environmental heterogeneity have been applied to metapopulations dynamics (Amarasekare, 2003, Amarasekare *et al.*, 2001, Hanski, 2008, Schmidt, Earnhardt, Brown and Holt, 2000), storage effects (Snyder and Chesson, 2003), and fugitive species (Holt, 1984, Seabloom *et al.*, 2005).

Given the intuitive appeal and theoretical support, numerous observational investigations have sought evidence of a link between environmental heterogeneity and plant diversity (Amarasekare, 2003, Deuschewitz *et al.*, 2003, Douda *et al.*, 2012, Dufour *et al.*, 2006, Lobel *et al.*, 2006, Schmidt, Earnhardt, Brown and Holt, 2000). In a

comprehensive review of the literature, Lundholm (2009) found 52 studies that investigated the influence of environmental heterogeneity on plant diversity. Forty-three of these were observational studies or “natural” experiments. These observational studies explored the influence of environmental heterogeneity across a broad array of habitats, including alpine systems (Loneragan *et al.*, 1984, Moser *et al.*, 2005), grasslands (Anderson *et al.*, 2004, Bakker *et al.*, 2003), forests (Balvanera *et al.*, 2006, Honnay *et al.*, 1999) and shrublands (Moody *et al.*, 2001). Investigators also considered a diverse spectrum of environmental factors in these studies ranging from soil based heterogeneity, measured across soil moisture (Freestone *et al.*, 2006, Richard *et al.*, 2000), organic matter (Desilets *et al.*, 2005, Gough *et al.*, 2000) or Nitrogen availability (Anderson, McNaughton and Ritchie, 2004, Palmer, 1991) to the influence of above-ground sources of environmental heterogeneity examined through variability in light (Bakker, Blair and Knapp, 2003), precipitation (Currie *et al.*, 1987, Rogers *et al.*, 2000) and temperature (Pausas *et al.*, 2003, Wohlgemuth, 1998).

This wide array of observational evidence suggests positive correlations between environmental heterogeneity and species richness in many of the investigated habitats including various types of wetlands, forests or grasslands. However, there are also studies in these same habitat types that reported negative and unimodal effects or no effect of heterogeneity on species richness. A similar situation arises in the types of environmental heterogeneity investigated where positive, negative, unimodal, and non-correlated relationships are described for a single factor during separate investigations (Lundholm, 2009). Although observational studies do not consistently correlate a specific source of heterogeneity to diversity, the majority report correlations between at

least one source of heterogeneity and diversity affirming the prediction that species richness should increase with environmental heterogeneity. However, the tendency to select study sites for their high species' richness (Balvanera and Aguirre, 2006, Lobel, Dengler and Hobohm, 2006, Thuiller *et al.*, 2006) or notable patterns in species' distribution (Desilets and Houle, 2005, Deutschewitz, Lausch, Kuhn and Klotz, 2003) and the presence of potentially confounding variables at the study area, such as site history (Dufour, Gadallah, Wagner, Guisan and Buttler, 2006), illustrate the need for controlled experimental evidence to corroborate these observational studies.

In contrast to the strong observational evidence, few experimental studies demonstrate a causal link between environmental heterogeneity and species richness. In a review of environmental heterogeneity investigations, Lundholm (2009) found that only nine of the fifty-two studies explicitly manipulated environmental heterogeneity. Since then, two more manipulations have experimentally investigated the influence of environmental heterogeneity. Of those, only five reported a significant effect of heterogeneity on plant diversity (Brandt *et al.*, 2013, Fitter, 1982, Questad and Foster, 2008, Vivian-Smith, 1997, Wilson, 2000). The five manipulations reporting significant results were widely varied in the type of environmental heterogeneity investigated and in the methods used to assess it. For example, Fitter (1982) performed a greenhouse manipulation on the impact of heterogeneous soil layers on competition between five common grassland plants. Vivian-Smith (1997) manipulated microtopography in constructed wetland terrariums and its influence on species sorting. Wilson (2000) and Questad and Foster (2008) conducted field experiments testing the consequences of heterogeneously applied disturbance on grassland community structure. Finally, Brandt

et al (2013) examined the influence of soil heterogeneity, generated through the plant-soil feedbacks, on species' recruitment dynamics in a common garden experiment. Vivian-Smith (1997) links microtopography to species sorting and increased species richness in artificial wetlands and the disturbance experiments (Questad and Foster, 2008, Wilson, 2000) report sorting between perennial and annual growth forms by disturbance level with increased overall richness in the community. However, no manipulation presents a link between increased species richness in intrinsically heterogeneous environments to its theoretical foundation, increased opportunity for species sorting.

The remaining six experimental manipulations of environmental heterogeneity described no significant effect on species richness. Aside from one manipulation of aboveground resources (Stevens *et al.*, 2002), all of these experiments addressed soil or substrate conditions examining various measures of soil or nutrient heterogeneity (Collins *et al.*, 1998, Reynolds *et al.*, 2007, Wijesinghe *et al.*, 2005), microsite texture (Grime *et al.*, 1987) and soil depth and Nitrogen (Baer *et al.*, 2004). Taken together, these results seem to suggest that there is a weak, if any, direct effect of soil heterogeneity on plant diversity despite soil heterogeneity being one of the largest sources of resource heterogeneity available to the plant community.

The disparity between observational evidence and field experiments is puzzling and reflects unanticipated artifacts either from the experimental designs or from species response to controlled heterogeneity. Likely, the gap between observational studies and experiments is a combination of both elements. Prominent hypotheses attributing this discrepancy to experimental design suggest that the experimental manipulations

had insufficient species pool sizes to saturate the available niche space (Lundholm, 2009, Questad and Foster, 2008, Reynolds, Mittelbach, Darcy-Hall, Houseman and Gross, 2007, VivianSmith, 1997) or there were inadequate differences in treatment parameters to induce species sorting and show treatment differentiation between homogeneous and heterogeneous environments (Lundholm, 2009, Questad and Foster, 2008). Other hypotheses propose that experimental design is not responsible for the gap between observation and experimental evidence; rather experimental results reveal differences in biological response mechanisms (clonal propagation, root foraging capacity, seed distribution) to environmental heterogeneity. One possibility is that clonal species are able to integrate resources across patch types giving species with strong vegetative growth a competitive advantage which leads to exclusion and depressed species richness within the community (Eilts *et al.*, 2011, Reynolds, Mittelbach, Darcy-Hall, Houseman and Gross, 2007). Another hypothesis proposes that the impact of environmental heterogeneity may vary between life stages, as younger individuals may be more sensitive to fine-scale resource conditions while mature plants are able to forage across patch types, leading to depressed richness in even-aged communities (Grubb, 1977, Lundholm, 2009, Lundholm, 2010, Questad and Foster, 2008).

Alternatively, the lack of an effect could also stem from the type of variables manipulated in the experimental studies and the disruption of plant-soil feedback cycles. Note that all six of the experimental investigations in Lundholm's (2009) review reporting no significant effect of heterogeneity on species diversity manipulated a resource directly associated with productivity, such as Nitrogen and light. Moreover, the five

studies that did report significant effects of heterogeneity on species diversity manipulated heterogeneity in parts of the environment not as strongly associated with productivity (microtopography, soil layers and allogenic disturbance). These findings are consistent with observational studies which suggests that soil enrichment expedites the formation of dense monospecific patches (Collins and Wein, 1998, Gough, Osenberg, Gross and Collins, 2000, Hutchings *et al.*, 2003, Suding *et al.*, 2005, Wedin *et al.*, 1996), often consisting of annuals with high seed output or perennials with strong vegetative propagation, such as clonal species (Collins and Wein, 1998, Dekroon *et al.*, 1995). The heterogeneity-diversity investigations appear to suggest that this is the case regardless of homogeneous or heterogeneous enrichment application (Baer, Blair, Collins and Knapp, 2004, Eilts, Mittelbach, Reynolds and Gross, 2011, Reynolds, Mittelbach, Darcy-Hall, Houseman and Gross, 2007, Wijesinghe, John and Hutchings, 2005). The use of major resources like Nitrogen and energy in these investigations may in fact homogenize the competitive space overshadowing or obscuring the influence of heterogeneity in other environmental conditions, such as soil type and microtopography. This is especially noteworthy in Wilson (2000), where heterogeneous disturbance increases species richness in unfertilized plots but has no effect on species richness in fertilized treatments.

Environmental heterogeneity is strongly expected to influence fine-scale diversity patterns and is supported by multiple observational investigations. However, experimental investigations provide limited empirical support for a direct link between environmental heterogeneity and community species richness and the existing experimental support is difficult to extend to field conditions. Because of the lack of

empirical field studies linking environmental heterogeneity to species richness, I manipulate the natural variation found in the soil profile to create spatially heterogeneous and homogeneous soil environments to investigate the effects of soil heterogeneity on plant community diversity and composition. The goal of this thesis is to examine how fine-scale soil heterogeneity influences plant community characteristics. In chapter 2, I test the influence of soil heterogeneity on species richness and species sorting in assembling grassland communities. Based on the previous theoretical and empirical work outlined above, I hypothesize first that communities with heterogeneous soil environments will have greater species richness than the homogeneous treatments. Second, species will sort between experimental patches. To test these hypotheses I conduct an annual census of the recreated communities for comparison between homogeneous and heterogeneous environments, specifically looking for differences in plant density, species richness and species sorting. The content in chapter 2 is a slight modification of my paper recently published in the *Journal of Plant Ecology* (Williams *et al.*, 2013).

In addition to influencing patterns of diversity and composition in plant communities, environmental heterogeneity may also influence plant flowering patterns as individuals in different environmental conditions accumulate the resources necessary to flower at different rates. However, few manipulations have created heterogeneous and homogeneous environments that differentially influence the plant community leaving the extended influence of soil heterogeneity on the greater community unexplored. In chapter 3, I test the influence of environmental heterogeneity on flower patterns in the grassland communities with heterogeneous and homogeneous soil

arrangements. Specifically, I ask first if soil type or soil heterogeneity influences patterns of flowering. Second, do patterns in phenology fluctuate or remain stable throughout the growing season? To address these questions, I conducted weekly censuses of flowering activity in the manipulated plots throughout the growing season to compare flowering phenology between heterogeneous and homogeneous plots and by flowering point in the growing season.

CHAPTER 2

EXPERIMENTAL EVIDENCE THAT SOIL HETEROGENEITY ENHANCES PLANT DIVERSITY DURING COMMUNITY ASSEMBLY

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Abstract

Aims. Environmental heterogeneity is a primary mechanism explaining species coexistence and extant patterns of diversity. Despite strong theoretical support and ample observational evidence, few experimental studies in plant communities have been able to demonstrate a causal link between environmental heterogeneity and plant diversity. This lack of experimental evidence suggests that either fine-scale heterogeneity has weak effects on plant diversity or previous experiments have been unable to effectively manipulate heterogeneity. Here, I utilize a unique soil manipulation to test whether fine-scale soil heterogeneity will increase plant richness through species sorting among experimental patch types.

Methods. This experiment was conducted in the tallgrass prairie region of south-central Kansas, U.S.A. I utilized the inherent variation found in the vertical soil profile, which varied in both biotic and abiotic characteristics, and redistributed these strata into either homogeneous or heterogeneous spatial arrangements in 2.4x2.4 m plots. After the soil manipulation, thirty-four native prairie species were sown into all plots. I conducted annual censuses at peak biomass to quantify species composition and plant density by species within the experimental communities.

Important Findings. After two years, species richness was significantly higher in heterogeneous relative to homogeneous plots and this pattern was independent of total plant density. In the heterogeneous plots, thirteen species had higher establishment in a specific patch type representing one of the three soil strata. Conversely, no species had greater establishment in the mixed stratum, which comprised the homogeneous plots, relative to the heterogeneous strata. These species sorting patterns suggest that fine-scale heterogeneity creates opportunities for plant establishment due to niche differences, which translates into increased plant diversity at the plot scale. Species richness was more strongly related to plant density among patches comprising homogeneous plots—where fine-scale heterogeneity was minimized, but weak in heterogeneous plots. This pattern is consistent with the idea that richness-density relationships dominate when neutral processes are important but are weak when niche-processes operate. Unlike many previous attempts, our results provide clear, experimental evidence that fine-scale soil heterogeneity increases species richness through species sorting during community assembly.

Introduction

Environmental heterogeneity, which includes spatial and temporal environmental variation, is one of the most intuitive explanations for differences in species diversity among communities. Presumably, fine-scale environmental variation within communities allows for resource partitioning and coexistence between competing species leading to differential species success among patch types (Bolker, 2003, Chesson, 2000a, Costanza, Moody and Peet, 2011, Melbourne, Cornell, Davies, Dugaw, Elmendorf, Freestone, Hall, Harrison, Hastings, Holland, Holyoak, Lambrinos, Moore and Yokomizo, 2007). Consequently, species diversity is predicted to increase with heterogeneity (MacArthur and Levins, 1964, Ricklefs, 1977). However, this niche-based perspective is at odds with neutral models that suggest stochastic process, immigration, and speciation render differences among species unimportant to species coexistence (Bell, 2001, Hubbell, 2001).

Given the intuitive appeal and theoretical importance, numerous observational studies have attempted to correlate environmental heterogeneity with species diversity. Because plants are sessile organisms that utilize similar essential resources, they would seem an ideal group to examine such relationships. Indeed, many studies have attempted to link fine-scale plant diversity to environmental heterogeneity across a broad array of habitats, including alpine systems, grasslands and forests utilizing a diverse set of environmental factors including soil moisture, organic matter, nitrogen availability, light, precipitation and temperature (reviewed in Lundholm, 2009). In general, observational evidence suggests positive correlations between fine-scale environmental heterogeneity and species diversity although some studies report

negative (Dufour, Gadallah, Wagner, Guisan and Buttler, 2006, Freestone and Harrison, 2006) and unimodal relationships (Loneragan and Moral, 1984) or no relationship. While observational studies do not consistently identify similar sources of heterogeneity across systems, the majority report correlations between at least one source of heterogeneity and species diversity, supporting the prediction that species richness should increase with environmental heterogeneity.

In contrast to observational evidence, few experimental studies in plant communities demonstrate a causal link between environmental heterogeneity and species richness. Most of these manipulations report no effect or even depressed species richness in heterogeneous treatments despite manipulating resources found to be important in observational studies such as: light (Stevens and Carson, 2002), nutrient heterogeneity (Collins and Wein, 1998, Eilts, Mittelbach, Reynolds and Gross, 2011, Reynolds, Mittelbach, Darcy-Hall, Houseman and Gross, 2007, Wijesinghe, John and Hutchings, 2005), microsite texture (Grime, Mackay, Hillier and Read, 1987), sedimentation depth (Stromberg *et al.*, 2011), and soil depth and nitrogen (Baer, Blair, Collins and Knapp, 2004). In fact, excluding experiments that use disturbance to create heterogeneous conditions (Questad and Foster, 2008, Wilson *et al.*, 2002), there is weak experimental evidence for a causal relationship between fine-scale soil heterogeneity and plant diversity despite what is reported in observational studies.

The disparity between observational evidence and field experiments is puzzling and suggests at least two key possibilities. First, attempts to manipulate soil resource heterogeneity do not mimic natural sources and may inadvertently favor species with strong competitive abilities across all levels of resource availability leading to no change

in diversity despite increased heterogeneity (Eilts, Mittelbach, Reynolds and Gross, 2011). This is a strong possibility particularly for nutrient addition experiments, which may disrupt plant-soil-microbe interactions or favor nitrophilic species that can capitalize on the highly mobile forms of nutrients not found in natural systems (Collins and Wein, 1998, Dekroon and Hutchings, 1995, Socher *et al.*, 2013) resulting in similar effects on plant communities whether applied in spatially uniform or heterogeneous patterns. For example, several observational studies found that soil nutrient enrichment leads to the formation of dense monospecific patches (Collins and Wein, 1998, Gough, Osenberg, Gross and Collins, 2000, Hutchings, John and Wijesinghe, 2003, Suding, Collins, Gough, Clark, Cleland, Gross, Milchunas and Pennings, 2005, Wedin and Tilman, 1996), often consisting of annuals with high seed output or perennials with strong vegetative propagation, such as clonal species (Collins and Wein, 1998, Dekroon and Hutchings, 1995). Thus far, heterogeneity-diversity experiments appear to suggest that this is the case regardless of homogeneous or heterogeneous enrichment application (Baer, Blair, Collins and Knapp, 2004, Eilts, Mittelbach, Reynolds and Gross, 2011, Reynolds, Mittelbach, Darcy-Hall, Houseman and Gross, 2007, Wijesinghe, John and Hutchings, 2005) and raises the possibility that the use of fertilizer to create heterogeneous conditions alters plant-soil interactions in ways that subvert positive heterogeneity-diversity relationships.

A second possibility is that any observed positive correlation between environmental heterogeneity and diversity occurs because the plants create environmental heterogeneity rather than responding to it. This perspective is consistent with neutral theory in which species colonization and extinction are driven

predominantly by stochastic processes rather than differential success rates among patch types (i.e. species sorting; Hubbell, 2005). The subsequent changes to environmental conditions are detectable but do not affect diversity patterns. This second hypothesis is important to understanding whether niche or neutral processes drive community assembly and composition. Yet, a clear dichotomy between the two may not be apparent because the relative importance of niche and neutral processes may fall along a continuum depending on system attributes such as organism size (Farjalla *et al.*, 2012), the degree of community isolation (Gravel *et al.*, 2006), environmental heterogeneity (Kadmon *et al.*, 2007), or successional state (Chu *et al.*, 2007). One way to discriminate between niche or neutral dominated processes is to use density-richness relationships which are predicted to be stronger under neutral than niche-controlled communities (Doncaster, 2009), but past studies have not examined this possibility.

To address the apparent discrepancy between observational and experimental evidence for heterogeneity-diversity relationships, I employ a novel manipulation of fine-scale soil heterogeneity by altering the natural variation found within the vertical soil profile to create spatially heterogeneous and homogeneous soil environments and quantify the effects on plant community diversity during early grassland community assembly. This manipulation was not intended to mimic a disturbance regime but rather to create fine-scale soil heterogeneity utilizing natural variation in soil characteristics. Specifically I ask, during community assembly, if 1) heterogeneous soils promote greater species richness within plant communities than homogeneous soils? 2) species

sort between experimental patches? 3) plant richness-density relationships vary between homogeneous and heterogeneous communities?

Materials and Methods

Study Site

The experiment was conducted at Wichita State University's Ninnescah Reserve (Latitude: 37.538, Longitude: -97.6782). The field was under agricultural use until the mid-1980s when it was restored to native grassland dominated by native perennial grasses (such as *Sorghastrum nutans* and *Panicum virgatum*) with scattered perennial forbs and legumes. The site has been managed with periodic burning and mowing to prevent invasion by woody species.

Experimental Manipulation

In preparation for the soil manipulation, a 9% glyphosate herbicide was applied over an 11x23m area in September 2010. The dead vegetation was cut with a weed whip and raked off the site. Seven weeks after the plant removal, ten, 2.4-meter square plots were established with 2m walkways between plots and assigned to either homogeneous (n=5) or heterogeneous soil treatments (n=5) in a randomized complete-block design. Heterogeneous treatments were created by excavating three 15cm strata from the vertical soil profile (Fig 1a) that were expected to vary in soil texture, nutrients, organic matter, and soil organisms, then redistributing each stratum into randomly located patches within the plot of origin to create plots with spatially heterogeneous soil conditions. For the homogenous treatment, all three strata were combined before allocation into patches within the plot of origin. In this way, the soil composition was kept constant for each plot while the natural spatial variation in soil conditions was

altered to reflect a known scale and create clear discrimination between heterogeneous and homogeneous soil treatments.

Each 15 cm stratum was extracted by first loosening and mixing the soil using a rototiller and then removing the soil with a small, front-end loader. Once removed, three soil strata from each heterogeneous plot were pooled in separate mixing containers for each stratum (1-3; Fig 2.1) and mixed again to control for the mixing effect in homogeneous treatments. All three strata from each homogeneous plot was placed in one large container and thoroughly mixed (i.e. "mixed stratum"). All mixing was done with a rototiller operated within the containers. The extraction, mixing and re-application for each plot was done independently so that soils were extracted then returned to the plot of origin. Prior to re-application of the soils, a plywood form was constructed to create a grid of thirty-six, 0.4x0.4 m patches within each plot. This patch size was selected because it was sufficiently large to include multiple individuals yet allow species to forage both within and among patches. In the heterogeneous plots, each of the three patch types were randomly assigned to twelve positions within the grid and filled with the soil of the specified strata (Fig 2.1). All patches within a plot were filled to the same level. Likewise, the homogeneous plots were filled the same way using the mixed stratum (Fig 2.1b). Upon completion of the soil manipulation, a polypropylene cloth was placed over the site to minimize movement of soil by winds during winter.

Soil Sampling

Following the removal of the polypropylene fabric in late April 2011, 15 cm soil cores were collected from the patches using a soil corer (inside diameter: 17.5mm). Two soil cores were taken from the center of each patch and homogenized for that

patch. After collection, samples were stored in the refrigerator (5° C) until sent for analysis at the KSU Soil Testing Lab. All samples were tested for sand-silt-clay composition, pH, and organic matter.

Seed Distribution

To prepare the plots for seed addition and to minimize a seedbank response, I applied glyphosate (2% and 6% respectively) on the 16 and 26 of May 2011. Following plant senescence, I removed senesced plants and sowed seeds of thirty-four native prairie species into each plot (Table 2.1). Seed addition rates were identical among plots based on recommended rates for prairie restoration (Diboll, 1997). Seeds were hand sown at a height of approximately 25 cm to reduce drift during low wind conditions and were added in multiple passes within each plot—with regular mixing of seed—to prevent spatial clumping of different species due to seed size or mass. To protect the seeds from granivory and wind displacement that often occurs in the absence of an established plant community, the plots were covered with a polypropylene fabric. This polypropylene fabric was replaced after one week with a biodegradable wood fiber germination mat to promote germination under severe drought conditions that began in late spring. All plots were watered three times a week to mitigate drought conditions. Supplemental watering was discontinued after 10 weeks and the total water received by the plots during the May-August period, 23.5 cm, matched the lowest precipitation received in a growing season over the previous 5 years (NOAA National Climatic Data Center).

Statistical Analysis

Soil Analysis

Soil Data from each patch were analyzed with multivariate analysis because the variables were expected to be interdependent. I used PERMANOVA and PERMDISP to test for differences in group centroids and dispersions (measures of central tendency and variance in multivariate space) among the four patch types. Euclidean distance was used to calculate the similarity matrix and statistical significance was calculated by generating pseudo *F*-values from 16000 permutations (Anderson *et al.*, 2008). I used Principle Components Analyses (PCAs) to visualize differences detected by the multivariate statistical tests (Fig 2.2). All multivariate tests were conducted with PerMANOVA+ within PRIMER 6.

Census Analysis

We quantified community composition through an annual census at peak biomass, recording the identity and density (number of stems) of each species within the community. I analyzed the census data for treatment effects on species richness at the patch scale using a one-way ANOVA in a randomized-complete block design accounting for repeated measures by year and adjusting for multiple comparisons with Tukeys adjustment (SAS 9.2). To examine the potential effect of density on richness (a sampling effect), I used a sample-based Coleman rarefaction to analyze accumulated richness across standardized densities within heterogeneous and homogeneous communities (EstimateS 9.0, Colwell, 2013). Next, I calculated species turnover among patches within each plot by subtracting average patch richness from plot richness (Questad and Foster, 2008). Differences in species richness and species turnover at

the plot scale were analyzed separately from the patch scale using a one-way ANOVA with the two levels of heterogeneity as the treatment effect in a randomized complete block design (SAS 9.2). To identify which species were associated with particular patch types (species sorting), I used an Indicator Species Analysis (ISA, PC-ORD 6.0). Species were identified as sorting when a significant indicator value among patch types was detected ($P < 0.05$) based on 16,000 randomizations. In addition to testing the effects of species turnover and species sorting on species richness, I regressed species richness on plant density across patches for homogeneous and heterogeneous plots to test whether this relationship reflected a strong or weak relationship as predicted by neutral or niche process, respectively (Doncaster, 2009).

Results

Soil Analysis

There were significant differences in soil composition among the three soil strata reflecting a decrease in organic matter and pH and an increase in clay content from the upper (stratum 1) to lower stratum (stratum 3; Table 2.2). Because soil includes numerous inter-related components, I used a multivariate analysis to test differences in the soil variables among patches and plots. A Principal Components Analysis (PCA) indicated that the first three axes accounted for 89.9% of the variation in the original variables, 44.1%, 31.8%, and 14.0% respectively. PerMANOVA tests indicated that soil conditions found in stratum 1-3 were significantly different from one another ($t_{118} \geq 3.2$, $P < 0.001$; Fig 2.2). Furthermore, PERMDISP analysis with Tukey's comparison indicated that the variability among patches in soil conditions comprising heterogeneous plots was substantially higher than in homogenous plots ($t_{358} = 6.68$, $P < 0.001$; Fig

2.2b). There was no difference in variability within the four patch types ($t_{118} \leq 1.403$, $P \geq 0.169$), but rather the difference was due to the variability created when multiple patch types were combined within heterogeneous plots.

Plant Response

In the first year of community establishment, repeated measures analysis revealed no significant differences in species richness ($t_{352} \geq -1.08$, $P \geq 0.7$) among patches from the three strata comprising the heterogeneous plots. However, these strata each had greater species richness ($t_{352} \geq 5.92$, $P < 0.0001$) than the mixed stratum comprising homogeneous plots. This pattern of species richness continues through two growing seasons, so that in the second year, strata 1-3 continued to sustain statistically similar levels of richness ($t_{352} \leq 1.12$, $P \geq 0.26$) while each patch type was significantly more species rich than in the mixed stratum ($t_{352} \geq 4.63$, $P < 0.0001$; Fig 2.3).

At the plot scale, I found that the communities with spatially heterogeneous soil conditions supported greater species richness than communities with spatially homogeneous soils in the first year ($t_8 = 2.3$, $P = 0.04$). The strength of this relationship appears to increase slightly over time, so that heterogeneous communities continue to maintain greater species richness than the homogeneous communities after the second year ($t_8 = 3.3$, $P = 0.01$ Fig 2.4). Sample-based Coleman rarefaction of heterogeneous and homogeneous plot types demonstrated that differences in plant density between communities did not explain the increased species richness within heterogeneous plots since heterogeneous plots had greater richness than homogeneous plots at equal densities (Fig 2.5).

To examine the mechanisms that promoted greater species richness in heterogeneous plots, I tested whether species turnover and species sorting among patches could explain differences in plot species richness. Analysis of species turnover indicated that communities in heterogeneous plots had greater turnover among patches than in homogeneous plots ($t_4 = 6.23$, $P = 0.003$). An Indicator Species Analysis (ISA) revealed that several species were significantly associated with particular soil types. In the first year, five species showed evidence of species sorting. Relative to other patch types *Chamaecrista fasciculata* and *Heliopsis helianthoides* had increased establishment in stratum 1, *Oligoneuron rigidum*, *Amorpha canescens* and *Eupatorium altissimum* in stratum 3; and no species had greater affinity for stratum 2 or the mixed stratum that comprised homogeneous plots. In the second year, the number of species significantly associated with a particular soil type increased from five to thirteen. In comparison to other patch types, *Amorpha canescens*, *Heliopsis helianthoides*, and *Schizachyrium scoparium*, had increased colonization in stratum 1, *Ambrosia psilostachya*, *Echinacea angustifolium*, *Helianthus maximilliani*, *Monarda fistulosa*, and *Ratibida pinnata* in stratum 2, and *Symphotrichum oblongifolium*, *Elymus canadensis*, *Lespedeza capitata*, *Panicum virginica*, and *Salvia azureas* in stratum 3. Once again, I found no species with significant associations to the mixed stratum in the second year (Fig 2.6).

In addition to patterns of species turnover and species sorting, I tested the strength of the density-richness relationships among patches within either heterogeneous or homogeneous communities to examine the relative dominance of niche or neutral processes in driving community richness as predicted by theory

(Doncaster, 2009). The density-richness relationship across patches was stronger in heterogeneous plots ($r^2 = .71$, $P = 0.0001$) than homogeneous plots ($r^2 = .66$, $P = 0.0001$) in the first year. However, by the second year plant density more strongly influenced species richness in homogeneous ($r^2 = .46$, $P = 0.0001$) than heterogeneous plots ($r^2 = .12$, $P = 0.0001$, Fig 2.7).

Discussion

Our results show that increased fine-scale soil heterogeneity has the capacity to enhance plant species richness (Fig 2.4). One possible reason for the increase might be differences in total plant density among treatments, but species rarefaction curves suggest that heterogeneous communities accumulate more species over standardized densities than homogeneous communities (Fig 2.5). The second possibility is that species richness increases within heterogeneous communities because of greater species turnover between patches generated by differential species success among patch types (species sorting). Although all species were capable of colonizing any patch type, a substantial number of species had increased establishment success in particular patch types. Specifically, 13 of the 34 added species had highest indicator values in one of the three soil types that comprised the heterogeneous soil treatment. By contrast, no species exhibited greater affinity for the mixed stratum in homogeneous plots compared to the soil patches found in the heterogeneous plots (Fig 2.6). This pattern of species sorting in heterogeneous plots increased from five species in the first year to thirteen species in the second indicating that the pattern strengthened over time. The increase in total richness within the heterogeneous communities is consistent with the view that heterogeneous soils increased opportunity for species colonization as

predicted by several niche-based models (Chesson, 2000a, MacArthur and Levins, 1964, Tilman, 1986), yet, there is limited experimental evidence in plant communities substantiating this response at fine scales (But see Questad and Foster, 2008, VivianSmith, 1997).

Not only was there a strong contrast in species sorting between homogenous and heterogeneous plots, but there were important differences in the density-richness relationships at the patch scale among homogenous and heterogeneous plots. A strong relationship between total plant density and richness is indicative of neutral-type processes (a sampling effect) where stochastic colonization and extinction lead to richness that is primarily dependent upon total plant density (Hubbell, 2005). In contrast, if niche-assembly strongly structures plant diversity, the density-richness relationship is likely to be less important (Doncaster 2009). By the second year, plant density accounted for only 12% of species richness in heterogeneous communities but 46% in homogeneous communities. This result, coupled with the reduced species turnover and lack of species sorting in homogeneous plots, suggests that reduced environmental heterogeneity leads to increased niche overlap while enhanced heterogeneity decreases niche overlap and thereby increases opportunities for the assembly of species rich communities. Our results suggest a distinction between niche and neutral controlled communities linked to environmental heterogeneity. However, levels of heterogeneity are likely to vary across landscapes depending on how geologic and anthropogenic factors influence the spatial grain and degree of variation within the system. Consequently, the plant responses in homogeneous-heterogeneous plots reported here may reflect a continuum where stochastic assembly primarily operates

under homogeneous soil conditions while niche-assembly occurs as environmental heterogeneity increases (Gravel, Canham, Beaudet and Messier, 2006).

Although heterogeneity-diversity relationships are pivotal to niche based community-assembly, support for such relationships is weak or absent. Previous experiments have often found that plant diversity does not increase following experimental manipulations of heterogeneity regardless if the source was soil nutrients (Baer, Blair, Collins and Knapp, 2004, Collins and Wein, 1998, Eilts, Mittelbach, Reynolds and Gross, 2011, Reynolds, Mittelbach, Darcy-Hall, Houseman and Gross, 2007, Wijesinghe, John and Hutchings, 2005), soil depth (Baer, Blair, Collins and Knapp, 2004, Stromberg, Butler, Hazelton and Boudell, 2011) or light (Stevens and Carson, 2002). Those experiments that have reported an increase in species richness were either greenhouse-style experiments manipulating soil type (Fitter, 1982) and topography (VivianSmith, 1997) or field manipulations that employ patchy disturbance to create heterogeneous environmental conditions in comparison to undisturbed communities (Questad and Foster, 2008, Wilson and Tilman, 2002). The use of patchy disturbance to create heterogeneity in the field has been a useful starting point, but may limit applicability because the response is likely dependent on the mortality of established species in disturbed patches. From these studies, one might conclude that fine-scale heterogeneity is important to plant species richness only if disturbance is part of the system.

A more compelling source of environmental heterogeneity in plant communities is from soils. For example, soil nitrogen, pH and water can vary simultaneously at a variety of scales and sites with naturally higher levels of heterogeneity in these

resources have been correlated with increased species richness (Richardson *et al.*, 2012). Since plants are sessile and share a common set of resource requirements (e.g. nitrogen or phosphorous, and water), soil heterogeneity is likely a fundamental opportunity for resource partitioning if niche-based coexistence operates within communities. However, soil heterogeneity experiments provide poor support for positive heterogeneity-diversity relationships (Baer, Blair, Collins and Knapp, 2004, Collins and Wein, 1998, Eilts, Mittelbach, Reynolds and Gross, 2011, Grime, Mackay, Hillier and Read, 1987, Reynolds, Mittelbach, Darcy-Hall, Houseman and Gross, 2007, Stromberg, Butler, Hazelton and Boudell, 2011, Wijesinghe, John and Hutchings, 2005) Our experiment shows that manipulations of fine-scale soil heterogeneity can lead to increased species richness in plant communities during community assembly through differential species establishment in the patch types that comprise heterogeneous plots.

The unique positive response of diversity to heterogeneity reported here raises interesting questions as to why responses may differ among experiments. One possibility is that our approach avoided the use of inorganic fertilizers (Collins and Wein, 1998, Eilts, Mittelbach, Reynolds and Gross, 2011, Wedin and Tilman, 1996), which may alter plant-soil-microbe interactions (Bever *et al.*, 2010, Klironomos, 2002) or obscure species responses to soil heterogeneity. For example, fertilizers provide highly mobile nutrients, which may not adequately mimic natural nutrient availability, and yields no change in species diversity despite the contrast between uniform and patchy application. A second possibility is that pre-treatment soil legacies created by plant-soil interactions or other sources found in intact communities may impede the response to the experimentally imposed heterogeneity. In our case, the soil manipulation required

resetting community assembly and this may increase the sensitivity of the response. However, resetting community assembly may not always be effective as Reynolds et al (2007) found that whether the resident community was severely disturbed or remained intact did not change the outcome—that heterogeneity created by the application of inorganic fertilizer did not increase plant diversity. Clearly new approaches are needed to identify both the experimental limitations and isolate the contribution of heterogeneity to diversity.

Although our experiment provides good evidence for heterogeneity-diversity relationships, these results should be viewed as part of a community assembly process rather than a community approaching a more stable condition. Clearly, long-term monitoring of these plots will be necessary to determine fully whether the heterogeneity-diversity effects will be sustained. However, the strength of sorting patterns and effects on species richness increased from the first to the second year suggesting that priority effects associated with the initial seeding are giving way to species-environment relationships. Furthermore, in grassland systems, such early establishment patterns of colonizing species are often critical to understand longer-term diversity patterns (Houseman and Gross, 2011, Martin *et al.*, 2012).

Our results suggest that the lack of support for heterogeneity-diversity field experiments is at least partly due to the strategies utilized to manipulate heterogeneity. Perhaps more importantly, our results provide some of the strongest experimental evidence to date that increased soil heterogeneity enhances species richness during community assembly due to increased species sorting among available patch types (Houseman and Gross, 2011). Finally, the variation in species sorting and richness-

density relationships suggest that strength of niche or neutral processes may shift along a continuum corresponding to the degree of spatial heterogeneity.

Acknowledgements

This project was supported by the NSF Award No. EPS-0903806 and matching support from the Kansas Technology Enterprise Corporation to G.R.H. We are grateful for the assistance of Anna Balthazor, Zach Quick, Suvi Samant, Bryant Wong, Evelyn Carlos and Tracie Harmon in the field. This is contribution 21 of the Wichita State University Field Station.

Tables

Table 2.1: Species Seeded List. Native prairie species and amount (mass) sown into each plot.

Species	Mass per plot (g)
<i>Amorpha canescens</i> Pursh	5.6
<i>Andropogon gerardii</i> Vitman 6	6.8
<i>Asclepias syriaca</i> L.	14.5
<i>Astragalus canadensis</i> L.	2.98
<i>Baptisia australis</i> (L.) R. Br.	17.76
<i>Dalea purpurea</i> Vent.	2.84
<i>Desmanthus illinoensis</i> (Michx.) MacMill. ex B.L. Rob. & Fernald	3.27
<i>Echinacea angustifolia</i> DC.	5.87
<i>Elymus canadensis</i> L.	24.2
<i>Eupatorium altissimum</i> L.	3.05
<i>Eupatorium perfoliatum</i> L.	3.06
<i>Helenium autumnale</i> L.	3.62
<i>Helianthus maximiliani</i> Schrad.	2.96
<i>Heliopsis helianthoides</i> (L.) Sweet	8.52
<i>Koeleria macrantha</i> (Ledeb.) Schult.	5.18
<i>Lespedeza capitata</i> Michx.	9.47
<i>Lespedeza virginica</i> (L.) Britton	2.87
<i>Liatris punctata</i> Hook.	4.27
<i>Monarda fistulosa</i> L.	3.06
<i>Oenothera macrocarpa</i> Nutt.	5.69
<i>Oligoneuron rigidum</i> (L.) Small var. <i>rigidum</i>	4.4
<i>Panicum virgatum</i> L.	3.17
<i>Penstemon cobaea</i> Nutt.	3.62
<i>Ratibida columnifera</i> (Nutt.) Woot. & Standl.	3.25
<i>Ratibida pinnata</i> (Vent.) Barnhart	3.05
<i>Rudbeckia hirta</i> L.	3.23
<i>Salvia azurea</i> Michx. ex Lam	11.18
<i>Schizachyrium scoparium</i> (Michx.) Nash	14.83
<i>Senna marilandica</i> (L.) Link	17.79
<i>Silphium laciniatum</i> L.	16.87
<i>Sorghastrum nutans</i> (L.) Nash	14.83
<i>Symphyotrichum novae-angliae</i> (L.) G.L. Nesom	4.2
<i>Symphyotrichum oblongifolium</i> (Nutt.) G.L. Nesom	2.98
<i>Verbena hastata</i> L.	2.96

Table 2.2: Patch Type Composition. These are the measured soil variables (mean \pm 1 standard error) for each stratum.

Soil Type	Organic Matter	pH	Sand	Silt	Clay
Stratum 1	2.78 \pm 0.08	5.59 \pm 0.03	40.80 \pm 0.43	47.57 \pm 0.41	11.63 \pm 0.23
Stratum 2	1.54 \pm 0.05	5.22 \pm 0.02	41.37 \pm 0.41	46.70 \pm 0.31	11.93 \pm 0.22
Stratum 3	1.22 \pm 0.04	5.09 \pm 0.01	39.73 \pm 0.54	47.33 \pm 0.33	12.93 \pm 0.31
Mixed stratum	1.81 \pm 0.04	5.35 \pm 0.01	41.52 \pm 0.25	46.60 \pm 0.20	11.88 \pm 0.13

Figures

Figure 2.1

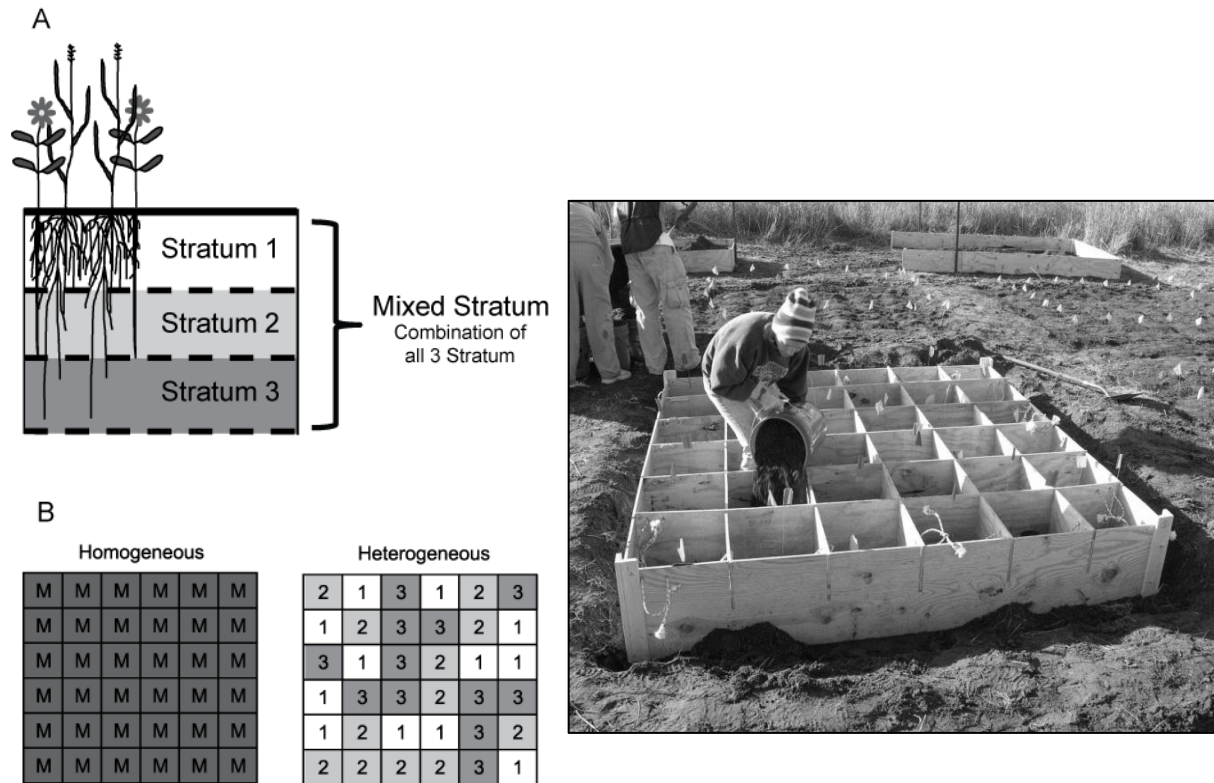


Figure 2.1: Illustrated Soil Manipulation (A) Hypothetical soil profile illustrating the three strata types (~15 cm each). (B) Aerial view illustrating patch randomization in homogeneous (left) heterogeneous (right) plots. The numbers represent strata 1–3 or the mixed stratum (M). (C) A picture illustrating the grid used to create the patch grid for each plot.

Figure 2.2

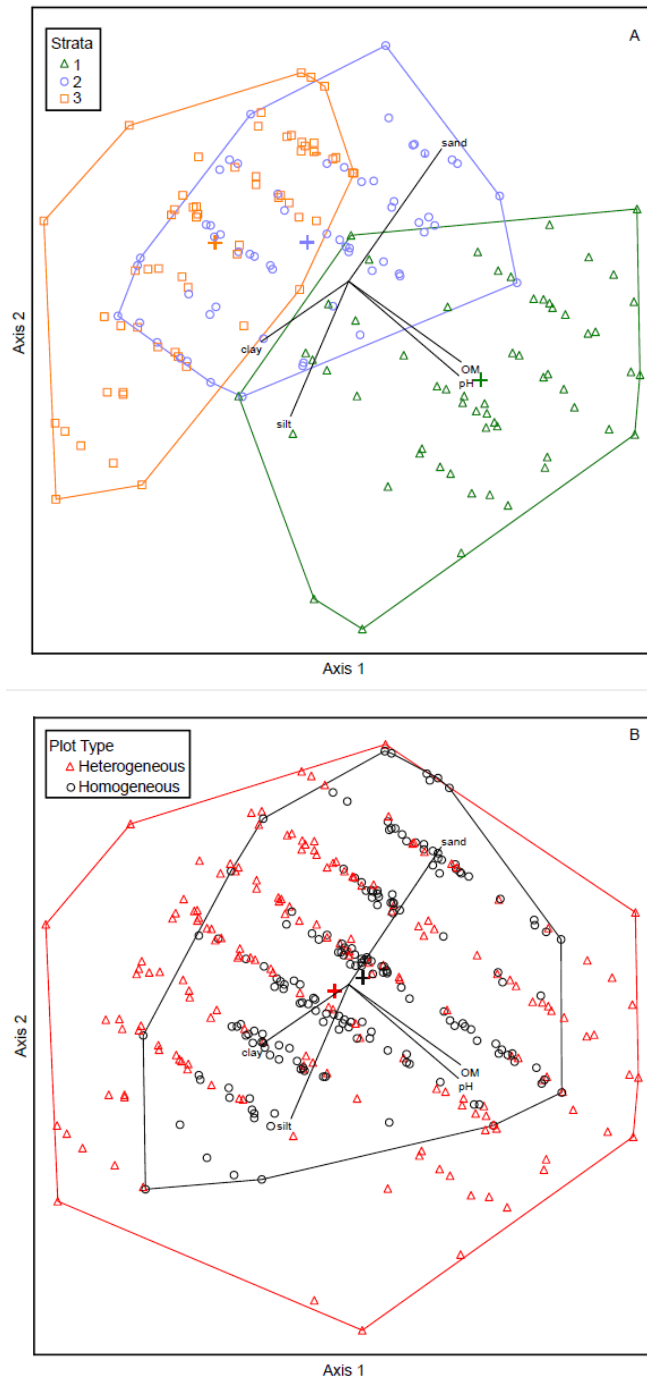


Figure 2.2: Soil Composition Ordination. Principle components analysis of patches based on five soil variables comparing patches with heterogeneous plots (A) or between heterogeneous and homogeneous plots (B).

Figure 2.3

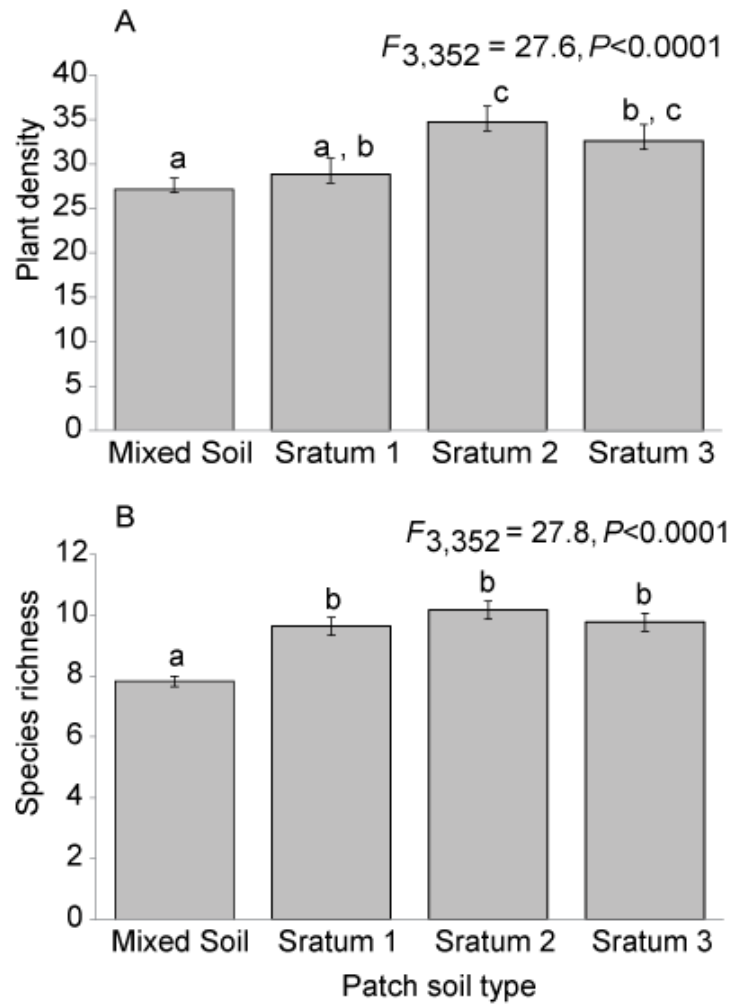


Figure 2.3: Patch-Scale Density and Richness Species richness after the second growing season at the patch scale (strata 1–3: $n = 60$; homogenized soils: $n = 180$). Distinct letter designators indicate significant differences in species richness (a: $b \ t_{352} \geq 4.63, P < 0.0001$; b: $b \ t_{352} \leq 1.12, P \geq 0.26$).

Figure 2.4

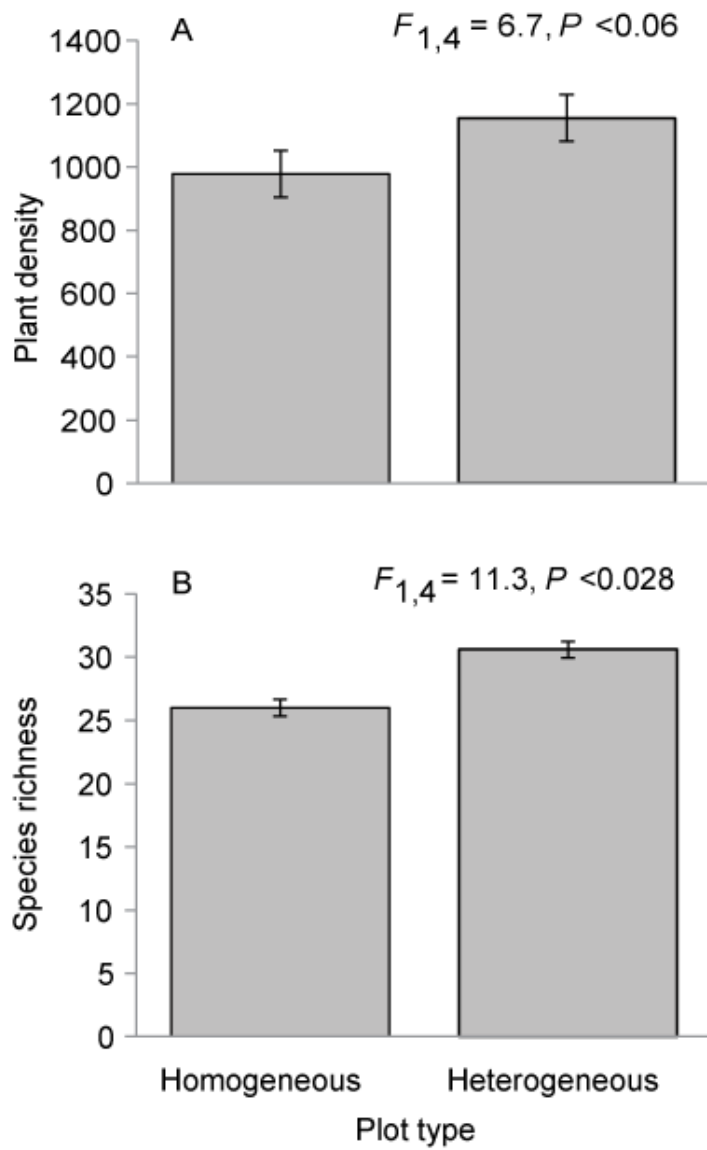


Figure 2.4: Plot-Scale Density and Richness Species richness after the second growing season at the plot scale (n = 5).

Figure 2.5

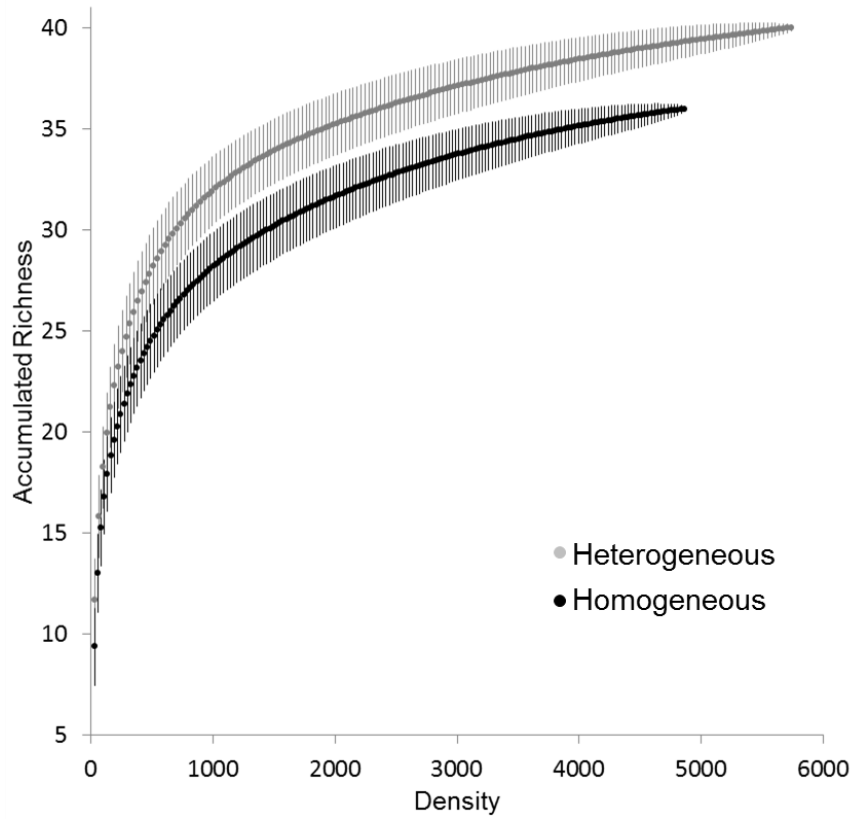


Figure 2.5: Species Accumulation by Density. Accumulated richness as a function of cumulative density after two growing seasons. Sample-based Coleman rarefaction of species richness (± 1 SD) in heterogeneous and homogeneous plots. When standardized by the cumulative number of individuals, heterogeneous plots had greater richness than homogeneous plots.

Figure 2.6

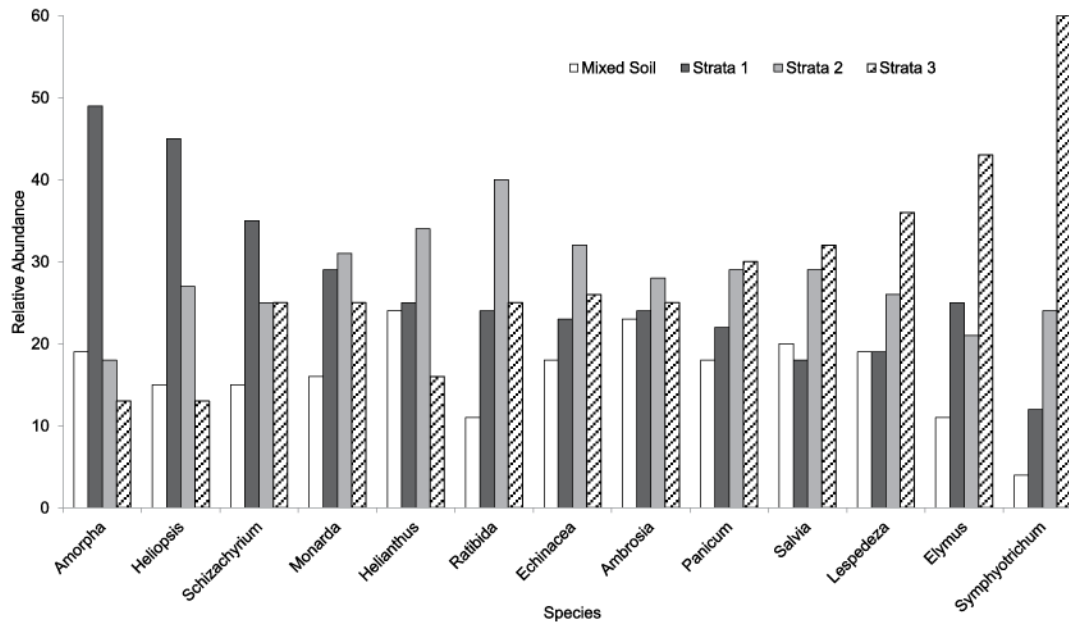


Figure 2.6: Species Sorting. Species sorting among patches of a specific soil type after two growing seasons as detected by ISA. Note the shift in peak abundance from left (stratum 1) to right (stratum 3) found in heterogeneous plots, but no species achieved peak abundance in the mixed soil strata comprising homogeneous plots.

Figure 2.7

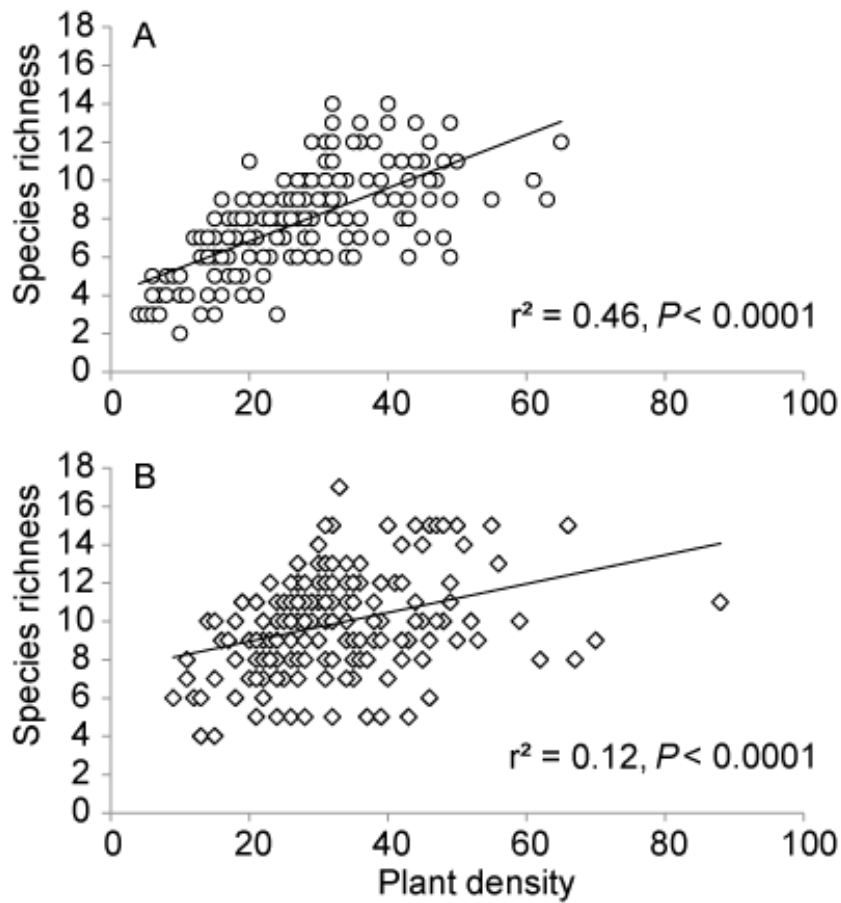


Figure 2.7: Species Richness Predicted by Density. Regression of species richness on plant density within patches of homogeneous (A) and heterogeneous (B) plots. After 2 years, plant density more strongly influences species richness in homogeneous than in heterogeneous plots.

CHAPTER 3

THE INFLUENCE OF SOIL HETEROGENEITY ON FLOWERING PATTERNS

Introduction

Soil heterogeneity is expected to influence plant species richness by increasing opportunities for resource partitioning and species sorting (Chesson, 2000a, Ricklefs, 1977, Williams and Houseman, 2013). This may create an additional indirect influence on consumer communities by increasing variation within the vegetative community and generating greater habitat heterogeneity (González-Megías *et al.*, 2007). However, previous studies investigating habitat heterogeneity examine variation in habitat quality or accessibility then consider responses in consumer diversity without examining the influence of underlying environmental heterogeneity, such as that found in the soil (Murdoch *et al.*, 1972, Tews *et al.*, 2004b). The research emphasis on habitat heterogeneity without considering the underlying soil heterogeneity is understandable because few studies have demonstrated a causal link between soil heterogeneity and changes in community composition (Questad and Foster, 2008, Williams and Houseman, 2013, Wilson and Tilman, 2002), but it leaves the mechanistic influence of environmental heterogeneity poorly understood.

In addition to influencing patterns of diversity and composition in plant communities, environmental heterogeneity may influence flowering phenology within and among communities (Dahlgren *et al.*, 2007). Although much recent work emphasizes potential responses to climate change (Brody, 1997, Colautti *et al.*, 2010, Etterson, 2004, Giménez-Benavides *et al.*, 2011), flowering phenology has been shown

to vary at fine spatial scales (Goulart *et al.*, 2005) and in accordance to abiotic conditions such as soil moisture (Dahlgren, von Zeipel and Ehrlén, 2007), nutrient levels (Wielgolaski, 2001) and light availability (Dahlgren, von Zeipel and Ehrlén, 2007, Galloway *et al.*, 2012). However, few manipulations have created heterogeneous and homogeneous environments that differentially influence the plant community (Questad and Foster, 2008, Williams and Houseman, 2013, Wilson and Tilman, 2002) and phenology studies focus on the responses of single species, typically herbaceous annuals (Dahlgren, von Zeipel and Ehrlén, 2007, Galloway and Burgess, 2012, Goulart, Lemos Filho and Lovato, 2005). This emphasis on annual populations coupled with the limited number of heterogeneity field manipulations leaves the relationship between soil heterogeneity and flowering phenology in establishing communities unexplored. To examine this relationship, I utilized the soil heterogeneity experiment described in chapter two and conducted weekly censuses of flowering activity in the manipulated plots throughout the growing season. Specifically, I ask first if soil heterogeneity influences patterns of flowering, such as flowering duration, peak number of plants in flower, cumulative flowering time, flower richness or cumulative flower richness. Second, do patterns in flowering phenology fluctuate or remain stable across the growing season?

Materials and Methods

Study Site

This investigation was conducted as Wichita State University's Ninnescah Reserve (Latitude: 37.538, Longitude: -97.6782) using reconstructed grassland communities assembling in either heterogeneous or homogeneous soil conditions

(Williams and Houseman, 2013). The field was under agricultural use until the mid-1980s when it was restored to native grassland dominated by native perennial grasses (such as *Sorghastrum nutans* and *Panicum virgatum*) with scattered perennial forbs and legumes. The site has been managed with periodic burning and mowing to prevent invasion by woody species.

Data Collection

To track flowering phenology within the plots, weekly surveys of flowering plants were performed throughout the growing season from April to October over the course of two years (2012-2013). During the weekly survey, all plants were examined and the number and identity of plants in flower was recorded. A plant was considered 'in flower' for the period following an opened bud until all flowers had dropped their petals. For species with modified or absent petals (such as the grasses), plants were considered in flower from the first appearance of the anthers until all anthers had dropped.

Analysis

To analyze the influence of heterogeneous or homogeneous soils on flowering patterns I examined flowering duration, peak number of plants in flower, an index of reproductive flowering time, flower richness and cumulative flower richness using a one-way ANOVA with the two levels of heterogeneity as the treatment effect in a randomized complete block design (SAS 9.2). The reproductive flowering time index creates a metric for quantifying and comparing the species' pollination opportunity across the season by summing the weekly number of flowering individuals across all weeks in flower. Following the analyses of treatment effects from the heterogeneity

manipulation, treatments groups were aggregated and phenology traits were examined as functions of time of first flower to investigate trends across the growing season.

Results

Heterogeneity and Phenology

During the second year (2012) of community assembly in the experimental plots, I had 28 species flower between April and October. As the community continued to develop in the third year of assembly (2013), the number of species to flower increased to 41 species across the entire site. The level of soil heterogeneity had no significant effect on the duration of any species in 2012 ($t_{1,4} \leq 1.45$, $P \geq 0.22$) or in 2013 ($t_{1,4} \leq 1$, $P \geq 0.37$). There was a trend for higher peak flowering in *Monarda fistulosa* in heterogeneous plots than homogeneous plots in 2012 ($t_{1,4} = 2.6$, $P = 0.08$). However, this association degraded in 2013 ($t_{1,4} = 0.95$, $P = 0.4$) and no other plants had significantly greater peak plants in flower between levels of heterogeneity. Using the index of reproductive flowering time, I again found no significant differences between heterogeneous and homogeneous soil types (2012: $t_{1,4} \leq 1.99$, $P \geq 0.12$; 2013: $t_{1,4} \leq 1.02$, $P \geq 0.37$). The patterns for peak flowering diversity (measured as the richness of species in flower) and cumulative flowering richness followed similar patterns as the flower traits. There were no significant differences in average weekly flowering richness among heterogeneous and homogeneous treatments in 2012 ($t_{1,4} = 0.21$, $P = 0.85$) or in 2013 ($t_{1,4} = -0.69$, $P = 0.53$). There was no difference in cumulative flowering richness between heterogeneous and homogeneous plots in 2012 ($t_{1,4} = 1.51$, $P = 0.21$). However by the third year of assembly, homogeneous plots had greater cumulative flowering richness than heterogeneous plots ($t_{1,4} = -2.25$, $P = 0.09$). Interestingly, this

increase in cumulative flower richness appears to be driven strongly by rare species (defined as species with only a single flowering plant across the entire site during peak production). In homogeneous plots, twelve of the thirty-four flowering species were rare (35%). Alternatively, only seven of the thirty flowering species in heterogeneous plots were rare species (23%).

Temporal Patterns in Flowering

Because there were no significant differences in flowering patterns by level of heterogeneity, I aggregated the data from homogeneous and heterogeneous treatments. I found that flowering duration followed a unimodal pattern in both years (Fig. 3.1). Species that began flowering early (April – May) and late flowering plants (August – September) flowered for similar periods ($t_{2, 39} = -1.64, P = 0.10$) and had shorter duration periods than species that began flowering in midsummer (June – July) (Fig 3.2, $t_{2, 39} \leq -3.33, P < 0.0001$). However, a species' maximum flowering plants (the peak number of plants in flower during any given week) was independent of the date of first flower (2012: $R^2 = 0.011$; 2013: $R^2 = 0.009$). Cumulative flowering time (index of flowering duration and flower production) was also independent of date of first flower. Patterns in flowering richness across the growing season revealed similar trends to flowering duration, where the greatest periods of richness occurred in midsummer. In contrast, flowering richness decreased and became more erratic for early and late season plants (Fig 3.3).

Discussion

Heterogeneity and Phenology

I found in chapter two that heterogeneous soils promote greater species richness than homogeneous soils during the first two years of community assembly. However, this increase in species richness did not translate into a significant increase in cumulative flowering richness for heterogeneous communities after two years. In contrast, homogeneous soils had a slight but non-significant ($p=0.09$) increase in cumulative flowering richness over heterogeneous soils in the third year of assembly. This increase in cumulative flower richness appears to be driven strongly by rare species. In homogeneous plots, twelve of the thirty-four flowering species were rare species (35%). Alternatively, only seven of the thirty flowering species in heterogeneous plots were rare species (23%). Without these rare species, homogeneous and heterogeneous soils accumulate near equal richness (22 and 23 species respectively).

As might be expected, total flowering richness increased between the second (28 species) and third (41 species) years of community assembly as more species reached reproductive maturity within the community. However, I found no effect of soil heterogeneity on the duration, peak weekly production of plants in flower or on the cumulative time spent in flower (see flowering index). *Monarda fistulosa*, a species shown to have a significant association with soils in heterogeneous plots had nearly significant peak production of weekly flowering plants in heterogeneous plots during the second year of assembly ($t_{1,4} = 2.6$, $P = 0.08$) but not in the third year ($t_{1,4} = 0.95$, $P = 0.4$). Suggesting that while heterogeneous soils did not increase the peak production of

plants in flower, they may have slightly reduced time required for some plants such of *Monarda fistulosa* to reach reproductive maturity (personal observation).

Temporal Patterns in Flowering

The maximum weekly flowering plants and cumulative flowering time were independent of date of first flower. However, flowering duration changes depending on when flowering occurs during the growing season (Fig 9, $F_{2,39} = 10.8$, $P = 0.0002$) and follows a unimodal pattern (Fig 3.1). Early-season and late-season plants typically flowered for less than four weeks while species mid-season plants frequently flowered upwards of six weeks and in some cases, flowered for as many as 16 weeks. This pattern fits with the intuitive expectation resource availability influences the length of time a plant can support reproductive organs. Species flowering early in the season may escape the stringent light and pollinator competition experienced later in the growing period, but this appears to come at the expense of longer flowering periods. Species flowering in the mid-season have accumulated resources for longer periods allowing them to flower for greater periods. Additionally, mid-season species may necessitate longer flowering periods to exploit the pollinator resource in an environment with greater interspecific competition due to the increased flowering richness of the midseason. Late-season plants have similar flowering durations to the early season plants and may represent species attempting to utilize an available resource window created after the mid-season plants have flowered and begun to senesce but before the close of the summer growing season.

Flowering richness across the growing season follows predictable patterns where richness is lower in the early season when only a few, early emerging species have the

resources to flower. As more species leave dormancy and accumulate resources, flowering richness increases and continues to follow a ramp-up pattern throughout the season. This pattern is apparent in year three of assembly (2013) but less so in year two (2012). Rather, richness peaks earlier then drops before another smaller peak in flowering richness. This pattern may be the result of the dryer conditions in 2012, decreasing the flowering duration and encouraging some mid-season or late season species to flower earlier as moisture levels continue to drop and temperature rises. However, the dryer conditions may emphasize three distinct windows of opportunity to flower. In limited resource conditions, species flowering may be constricted to one of these optimal opportunities to flower. Alternatively, years with greater resource availability may allow species to continue to accumulate resources for longer periods before flowering, increasing the duration of flower and overlap between flowering species contributing to the ramp-up pattern observed in year 3 (2013).

Our results do not demonstrate any significant influence of soil heterogeneity on the flowering patterns during early grassland assembly. However, I found differences in flowering composition between heterogeneous and homogeneous communities. Homogeneous and heterogeneous communities sustained comparable richness for species with abundances greater than one. Alternatively, homogeneous communities had greater richness in single instance or rare species than heterogeneous communities did. Furthermore, our findings demonstrate patterns in flowering pattern throughout the growing season for both flowering duration and flowering richness, where flowering duration was greatest for species beginning flower production in June and July, Flowering richness demonstrated a ramp-up effect in 2013 with more species

flowering consecutively as the season progresses. This pattern differs from the previous year (2012), where flowering richness has peaks and dips throughout the season. Further monitoring will be necessary to determine if heterogeneity influences flowering patterns later in community assembly and to determine if temporal patterns in flowering duration and flowering richness change with community stage.

Figures

Fig 3.1

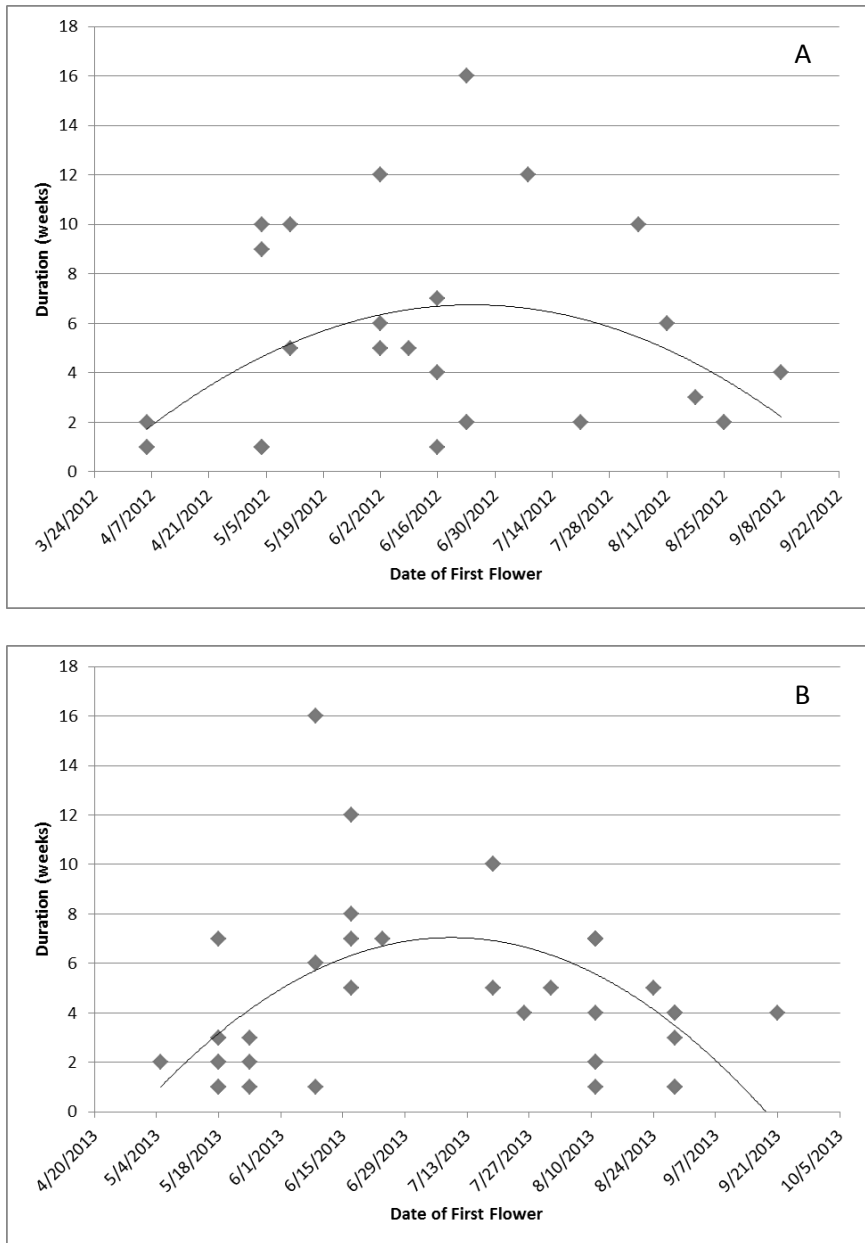


Fig 3.1. Flowering Duration across the Growing Season. Flowering duration by date of first flower during the second (A) and third (B) years of community assembly (2012, 2013 respectively). Flowering duration is typically highest for species flowering in June (2012: n = 28; 2013: n=41).

Fig 3.2

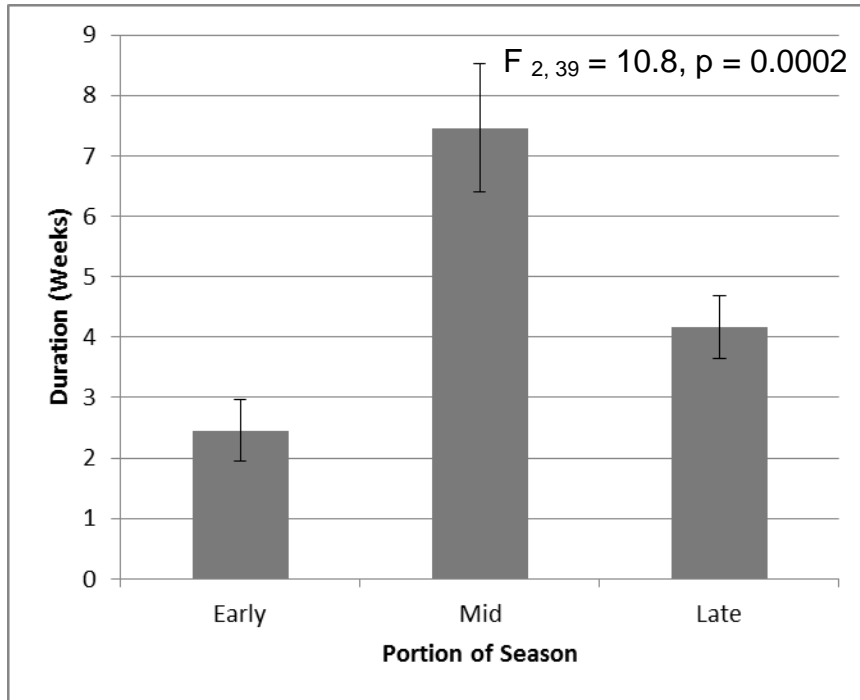


Fig 3.2. Differences in Flowering Duration by Period in Season. The average number of weeks in flower throughout the growing season, Early-season plants begin flowering in April and May. Mid-season plants begin flowering in June and July. Late season plants begin flowering in August and September.

Fig 3.3

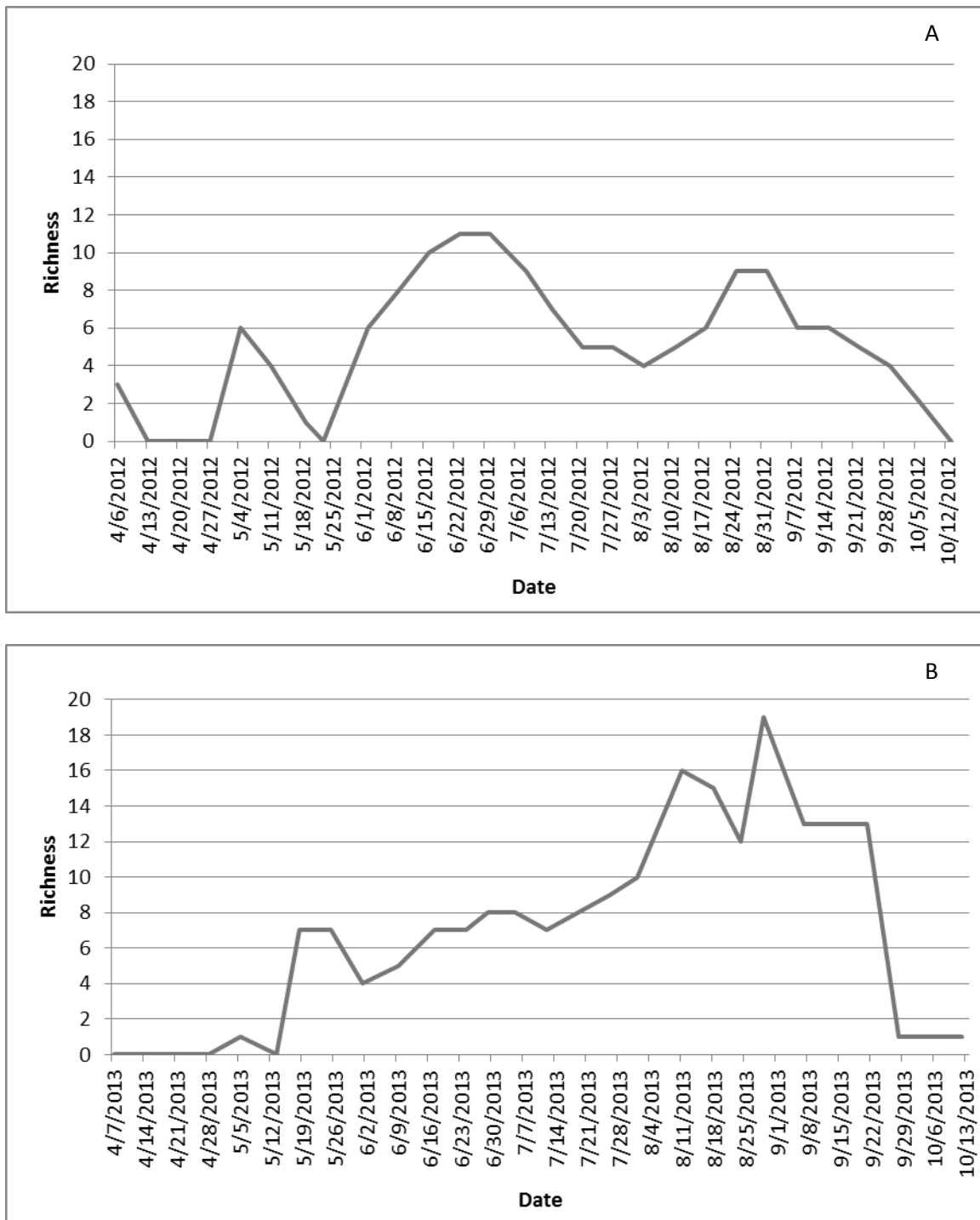


Fig 3.3. Flowering Richness across the Growing Season. Weekly flowering richness throughout the growing season in the second (A) and third (B) years of community assembly.

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