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Effects Of Plant Community Structure, Soil Components, And Aboveground Fungal Presence On Grassland Productivity

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EFFECTS OF PLANT COMMUNITY STRUCTURE, SOIL COMPONENTS, AND ABOVEGROUND FUNGAL PRESENCE ON GRASSLAND PRODUCTIVITY

by

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This dissertation, submitted by Thomas McKenna in partial fulfillment of the requirements for the degree of Doctor of Philosophy from the University of North Dakota, has been read by the Faculty Advisory Committee under whom the work has been done and is hereby approved.

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Thomas P. McKenna
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ABSTRACT

This dissertation investigates how aspects of the resident plant community affect grassland productivity. Chapter One gives an overview of how grassland productivity can be affected by the structural components of a plant community, abiotic and biotic soil components, and the presence of aboveground fungi. In Chapter Two, I present results of an experiment where the frequency of interspecific interactions in plant communities was altered along richness and evenness gradients by either randomly placing species in plots (dispersed plots) or by aggregating species in groups of four individuals (aggregated plots). Results suggest aggregation decreased productivity by promoting species coexistence and not by decreasing niche partitioning and facilitation. In Chapter Three, I compare two diversity effect modeling approaches (additive partitioning model and Diversity Interaction models) and show how using sown and realized proportions may alter outcomes and interpretations of diversity effects analyses. In Chapter Four, I describe a set of experiments to determine whether soil feedbacks affect grassland species monoculture yields. To determine the mechanism (abiotic or biotic), focal species were grown in soil cores from conspecifically conditioned plots that removed soil biota by two different heating treatments. Results reinforce the facilitative effect of legumes and suggest nutrient limitation may be more important than soil biota effects in the early years of grassland establishment. In Chapter Five, I evaluated the effects of aboveground fungal presence in *Pascopyrum*

smithii (western wheatgrass) and one of its' cultivars, Rodan wheatgrass. Results suggest fungal presence affects multiple above- and belowground responses. However, the lack of specificity of the fungal presence testing method created difficulty in interpreting the results. I recommend the use of multiple methods to determine specific fungal presence as to ensure the identity of treatments being applied in experiments.

CHAPTER I:
INTRODUCTION

Diverse grassland plant communities provide many ecosystem services (Hooper et al. 2005). Although the many benefits of diversity are known, much is to still to be learned about the underlying mechanisms that drive functions of interest. For more insight, researchers perform field and greenhouse experiments at the community, population, and species scales and monitor above- and belowground responses to these manipulations.

At the community level in the experimental grassland context, the number (richness) and abundance (evenness) of species are often altered at the plot-scale, and productivity (aboveground biomass production) is the functional response of interest (reviewed in Tilman et al. 2014). Species richness receives the most attention and tends to positively affect biomass production (Tilman *et al.* 2001, Balvanera *et al.* 2006, Isbell *et al.* 2009). Increasing evenness has been shown to also increase biomass production (Wilsey and Potvin 2000, Orwin *et al.* 2014), but the initial relative abundance of species may not be as influential as species richness over time (Schmitz *et al.* 2013). An interaction component often overlooked and confounded with richness and evenness is the arrangement of species (species pattern) in an area. As plot-scale richness and evenness change, or even if they are held constant, the frequency of less

than plot-scale intra- and interspecific interactions can be altered by the placement of species in a plot. Plant interactions occur over relatively small distances (Vogt *et al.*2010), so the outcome of facilitative or competitive interactions can hinge on whether the majority of an individual's adjacent neighbors are conspecifics or heterospecifics. Therefore neighborhood pattern may affect plot-scale responses. Experimental alteration of planting pattern is needed to investigate this response and would allow for a greater understanding of the spatial scales over which plant community structure affects productivity. In Chapter Two, results from a three year field study in which species pattern was altered along richness and evenness gradients are presented. This study was recently published in *Ecology and Evolution* (McKenna & Yurkonis 2016).

A variety of mathematical models are available for the examination of the mechanisms and patterns driving the diversity–productivity relationship (Hector et al. 2009). Two modeling approaches often used are the additive partitioning model (Loreau and Hector 2001) and Diversity-Interactions modeling (Kirwan et al. 2009). In both approaches either the sown proportion of individuals (experimental density at planting) or realized proportions (proportion of biomass contributed by each species in the previous growing season; sensu Finn et al. 2013) may be used as model inputs. Although either proportion may be used there has been little discussion on how the proportion used alters model outcomes and interpretation. If sown proportions are used, successive year diversity effects are calculated based on the number of individuals of each species (density) at planting. Because species abundances may change as

species establish and with changes in abiotic factors, this approach may not allow for the examination of the variation in species dynamics over time. One way to account for these variations is to use the contribution to biomass of each species from the end of the previous year (realized proportions; sensu Finn et al. 2013). However, when realized proportions are used, biomass production of species is substituted for the density of species. Some species may increase in their contribution to plot biomass by increasing in size and others may increase their contribution by increasing in the number of individuals (Marquard et al. 2009). This may lead to differences in model outcomes between sown and realized proportions, as variation in species biomass production may not be indicative of a change in species density. Another possible drawback to sown proportions is that different species planted in the same proportion are expected to contribute equally to total plot biomass (Hector 1998; Connolly et al. 2001), which is often not the case in experiments using a diverse species pool. This may lead to a bias in the calculation of diversity effects using sown proportions. Using realized proportions, the bias may be removed as biomass values are used to calculate diversity effects instead of the experimental sown density. To encourage more discussion about these drawbacks and inform ecologists interested in using these statistical tools, outcomes and interpretation of the additive partitioning model and Diversity-Interactions modeling with sown and realized proportions are compared in Chapter Three. All analyses are done with the dataset from Chapter Two.

Although much attention is paid to the responses of plant communities aboveground, species interactions with belowground biota may affect productivity

responses of interest. Recent studies have shown that negative soil feedbacks driven by soil biota can partly explain the relationship between plant community diversity and productivity (Schnitzer et al. 2011, Maron et al. 2011) and species-specific overyielding in mixtures (Hendriks et al. 2013). These effects can arise as soil pathogens may increase over time in monocultures and low diversity mixtures, causing decreased productivity (Kulmatiski et al. 2012). Planting species in mixtures may provide relief from these detriments, as pathogen loads may decline with decreasing host density (Schnitzer et al. 2011). This suggests complementarity effects may come about from different species utilizing segregated resource pools (differing root growth patterns) and from dilution of species-specific pathogens in mixtures (Kulmatiski et al. 2012; Van der Putten et al. 2013). The knowledge of species-specific feedbacks, along with insight into the feedback mechanism (biotic or abiotic), may lead to insights on the how feedbacks lead to community responses in biodiversity studies and lead to better predictions of plant performance (Maron et al. 2011; Schnitzer 2011; Hendriks et al. 2013). In Chapter Four, results from an in-field soil feedback study and a greenhouse study with soil biota removal to determine feedback effects are presented. Soils for this study were conditioned with the same species used in Chapter Two, and focal species were chosen based on monoculture performance in the experiment from Chapter Two. This was done in an attempt to better understand the role of soil feedbacks in results observed in the main biodiversity experiment.

Species-specific feedbacks and contributions to diversity effects may be altered in the presence of plant-specific symbionts (i.e., fungi or bacteria). For example,

endophytic fungi of the genus *Epichloe* live in the above-ground intercellular space of leaf sheaths, stems, inflorescences, and seeds of cool season grasses (Kuldau & Bacon 2008). In introduced forage grasses, presence of *Epichloe* has shown to decrease herbivory (Clay & Schardl 2002; Richmond et al. 2004), increase drought tolerance and growth (reviewed in Malinowski & Belensky 2000), and increase ability of the host to invade diverse communities (Rudgers et al. 2005). These advantages could lead to substantial changes in the components of community diversity. Endophytes may also affect the host grass by altering root morphology (Malinowski et al. 1999) and the quantity and quality of root exudates (Franzluebbers & Stuedemann 2005). These changes may be the mechanism that leads to endophyte presence negatively affecting herbivorous soil nematodes (Kimmons et al. 1990, West et al. 1988), creating differences in soil microbial communities (Rudgers & Orr 2009), and impacting carbon and nitrogen pools (Franzluebbers & Stuedemann 2005, Franzluebbers et al. 1999). The majority of endophyte studies have focused on only a few grass species (Saikkonen et al. 2006; Cheplick & Faeth 2010), so it is unclear what effects endophytes have in this region. Understanding the impacts of endophyte presence would allow researchers to determine whether such plant symbioses are necessary to take into account when assessing plant diversity-productivity relationships. In Chapter Five, a field experiment investigating above- and belowground fungal presence effects on native western wheatgrass (*Pascopyrum smithii*) and one of its' cultivars, Rodan, is presented.

By investigating the effects of species pattern, soil feedbacks, and aboveground fungal presence on above- and belowground responses, more insight will be gained on

the mechanisms of diversity effects. This knowledge could be used for improving restoration and reconstruction techniques and for creating communities to perform a desired function. In agricultural settings, this information may assist in creating diverse multifunctional communities that are productive, have lower levels of inputs, and are more resilient.

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CHAPTER II:
ACROSS SPECIES-POOL AGGREGATION ALTERS GRASSLAND PRODUCTIVITY AND DIVERSITY

Abstract

Plant performance is determined by the balance of intra- and interspecific neighbors within an individual's zone of influence. If individuals interact over smaller scales than the scales at which communities are measured, then altering neighborhood interactions may fundamentally affect community responses. These interactions can be altered by changing the number (species richness), abundances (species evenness), and positions (species pattern) of the resident plant species and we aimed to test whether aggregating species at planting would alter effects of species richness and evenness on biomass production at a common scale of observation in grasslands. We varied plant species richness (2, 4, or 8 species and monocultures), evenness (0.64, 0.8, or 1.0), and pattern (planted randomly or aggregated in groups of four individuals) within 1 × 1 m plots established with transplants from a pool of 16 tallgrass prairie species and assessed plot-scale biomass production and diversity over the first three growing seasons. As expected, more species rich plots produced more biomass by the end of the third growing season, an effect associated with a shift from selection to complementarity effects over time. Aggregating conspecifics at a 0.25 m scale

marginally reduced biomass production across all treatments and increased diversity in the most even plots, but did not alter biodiversity effects or richness-productivity relationships. Results support the hypothesis that fine-scale species aggregation affects diversity by promoting species co-existence in this system. However, results indicate that inherent changes in species neighborhood relationships along grassland diversity gradients may only minimally affect community (meter) - scale responses among similarly designed BEF studies. Given that species varied in their responses to local aggregation, it may be possible to use such species-specific results to spatially design larger-scale grassland communities to achieve desired diversity and productivity responses.

Introduction

Although there has been a long-standing interest in elucidating the mechanisms that contribute to grassland Biodiversity-Ecosystem Function (BEF) relationships, it is still relatively unclear to what extent plant community responses are sensitive to variation in plant neighborhood composition. Plants exist in spatially limited neighborhoods that are defined by the distances over which individuals interact. If the scales of interaction among neighbors are sufficiently smaller than the scales over which communities are measured, then changing neighborhood interactions may affect community productivity and overall plant species diversity – productivity relationships (Lamošová *et al.* 2010; Zhang *et al.* 2014).

The effect of altering neighborhood composition on community productivity depends on the competitive relationships among species in focal pool. When strong competitive differences occur among species in the pool, increasing the frequency of conspecific neighborships through aggregation may allow weaker competitors to persist as a result of delayed competitive exclusion (Stoll and Prati 2001; Monzeglio and Stoll 2005) and temporal priority effects (Porensky *et al.* 2012). This is well established theoretically (Chesson and Neuhauser 2002; Rácz *et al.* 2006) and at least experimentally within annual or newly establishing communities (Stoll and Prati 2001; Monzeglio and Stoll 2005; Porensky *et al.* 2012). In this case, if aggregation benefits less competitive and presumably less productive species, then aggregated communities would be less productive and more diverse than non-aggregated counterparts due to greater abundances of subordinate species.

Alternatively, when species benefit more from adjacency with heterospecifics than conspecifics, increasing the frequency of conspecific neighborships through aggregation may reduce the contribution of positive heterospecific effects to community scale responses (Naeem *et al.* 1999; Mokany *et al.* 2008). This advantage toward heterospecific neighbors can arise as a result of interspecific niche partitioning and facilitative interactions (complementarity effects) which have been shown to be increasingly important in driving community biomass production over time (Fargione *et al.* 2007; Cardinale *et al.* 2007). In this case, if aggregation reduces beneficial heterospecific interactions, then aggregated communities would be less productive and

less diverse than non-aggregated counterparts because of a decrease in complementary interactions and a greater disparity in species abundances.

Within experimental settings, conspecific aggregation appears to affect species coexistence during grassland establishment (Porensky *et al.* 2012; Yurkonis and McKenna 2014; Orwin *et al.* 2014; Seahra *et al.* 2015), but it is unclear whether these effects are ubiquitous and persistent within increasingly diverse communities. To date, aggregation studies have mostly assessed aggregation effects at sub-meter scales over a single growing season (Monzeglio and Stoll 2005; Mokany *et al.* 2008; Orwin *et al.* 2014; Yurkonis and McKenna 2014). This limits our ability to assess how changing neighborhood relationships affect grasslands at common scales of observation (but see Yurkonis *et al*. 2012; Seahra *et al.* 2015) and prevents us from elucidating how species aggregation affects the development of grassland complementarity effects which typically arise after several growing seasons (Cardinale *et al.* 2007). Lamošová *et al.* 2010 and Zhang *et al*. 2014 are the only studies to date that have asked whether aggregation alters grassland diversity-productivity relationships. In both cases, conspecific aggregation reduced development of complementarity effects in the most species-rich communities over a single season, but it is unclear whether these effects would persist within diverse, perennial grasslands.

Our goal was to ascertain how changes in neighborhood interspecific relationships affect diversity and productivity responses along perennial grassland richness and evenness gradients. We increased intraspecific interactions along richness

and evenness gradients to determine how sub-meter neighborhood composition affects meter-scale biodiversity-ecosystem function relationships within a three- year manipulative field experiment with an extensive species pool. We test the hypotheses that conspecific aggregation reduces community biomass production either by promoting species coexistence and, thus, increasing diversity or by reducing niche partitioning and facilitative interactions (complementarity) and, thus, decreasing diversity. Findings help to elucidate the effect of changing neighborhood relationships on biomass production responses in BEF studies.

Materials and Methods

Experimental design

The Species Pattern and Community Ecology (SPaCE) experiment consists of plots arranged in a randomized complete block design with 5 blocks established at the University of North Dakota's Mekinock Field Station (Lat 47.9620/Long -97.4517) in May 2012. Greenhouse grown transplants (16 weeks old) were planted into 1 × 1 m plots (2 m spacing) divided into an 8 × 8 grid (64 individuals $plot^{-1}$). Plots varied in richness (2, 4, 8 species, and monocultures), evenness (0.64, 0.8, and 1), and species pattern (random or aggregated) (3 levels richness × 3 levels evenness × 2 levels pattern = 18 mixtures; (18 mixtures + 16 monocultures) × 5 blocks = 170 plots). Abundances at low, intermediate and high evenness within each richness level were: 8:56, 16:48, and 32:32 in two species plots, 4:4:28:28, 8:8:24:24, and 16 individuals per species in four species plots, 4:4:4:4:4:8:16:20, 4:4:4:4:12:12:12:12, and 8 individuals per species in eight species

plots. Species were randomly allocated to the low, medium and high abundances within evenness treatments. The pattern treatment was applied at the plot level, and each species was assigned independently (dispersed) to planting positions or to a group of four adjacent planting positions (aggregated) a plot. The site was in continuous agriculture for the previous 15 years, and a cultivator was used to remove weed seedlings prior to planting into bare soil. Soils at the site are moderately well drained LaDelle silt loam with 0 to 2 % slopes. Transplants were watered as needed for two weeks to aid in plot establishment. Misplants and dead individuals were replaced during this two week establishment period. Plot species composition was maintained with monthly weeding during the growing season, and aisles were mowed as needed.

The species composition of each plot was determined by randomly selecting species from a pool of 16 common tallgrass prairie species with four representatives from each functional group (warm and cool season grasses, forbs, and legumes). Species functional diversity was constrained as follows: two species plots contained a grass (warm or cool season) and a legume or a forb, four species plots contained one species from each functional group, and eight species plots contained two species from each functional group. The cool-season grasses: *Pascopyrum smithii* (PS; western wheatgrass), *Elymus canadensis* (EC; Canada wildrye), *Elymus trachycaulus* (ET; slender wheatgrass), and *Nassella viridula* (NV; green needle grass), the warm-season grasses: *Andropogon gerardii* (AG; big bluestem), *Panicum virgatum* (PV; switchgrass), *Schizachyrium scoparium* (SS; little bluestem), and *Sorghastrum nutans* (SN; Indian grass), the forbs: *Helianthus maximiliani* (HM; maximilian sunflower), *Monarda fistulosa*

(MF; wild bergamot), *Ratibida columnifera* (RC; yellow coneflower), and *Solidago rigida* (SR; stiff goldenrod), the legumes: *Desmodium canadense* (DC: showy tick trefoil), *Astragalus Canadensis* (AC; Canada milkvetch), *Dalea purpurea* (DP; purple prairie clover), and *Glycyrrhiza lepidota* (GL; American licorice) were used in this experiment (seed obtained from Prairie Restorations Inc., Princeton, MN). Seed was stored at -20^{o} C and legume seeds were mixed with genus specific inoculant (Prairie Moon Nursery, Winona, MN) prior to seeding in the greenhouse.

Data collection

During the first three growing seasons (May – August) soil surface light and soil moisture was recorded every two weeks. Above and below canopy photosynthetic active radiation (PAR) was recorded (AccuPAR LP-80, Decagon Devices, Inc.; Pullman, WA, USA) between 10 am and 3 pm (daylight savings time) on cloudless days and used to calculate the proportion of available PAR reaching the soil surface. Percent volumetric soil moisture measurements (Scout TDR 100 with 20 cm probes; Spectrum Technologies, Inc.; Aurora, IL, USA) 0.25 m were made inside the north and south plot edges and averaged for each plot.

At the end of each growing season (September), aboveground biomass was cut to 5 cm above the soil surface, sorted to species, dried to a constant mass (60 ^{o}C), and weighed. Plot Simpson's diversity (*D*) was calculated as $D = \frac{1}{\sum_{i=1}^{S} {p_i}^2}$, where *S* is the number of species in the community and p_i is the proportional biomass of species *i*. Selection and complementarity effects were calculated using the additive-partitioning

model of Loreau and Hector 2001 based on species mixture and monoculture biomass production. This was based on species proportions by individuals (number of individuals of species *i*/ 64 individuals) at planting for year one and previous year proportion of plot biomass for each species in years two and three. Species proportions and species richness were adjusted (7 % of plots) for persisting misplants not corrected in the establishment period. If species not in the assigned species pool was planted (6 % of plots), the individual was removed, and the total biomass for the plot was adjusted by adding the appropriate number of average individual weights for that species.

Plot interspecific interactions were quantified as the summed proportion of all possible neighborships that occurred among heterospecific neighbors. The program QRULE (Gardner and Urban 2007) was used to calculate species proportional neighborships for each initial planting map based on the closest neighbors for each individual (four neighbor rule with no diagonals). The proportion of heterospecific neighborships increased with increasing species richness (Fig.2.1A) and species evenness (Fig. 2.1B), and in both instances dispersed plots had greater heterospecific association than aggregated plots.

Data analysis

Species richness, evenness, and pattern effects on biomass production, selection, and complementarity were compared across the three growing seasons with Repeated Measures ANOVA (proc mixed; SAS v9.3, Cary, NC) with fixed block effects. Soil surface light and volumetric soil moisture were similarly compared across sample

dates within each growing season. Plot biomass, Simpson's Diversity, and soil moisture were natural log transformed, percent PAR was arcsine square root transformed, and selection and complementarity were square root transformed with the original sign maintained (Loreau and Hector 2001) to meet assumptions. Significant ANOVA tests were followed by least significant difference (LSD) tests to identify differences among treatment groups.

Species-specific performance in dispersed and aggregated plots was quantified by calculating per individual performance. This was calculated for each year by dividing the biomass of each species in a plot by the number of individuals originally planted of that species. Because of unequal and low sample size an Exact Wilcoxon two-sample test (npar1way; SAS v9.3) was used to compare species differences between dispersed and aggregated plots within each year.

Results

Biomass production varied among species and over time (Fig. 2.2). The forbs *H. maximiliani* and *S. rigida* consistently produced the most aboveground biomass when grown solely in the presence of conspecifics (monoculture) while legumes produced the least (Fig. 2.2). Warm and cool season grasses were intermediate in their monoculture biomass production. In the presence of heterospecific effects (mixture), *M*. *fistulosa* and *R. columnifera* were the only species that consistently produced more biomass than would have been expected based on their monoculture performance (deviation in mixture; Fig. 2.2). Additionally, there was a temporal shift in the type of species that

performed well in the presence of heterospecific effects. Species that produced less biomass in the presence of conspecifics (low monoculture yields) shifted from producing less biomass than expected to producing more biomass than expected in the presence of heterospecific effects (year one vs. year three; Fig. 2.2). In particular, all of the grasses overyielded in mixture relative to monoculture by the third year.

As is common in BEF experiments, community-scale biomass production was most strongly affected by the richness manipulation and was variable among years (Table 2.1; Fig. 2.3). Differences in biomass production among richness treatments were driven by selection effects. Selection effects initially increased with species richness (Fig. 2.4A). In years two and three, selection effects were negative and decreased with species richness. Selection also marginally differed between the most even (LS transformed mean ± SE = -1.12 ± 0.95) and intermediate (-4.20 ± 0.95) evenness plots. Complementarity effects developed within the four and eight species plots by the end of the third growing season (Fig. 4B), but these outcomes were not affected by evenness treatments.

Species aggregation marginally affected biomass production and species diversity responses. Aggregated plots produced marginally less biomass than dispersed plots across richness and evenness treatments (Fig 2.5A). Additionally, aggregated plots were more diverse in the most even treatment (Fig. 2.5B). Aggregation did not affect community-scale selection or complementarity effects.

Species varied in their responses to the aggregation treatment within mixed communities. In all three years, the per individual yield of *D. purpurea* ($p < 0.05$ for all three years) and *S. scoparium* ($p < 0.10$ for all three years) was greater in aggregated plots than dispersed plots (Fig. 2.6). *S. rigida* yielded marginally more per individual in aggregated plots than in dispersed plots in the first and second year ($p < 0.10$ in both years). *E. canadensis* yielded less per individual in aggregated plots than dispersed plots in the second year ($p = 0.0248$), and *E. trachycaulus* yielded more per individual in aggregated plots than in dispersed plots in the third year ($p = 0.0399$; Fig. 2.6).

Aggregation affected some measures of community-scale resource use. Aggregated two species plots (0.80 ± 0.02 %) intercepted less light than two species dispersed plots (0.74 ± 0.02 %) in the first growing season (Richness × Pattern: $F_{2,65.6} = 3.20$, $p = 0.0474$). In early June of the third growing season, aggregated intermediate evenness plots intercepted marginally less light than dispersed counterparts, but this effect disappeared thereafter (Date × Evenness × Pattern: $F_{8,96.7} = 2.29$, $p = 0.0271$). Additionally, aggregated plots (LS transformed mean ± SE = 3.98 ± 0.021 %) were marginally drier than dispersed plots (4.04 ± 0.021 %; Pattern: $F_{1,65.4} = 3.76$, $p = 0.0567$) in the third growing season.

There were also treatment effects on resource use that were not affected by the pattern manipulation. In year one, early season effects of evenness in two species plots declined over the season and evenness effects developed over the season in four species plots (Date × Richness × Evenness: $F_{16,131} = 2.17$, $p = 0.0089$). In early July of the

second season there was an effect of evenness on soil moisture along the richness gradient (Date × Richness × Evenness: $F_{20,142} = 2.08$, $p = 0.0072$). In the third growing season, four and eight species plots intercepted more light than two species plots (Richness: $F_{2,63.2} = 6.73$, $p = 0.0022$).

Discussion

We tested whether or not neighborhood conspecific aggregation affected community (meter)-scale biomass production and diversity along richness and evenness gradients within a tallgrass prairie experimental system. As with previous studies, conspecific aggregation decreased (marginally) biomass production (Lamošová *et al.* 2010; Zhang *et al.* 2014) along richness and evenness gradients and increased diversity (Houseman 2013; Yurkonis and McKenna 2014) within the most even plots. While complementarity increased over time, these effects were not affected by aggregation at the 0.25 m scale. Findings indicate that fine-scale species aggregation decreased productivity by promoting species coexistence rather than by decreasing complementarity effects. Although sub-meter aggregation may contribute to initial diversity maintenance in this system, species aggregation and inherent changes in species spatial relationships along diversity gradients are not likely to substantially affect productivity and diversity responses in this setting.

Species varied in their responses to aggregation and more investigation is needed into the scales over which individual species interact and whether aggregation can be manipulated on a species-by-species basis to alter grassland productivity and

diversity. Conspecific aggregation benefitted a select group of species and did not limit growth of the most productive species in this system. The only species hindered by aggregation was the most productive cool-season grass in this study (*E. canadensis*), but this effect was limited to the second growing season. In contrast, four of the 16 species showed some evidence of improved yields with aggregation. This group included more and less productive species within each functional group. Most notably, *S. scoparium* and *D. purpurea* performed better in aggregated plots in all three years. For these species, aggregation may improve yields due to temporal dynamics that alter species access to light and spatial resources. For *S. rigida* and *E. trachycaulus*, the benefits of aggregation were temporally variable, which indicates that the outcomes of these species interactions with neighboring individuals are potentially context dependent.

After three years, there was no strong evidence that fine-scale species pattern affected the development of grassland complementarity effects. Although the frequency of intra-and interspecific interactions was substantially altered with our treatments, our 0.25 m aggregation treatment was not sufficient enough to reduce community (meter)-scale measures of niche partitioning and facilitation. This outcome likely arose as a majority of the species were interacting with neighboring individuals on scales larger than 0.25 m. Although we were unable to effectively isolate these species from heterospecific effects in mixtures, these species may need manipulations on larger scales (>0.25 m) to affect their yields and interactions with other species (Seahra *et al.* 2015).

Given that a majority of the species in the pool were not affected by sub-meter patterning and that there was little effect on species heterospecific interactions, it appears that species pattern changes along diversity gradients may only minimally affect diversity and productivity outcomes in similarly designed BEF studies. However, it may still be possible to affect diversity, productivity, and related community-scale responses by manipulating neighborhood composition on a species basis and with attention to species neighborships (e.g. positioning of legumes relative to grasses). Additionally, such aggregation may alter other functions such as invasion (Yurkonis *et al.* 2012), root biomass production (Orwin *et al.* 2014), soil microbe community structure (Massaccesi *et al.* 2015), or insect interactions (Parachnowitsch *et al.* 2014) due to changes in the patterning and overall resource use. Future studies need to consider to what extent fine-scale species pattern affects these processes and functions and to what extent our species-specific results could be used to design spatially structured communities (e.g. in grassland reconstruction settings or within larger experimental plots) to achieve desired grassland diversity and productivity goals.

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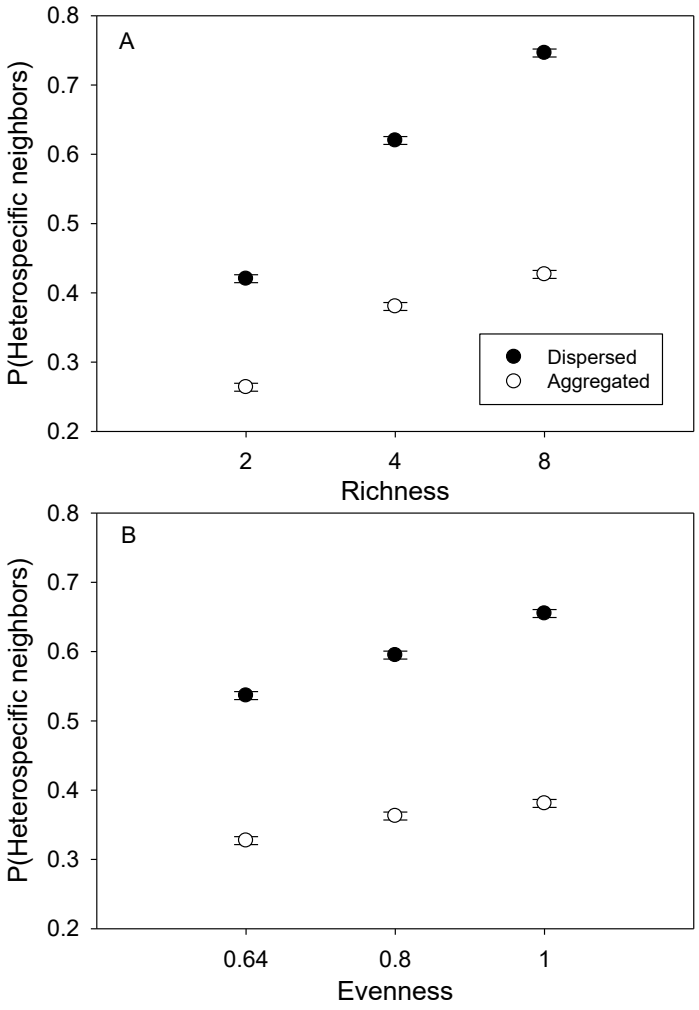

Figure 2.1. The proportion of all possible neighborships that occurred among heterospecific neighbors (mean ± SE) initially varied across plots planted at differing species richness (A) and evenness (B) levels. Plots were planted with individuals randomly assigned to 64 planting positions (dispersed) or in groups of four conspecific individuals (aggregated).

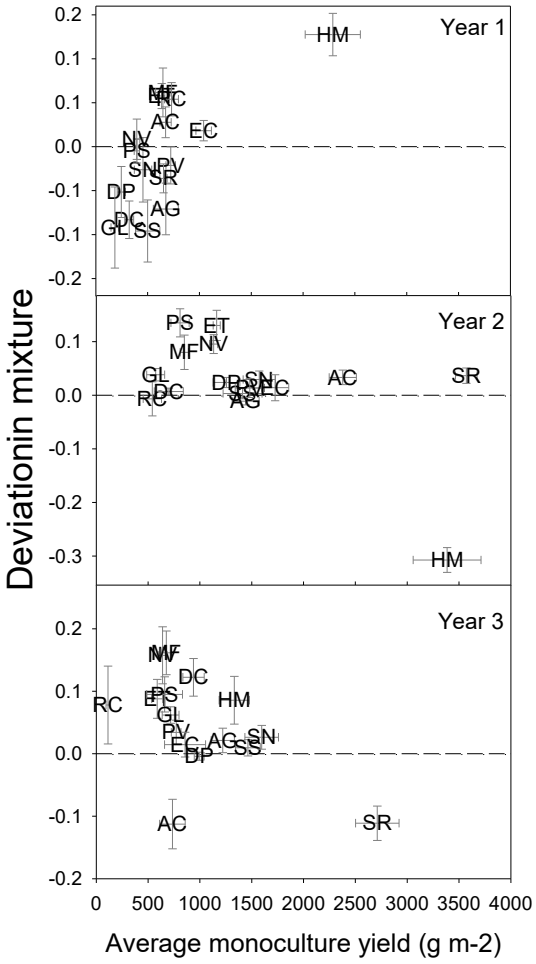

Figure 2.2. Difference in species relative yields ΔRY = $RY_{Observed}$ – $RY_{Expected}$) across all treatments (mean ± SE) and in relation to their monoculture yields (mean ± SE) in each growing season. Species labeled with the first letter of their genus and specific epithet: *Pascopyrum smithii* (PS), *Elymus canadensis* (EC), *Elymus trachycaulus* (ET), *Nassella viridula* (NV), *Andropogon gerardii* (AG), *Panicum virgatum* (PV), *Schizachyrium scoparium* (SS), *Sorghastrum nutans* (SN), *Helianthus maximiliani* (HM), *Monarda fistulosa* (MF), *Ratibida columnifera* (RC), and *Solidago rigida* (SR), *Desmodium canadense* (DC), *Astragalus Canadensis* (AC), *Dalea purpurea* (DP), *Glycyrrhiza lepidota* (GL).

Table 2.1. Results from Repeated Measures ANOVA of planted richness, evenness, and pattern effects on biomass production, biodiversity effects, and Simpson's Diversity over three growing seasons. Values are F- statistics and degrees of freedom (df).

	Biomass		Selection		Complementarity		Simpson's Diversity	
Effect	df	F	df	F	df	F	df	F
Block	4,68	0.55	4,68	0.97	4,68	3.84**	4,68	1.73
Richness (R)	2,67.9	3.15*	2,68	2.09	2,65.6	0.75	2,68.2	127.29**
Evenness (E)	2,67.9	0.65	2,68	2.91†	2,65.6	1.16	2,68.2	0.25
Pattern (P)	1,67.9	3.79†	1,68	0.00	1,65.6	1.32	1,68.2	2.69
R × E	4,67.9	0.28	4,68	0.56	4,65.6	1.52	4,68.2	2.04†
R × P	2,67.9	1.70	2,68	0.40	2,65.6	0.16	2,68.2	0.18
E × P	2,67.9	2.30	2,68	0.30	2,65.6	0.94	2,68.2	5.60**
R × E × P	4,67.9	0.85	4,68	0.31	4,65.6	1.13	4,68.2	2.34†
Year (Y)	2,71	363.47**	2,71	78.64**	2,71	17.43**	2,71	1.99
Y × R	4,84.4	1.67	4,84.4	9.10**	4,84.4	3.70**	4,84.4	1.37
Y × E	4,48.4	1.41	4,84.4	1.39	4,84.4	0.92	4,84.4	1.89
Y × P	2,71	0.07	2,71	1.33	2,71	0.60	2,71	2.92†
Y × R × E	8,98.5	3.04**	8,98.5	0.93	8,98.5	1.08	8,98.5	0.93
Y × R × P	4,84.4	0.76	4,84.4	0.89	4,48.4	1.25	4,84.4	0.81
Y × E × P	4,84.4	1.52	4,84.4	1.16	4,48.4	0.33	4,84.4	0.90
Y × R × E × P	8,98.5	1.16	8,98.5	0.66	8,98.5	1.53	8,98.5	1.60

(* = $p < 0.05$, ** = $p < 0.01$, † = $p < 0.1$)

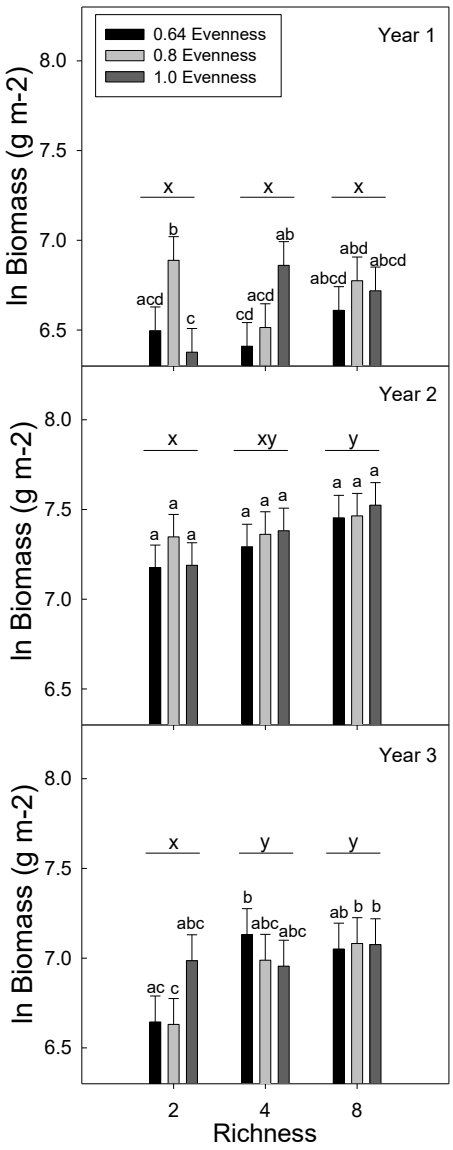

Figure 2.3. Effects of richness and evenness on aboveground biomass production (LS ln transformed mean ± SE) for each year of the experiment. Different letters above the lines (x and y) indicate differences among richness levels within year, and different letters above the bars (a-d) indicate differences within year among richness and evenness levels (LSD test).

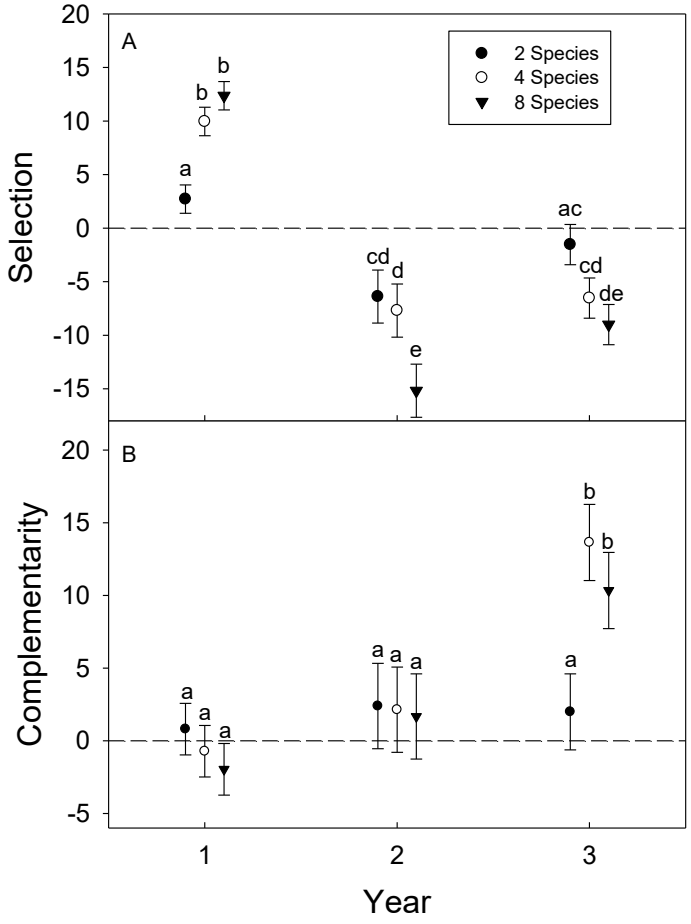

Figure 2.4. Selection effects (A) and complementarity effects (B) at each richness level for the first three growing seasons (LS square root transformed mean ± SE). Within each graph different letters indicate a significant difference (LSD test).

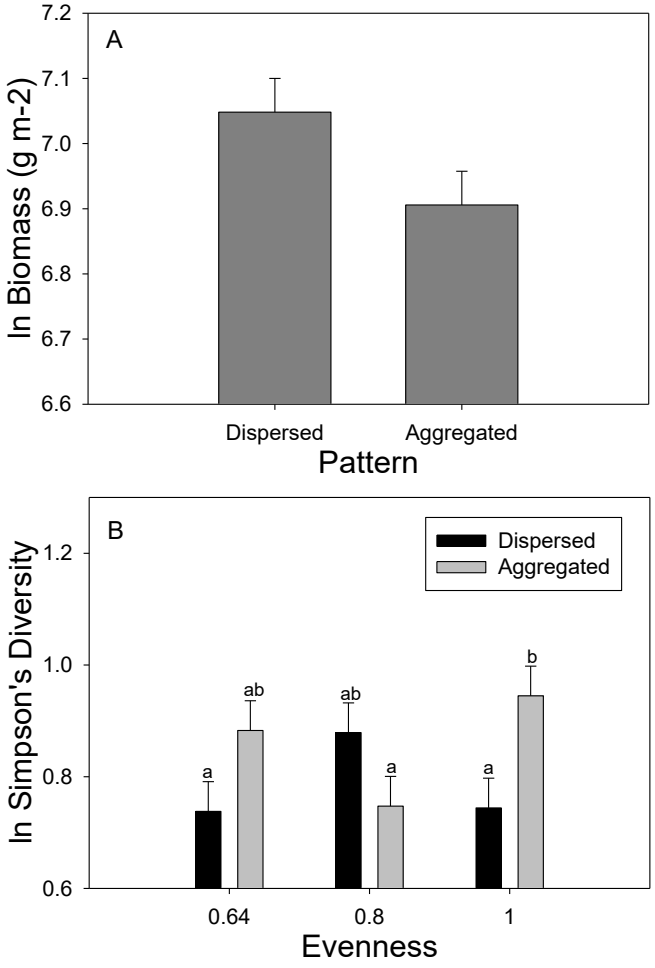

Figure 2.5. Effects of planted species pattern on aboveground biomass production (A; LS ln transformed mean ± SE) and effects of species pattern at each evenness level on Simpson's Diversity (B; LS ln transformed mean ± SE). Bars with different letters are significantly different (LSD test).

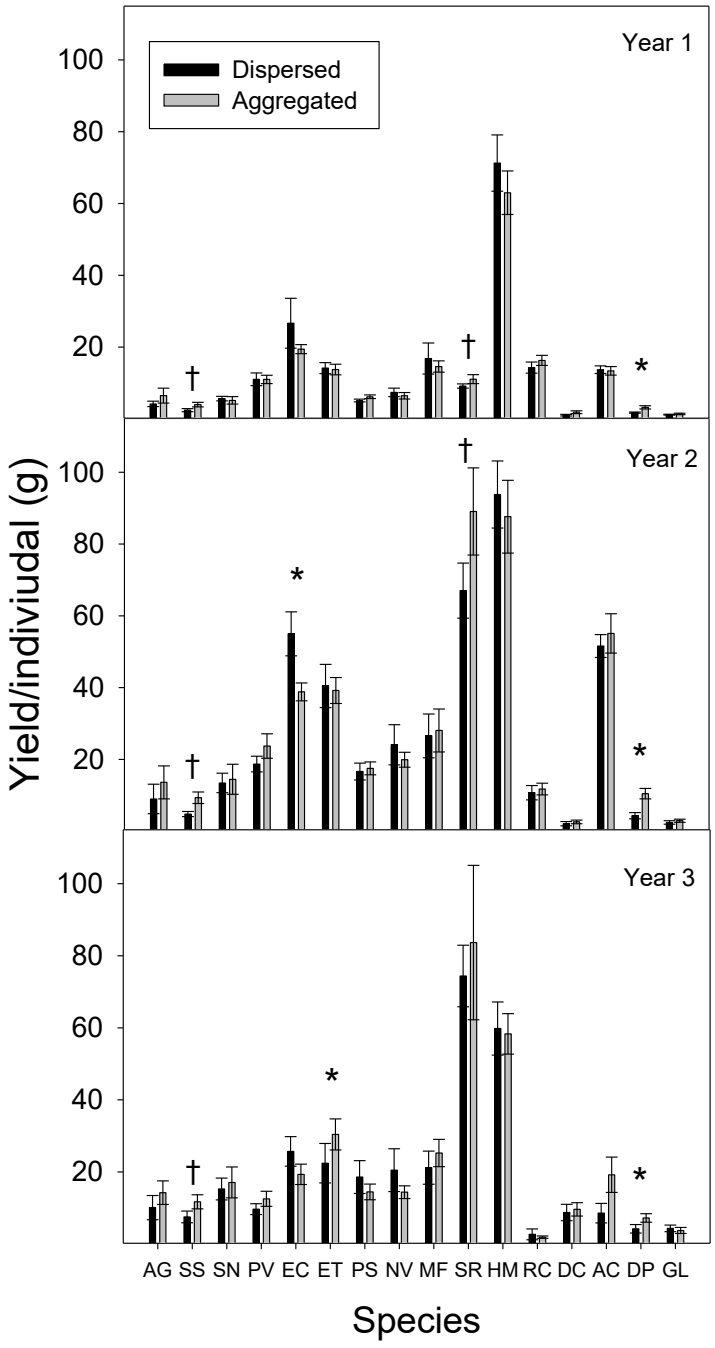

Figure 2.6. Yield per individual (mean ± SE) for each species in dispersed and aggregated plots for all three years of the experiment. An exact Wilcoxon two-sample test was used to compare yields within species and year between pattern treatments (* = $p < 0.05$, † = $p < 0.10$).

CHAPTER III:
A COMPARISON OF SOWN AND REALIZED PROPORTIONS IN THE CALCULATION OF DIVERSITY EFFECTS

Abstract

Researchers use a variety of models to better understand the mechanisms and patterns driving diversity-productivity relationships. For the additive partitioning model and Diversity-Interactions modeling, the sown (initial) proportions of plant species or the proportion each species contributes to plot biomass in the previous year (realized proportions) may be used as inputs for the calculation of diversity effects. Using sown proportions places emphasis on the experimental densities of each species, whereas using realized proportions tells us whether diversity benefits communities in response to changes in species contributions to plot biomass over time. To better understand the outcomes associated with these approaches in a grassland system, we compared results from using each proportion in the additive partitioning model and Diversity-Interactions modeling. We used three years of data from a long term field experiment at the University of North Dakota. Plots (1 x 1 m) were planted from a pool of 16 native grassland species and varied in species richness (monocultures, 2, 4, and 8), Simpson's evenness (low, medium, and high), and species pattern (planted randomly or aggregated in groups of 4 conspecifics). Plots were weeded monthly, and at the end of each growing season aboveground biomass was clipped, dried, and weighed. Using

different proportions in the additive partitioning model altered the magnitude and direction of selection effects and the relationship between selection effects and treatments. In the Diversity-Interactions models, diversity effects on productivity were present in years two and three in the sown proportions models, but diversity effects were absent in the realized proportion models. Results indicate that the variation in density-biomass production relationships among study species may contribute to the differences in model outcomes. In similar experiments with a diverse species pool, realized proportions should be used in the additive partitioning model to more adequately capture species expected values as the calculated diversity effects are based on species biomass production and not initial abundance. Using Diversity-Interactions modeling, variation in the relationship between productivity and density among species in the pool make it difficult to relate outcomes of sown and realized models. To advance our understanding of this modeling approach we need to determine how species density-productivity relationships relate to the potential interactions within a plot.

Introduction

The positive effect of diversity on productivity in grassland plant communities is supported by studies that manipulate the components of community diversity (Cardinale et al. 2006). A variety of mathematical models and statistical approaches (as reviewed in Hector et al. 2009) have been developed to gain insight on the mechanisms and patterns driving these relationships. Although these models can provide good

information about components driving functioning within a system, interpretation of results independent of model inputs (e.g. using the sown or initial proportion of each species versus using species proportions as calculated from their previous year biomass) may lead to different conclusions.

The additive partitioning model of Loreau and Hector (2001) separates the net diversity effect into selection and complementarity effects. Selection effects are related to the physical characteristics of the species present, and complementarity effects are related to the interspecific interactions (facilitation and niche partitioning). This model requires that all species used in mixtures must also be grown in monocultures, and biomass harvested from mixture plots must be separated to species in order to calculate relative yields. The difference in the relative yield observed (yield in mixture/average monoculture yield) and the relative yield expected (performance in monoculture adjusted for planted proportion) are used to calculate selection and complementarity. In single season experiments, the sown (seeded or planted) proportion of each species is often used to quantify species expected contribution to biomass production. In experiments that span several growing seasons species expected contributions can also be quantified by the proportion of total plot biomass for each species at the end of the previous season (realized proportions sensu Finn et al. 2013).

Other modeling approaches, such as Diversity-Interactions models (Kirwan et. al 2007; Kirwan et al. 2009), do not require the separation of species at harvest. Diversity-Interactions models use linear methods to fit models describing the relationship

between the relative abundance of species in a community and an ecosystem response. Species identity effects can be separated from species interaction effects (diversity effects), and biological hypotheses can be tested by comparing models that vary in the way interspecific interactions are assumed. As with the additive partitioning approach, when analyzing productivity, initial proportions of species planted or realized proportions can be used as the relative abundance of species in multiple-year experiments.

The decision of what proportions are used in these models will change the reference point for comparison. If the sown proportions are used, the results from each successive year are relative to the allotted density of each species at the beginning of the experiment. With this approach variation in species dynamics from previous years are included in the calculations of diversity effects (Finn et al. 2013). This variation could be substantial if species have delayed establishment or are involved in soil feedbacks. By using realized proportions, the within year variation in species contributions to plot biomass arising from interactions and abiotic changes may be assessed (Finn et al. 2013).

Also, if the sown proportions are not adjusted for the physical characteristics of the species planted, the proportions and expected relative yields may not be representative of species potential contributions to biomass production (Huston 1997; Connolly et al. 2001; Kirwan et al. 2009). All species are expected to contribute equally, but a very tall species will contribute more biomass than a small species at a given

proportion planted (Hector 1998). Adjustments to the sown proportions may be necessary to account for species differences, especially when using a diverse species pool (Polley et al. 2003). Differences have been accounted for by including a specific treatment structure in the experimental design (Roscher et al. 2004) or by creating an index (Grace et al. 1992; Connolly et al. 2001), yet adjustments are not often implemented. Using realized proportions, adjustments are incorporated into the calculations in successive years because expected values are based on biomass production in previous years rather than initial sown density.

Studies have compared the modeling approaches described above (Fibich et al. 2015) and have outlined when to use each model (Hector et al. 2009; Fibich et al. 2015), but there has been little discussion (Finn et al. 2013) on how interpretation of model outcomes and interpretations change using sown and realized proportions. The objectives of this study were to compare outcomes of the additive partitioning model and Diversity-Interactions models using sown and realized proportions. The two model approaches with varying proportions were applied to data collected from a three year in-field biodiversity experiment at the University of North Dakota. Results demonstrate the sensitivity of the modeling approaches to species expected values when using a diverse species pool, and the importance of clarifying effects within the interpretation of biodiversity outcomes.

Methods

Experimental Design

The data used for this analysis were collected from the Species Pattern and Community Ecology (S.P.a.C.E.) field experiment at the University of North Dakota over the first three field seasons (2012, 2013, and 2014). The experiment consists of grassland plots (1 x 1 m; 2 m spacing) arranged in a randomized complete block design with 5 blocks. Transplants were planted into plots (June 2012) that were divided into an 8 x 8 grid (64 individuals per plot) and varied in richness (2, 4, 8 species and monocultures), Simpson's evenness (low, medium, high), and species pattern (random or aggregated) (3 levels richness x 3 levels evenness x 2 levels pattern = 18 mixtures + 16 monocultures = 34 plots * 5 blocks = 170 plots). The pattern treatment was applied at the plot level in mixtures, so every species was assigned to a random position (dispersed) in the 64 squares of the plot or was clumped into groups of four (aggregated). For further details of experimental design see McKenna and Yurkonis (2016).

The species composition of each plot was determined by selecting species from a pool of 16 common prairie species (4 species from each functional group). The cool-season grasses: *Pascopyrum smithii* (western wheatgrass), *Elymus canadensis* (Canada wildrye), *Elymus trachycaulu*s (slender wheatgrass), and *Nassella viridula* (green needle grass), the warm-season grasses: *Andropogon gerardii* (big bluestem), *Panicum virgatum* (switchgrass), *Schizachyrium scoparium* (little bluestem), and *Sorghastrum nutans*

(Indian grass), the forbs: *Helianthus maximiliani* (maximilian sunflower), *Monarda fistulosa* (wild bergamot), *Ratibida columnifera* (yellow coneflower), and *Solidago rigida* (stiff goldenrod), the legumes: *Desmodium canadense* (showy tick trefoil), *Astragulus canadensis* (Canada milkvetch), *Dalea purpurea* (purple prairie clover), and *Glycyrrhiza lepidota* (American licorice) were used in this experiment. Transplants of each species were grown separately in the UND greenhouse for 16 weeks prior to planting. Species were randomly selected for each plot with the following constraints: in two species plots, one species was grass (warm or cool season) and the other was either a legume or a forb, in four species plots, one species from each functional group was selected, and in 8 species plots, two species from each functional group were selected.

Plots were weeded of non-focal plants and aisles were mowed as needed to avoid competition with planted species. At the end of each growing season (September), aboveground biomass was cut to 5 cm above the soil surface, sorted to species, dried to a constant mass (60 ^{o}C), and weighed.

Additive partitioning model

Selection and complementarity effects were calculated using the additive-partitioning model of Loreau and Hector 2001. The sum of selection and complementarity effect equals the net biodiversity effect (ΔY = observed yield – expected yield):

ΔY = Selection effect + Complementarity effect

Selection effects are based on the covariance of species performance in mixture and monoculture:

Selection effect = $N\text{cov}(\Delta RY, M)$

where N is the number of species in mixture and M is the yield of species in monoculture. ΔRY is the difference in the relative yield observed (RY_O) and relative yield expected (RY_E) of a species. RY_O is the biomass production of a species observed in mixture divided by that species monoculture yield. RY_E is either the sown or realized proportion multiplied by that species monoculture yield. Positive selection occurs when a species that does well in monoculture also performs well in mixture. Negative selection can occur when species that have low monoculture yields do well in mixture or when species with high monoculture yields perform poorly in mixture.

Complementarity is calculated using the average deviation in relative yield ($\overline{\Delta RY}$) and the average monoculture yield of all species in that mixture ($\overline{M}$):

Complementarity effect = $N(\overline{\Delta RY})(\overline{M})$

Positive complementarity effects suggest that species are performing better in mixture than monoculture because of niche partitioning and facilitation, and negative values suggest species are performing better in monoculture than mixture due to competition between species. However, complementarity effects do not change when the relative yield expected (RY_E) is altered. This is because the above equation can be rewritten as:

Complementarity effect = $(\sum_i RY_{O,i} - 1)(\overline{M})$

We applied the additive partitioning method in two ways to our data, (1) using the sown proportion of individual of each species (*i*/64 individuals) at planting in each year; and (2) using realized proportions: the proportion of individuals at planting was used for year one, and the previous year proportion of annual total plot biomass for each species in years two and three.

Treatment effects (year, richness, evenness, pattern and their interactions) on selection and complementarity for all three years and each proportion calculation were assessed with Repeated Measures ANOVA (proc mixed; SAS v9.3, Cary, NC) with fixed block effects. Selection and complementarity were square root transformed with the original sign maintained to meet assumptions. Significant ANOVA results were followed by Least Significant Difference (LSD) multiple-comparison test to distinguish differences between treatment groups.

Diversity-Interaction models

Multiple Diversity-Interaction models (Kirwan et al. 2007; Kirwan et al.2009) were fit (proc glm; SASv9.3) to determine species identity effects and to test biological assumptions about how species interact to contribute to biomass production. Models were fit within each year using sown proportions, and then the analysis was repeated using previous year proportions.

Identity Model:

In the identity model, species contributions are based on monoculture yield weighted by the proportion in mixture, and it is assumed that species do not interact:

$$y = \sum_{i=1}^{s} \beta_i P_i + \alpha_k + \varepsilon$$

P_i is the proportion of species *i*, β_i is the estimated performance of species *i* in monoculture, *s* is the number of species in the species pool, α_k is the effect of block (k = 1 to 5), and ε is the residual.

Average pairwise interaction model:

The average pairwise interaction model includes a single interaction term (δ_{AV}) for all the pairwise species interactions in a community and assumes that all pairwise interspecific interaction strengths are the same:

$$y = \sum_{i=1}^{s} \beta_i P_i + \alpha_k + \delta_{\mathrm{AV}} \sum_{\substack{i,j=1 \\ i<j}}^{s} P_i P_j + \varepsilon$$

$P_i P_j$ is the proportion of species *i* times the proportion of species *j.*

Additive species-specific contributions to interactions model:

In the additive species-specific contributions to interactions model, each species contributes the same additive component (λ_i) to every pairwise interaction it is involved in regardless of the identity of the other species in the interaction:

$$y = \sum_{i=1}^{s} \beta_i P_i + \alpha_k + \sum_{\substack{i,j=1 \\ i<j}}^{s} (\lambda_i + \lambda_j) P_i P_j + \varepsilon$$

To calculate the expected interaction effect of species *i* and *j*, the fixed effect for species *i* (λ_i) is added to the fixed effect of species *j* (λ_j). The species interactions can be estimated by

$$\sum_{i=1}^{s} \lambda_i P_i(1 - P_i) + \varepsilon$$

Functional group model:

The functional group effect model categorizes species by functional group, and interactions between $(\delta_{ab} P_a P_b)$ and within functional group $(\delta_{\mathrm{aa}}$ and $\delta_{bb})$ are the diversity effect. The model when there are two functional groups is:

$$y = \sum_{i=1}^{s} \beta_i P_i + \alpha_k + \delta_{\mathrm{aa}} \sum_{\substack{i,j=1 \\ i<j}}^{s} P_i P_j + \delta_{bb} \sum_{\substack{i,j=t+1 \\ i<j}}^{s} P_i P_j + \delta_{ab} P_a P_b + \varepsilon$$

The formula above is for a pool that contains *s* species with *t* species of functional group *a,* and *s – t* species of functional group *b* (two functional groups). P_a and P_b are the proportion of the community that are in functional group *a* and functional group *b*. In the analyses for this experiment, there are four functional groups so there are 10 terms in the diversity effect (four within functional group interactions and six between functional group interactions).

All pairwise interaction model:

The all pairwise interaction model includes a separate pairwise interaction for all species in a mixture as the diversity effect:

$$y = \sum_{i=1}^{s} \beta_i P_i + \alpha_k + \sum_{\substack{i,j=1 \\ i<j}}^{s} \delta_{ij} P_i P_j + \varepsilon$$

The coefficient δ_{ij} is the potential for two species to interact, and $\delta_{ij} P_i P_j$ is the contribution to biomass production from the interspecific interaction of species *i* and *j*. A drawback to this model is that the number of pairwise interactions increases very rapidly with increasing species richness in a community, which can lead to difficulties in model fitting. If there are not issues with model fitting, there may be difficulty in interpreting the high number of interaction coefficients.

Due to the large number of species in this study (16 species means there are 16C2 = 120 pairwise interactions), there is not sufficient data to estimate all of the pairwise interaction terms and therefore it is not possible to fit the all pairwise model. A possible way of bridging the gap between the all pairwise model and the other Diversity-Interaction models is to include the pairwise interactions as a random term in the model (Brophy et al. 2016 IN REVISION). Below is an example of including the random term in the average pairwise model.

$$y = \alpha_k + \sum_{i=1}^{s} \beta_i P_i + \delta_{\mathrm{AV}} \sum_{\substack{i,j=1 \\ i<j}}^{s} P_i P_j + \sum_{\substack{i,j=1 \\ i<j}}^{s} d_{\mathrm{ij}} s P_i P_j + \varepsilon$$

where $\varepsilon \sim N(0, \sigma_1^2)$ and $d_{\mathrm{ij}} \sim N(0, \sigma_2^2)$. The inclusion of the random term provides a lack-of-fit test for the fixed diversity effect. If random effects improve the model fit, the fixed effect does not explain the entirety of the variability caused by all possible

pairwise interactions. Additional variability is accounted for by the random term, and this variability is included in the fixed effect coefficients and standard errors.

Species pattern interaction models:

Pattern was included as an interaction with the diversity effects, as this treatment was only applied to mixture and not monoculture plots. An example of how pattern was incorporated is given for the average pairwise model below:

$$y = \sum_{i=1}^{s} \beta_i P_i + \alpha_k + \delta_{\mathrm{AV}} \sum_{\substack{i,j=1 \\ i<j}}^{s} P_i P_j + \gamma_{AV} \sum_{\substack{i,j=1 \\ i<j}}^{s} P_i P_j * \mathrm{Pattern} + \varepsilon$$

Pattern was coded 1 for aggregated plots and 0 dispersed plots. The term δav is the diversity coefficient for the random pattern, while $\delta_{av} + \gamma_{av}$ is the diversity coefficient for the structured pattern. If γ_{av} is zero, then the diversity effects do not differ for the two sowing treatments.

Model comparisons:

Initially, all models were compared (proc glm; SAS v9.3) based on the hierarchy in Kirwan et al. 2009. Model comparisons were done using an F-test within each year of data collection. Because the all pairwise cannot actually be fit, the pairwise interactions were included as a random term in the best model for each year to see if there is any additional variability in the model that can be explained. The random term model was fitted using REML so it could be compared to the best fit model without the random term included using a likelihood ratio test (LRT).

Results

Species varied in their contribution to plot biomass over time (Fig. 3.1). This led to differences in the relative yield expected based on sown proportions and realized proportions (Fig. 3.2).

Additive partitioning model

Complementarity effects increased over time, and were only affected by species richness in year three (Table 3.1; Fig 3.3A, 3.3B). With sown proportions, mean selection decreased over time to close to zero in the third year (Fig. 3.3A). The change in selection effects is due to the change in deviation in relative yields of species. In year one, species that had low monoculture yields tended to produce less biomass in mixtures, and the highest producing monoculture performed very well in mixture (Fig. 3.4A). This produced positive selection results. In year two using sown proportions, some lower yielding species did better in mixture than in monoculture, which would reduce selection effects. In year three using sown proportions, more low yielding species did better in mixture than monoculture, and the species with the highest biomass did not have the greatest deviation in mixture. This would cause selection to decrease even more.

Using realized proportions, mean selection decreased substantially from year one to year two and stayed negative in year three (Fig. 3.3B). Using realized proportions in year two, a majority of the low yielding species did better in mixture than monoculture and the second highest producing species had a dramatically lower

proportion of plot biomass than in year one (Fig. 3.4B). These results gave rise to the negative selection effects observed. In year three using realized proportions, all lower yielding species except one did better in mixture than monoculture, and the highest producing species performed poorly in mixture. This would also cause selection effects to be negative.

The changes in selection effects calculated with the proportion used caused the net diversity effect to be lower using realized proportions than using sown proportions in years two and three. Using sown proportions, the net diversity effect increased over time and was positive all three years (Fig 3.3A). When realized proportions were used, the net diversity effect was negative in year two and positive in year three (Fig.3B).

The relationship between treatments and selection effects were also altered substantially. Using sown proportions, selection tended to increase with species richness in years one and two but not year three (Table 1; Fig. 3.5A). Overall richness did not affect selection when using realized proportions, but selection decreased with increased species richness in years two and three (Fig. 3.5B). Evenness effects were similar for sown and realized proportions, but overall selection calculated with realized proportions was much lower (Fig. 3.5C and 3.5D). Species pattern had no effect on selection or complementarity using sown or realized proportions.

Diversity-Interaction modeling

In year one, the average pairwise model was a better fit than the identity model, and the additive species contribution model was a better fit than the average pairwise

model (Table 3.2). *Helianthus maximilliani* had the largest estimated positive contribution to interaction effects, and it had the only significant interaction effect (Table 3.3). Because the *H. maximilliani* estimate was so large (Table 3.3; $\widehat{\lambda}_{HM} = 1620.60$), its interaction with every other species ($\widehat{\lambda}_{HM} + \widehat{\lambda}_{i}$) was strong regardless of the identity of the other species in the interaction (Fig. 3.6A). *H. maximilliani* had the highest monoculture yields, and the majority of mixtures that did better than average at each richness level had *H. maximiliani* present (Fig. 3.6B). The inclusion of random effects for pairwise interactions in the diversity models did not improve fit.

Using the sown proportions in year two, the average pairwise effect model was a better fit than the identity model, and no other diversity model was a better fit (Table 3.2). This indicates that the strength of all pairwise interactions is the same regardless of the identity of the species in the interaction, and the diversity effect should be greatest when species are equally represented at the highest richness level. The inclusion random effects for pairwise interactions in the diversity models did not improve fit. Using sown proportions in year three, the average pairwise model was a better fit than the identity model, and the additive species-specific contribution model was a better fit than the average pairwise model. Therefore, the best fit model was the additive species-specific contribution to interactions model. *R. columnifera* was the only species that did not have a significant identity effect (Table 3.3). The warm-season grass *P. virgatum* and the legume *A. canadensis* had significant positive estimated pairwise interactions, and the legume *D. purpurea* had a marginal positive estimated pairwise

interaction (Fig. 3.7). The inclusion of the random term and species pattern did not improve model fit.

When realized proportions were used in year two, the inclusion of diversity effects did not improve model fit, so the identity model was the best (Table 3.2). This suggests that the contribution of each species to plot biomass is relative to the proportion of monoculture yield. *H. maximiliani* and *S. rigida* would contribute the most biomass per individual planted, and *R. columnifera* would contribute the least (Table 3.3). The inclusion of the random interaction term did not improve fit. If realized proportions were used for year three, no model is better than the identity model. This again suggests there is a lack of diversity effects and the contribution of each species to productivity in mixture would be based on species yield in monoculture. The only species without a significant estimate was the forb *R. columnifera*. *S. rigida* and *S. nutans* would have the greatest contribution to plot biomass. The inclusion of the random term with species interactions based on the abundance of species improved model fit (Table 3.4)

The inclusion of species pattern did not improve model fit using sown proportions or realized proportions in any of the three years. For brevity, the results were not included.

Discussion

The main objectives of these analyses were to understand the possible drivers of productivity in three years of a biodiversity experiment and to determine how using sown or realized proportions affects outcomes from two modeling approaches. Varying the proportions in the additive partitioning model altered the magnitude and direction of selection effects and the relationship between selection effects and study treatments. Using sown proportions in the Diversity-Interaction modeling approach, diversity effects were present in years two and three, but when realized proportions were used only species identity was important. Results indicate that the decision to use sown or realized proportions affect how diversity effects are perceived in grassland experiments.

In the additive partitioning model, the variability in the selection effects is partly due to two factors. First, the proportion of sown individuals assigned to a species does not necessarily reflect how that species would proportionately contribute to a plots biomass (Hector 1998; Connolly et al. 2001). Second, species expected yield changed over time using realized proportions. For example, the most productive species in the first growing season, the fast growing, tall statured *H. maximiliani* (HM), also did very well in mixture in year one (Fig. 3.2 and 3.4A). This species contributed substantially more to plot biomass than would have been predicted based on the proportion it was sown. In the second growing season, slower establishing species contributed more to plot biomass than they did in the first growing season. This proportional increase in

slower-establishing species reduced the proportional contribution of the high yielding HM in mixtures. In the sown proportion model for the second growing season, HM did better in mixture than monoculture even though HM observed relative yield decreased from year one to year two. This occurred because the expected HM proportion (sown proportion in mixture) was much lower than HM's potential contribution to plot biomass. Using sown proportions to estimate species contributions to mixture did not account for inherent differences in species biomass production, which were substantial across our species pool. In the realized proportion model, the calculated expected relative yield of HM for the second growing season was so great that HM performed worse in mixture than monoculture (Fig. 3.2 and 3.4B). Using realized proportions, expected yields were more representative of species expected biomass contributions. In systems with less variation among species in the pool, results may be more consistent between the approaches.

In the Diversity-Interactions models, using the two proportion approaches resulted in different outcomes. For example, with the sown proportions approach the additive species model was the best fit in year three. This indicates that diversity effects across three growing seasons can be explained by species contributing fixed interaction strengths to pairwise interactions (Kirwan et al. 2009). Changes in species yields resulting from abiotic and biotic interactions within the first and second growing seasons are included in third growing seasons outcome, so it is difficult to isolate the interactions that determined the outcome in just year three. Each harvest has to be looked at independently, and the previous years' outcomes may have to be ignored.

The results of sown analyses give insight on how species would have to interact in order to reach three different biomass production end points (three growing seasons). What still needs clarification is in what way outcomes from within each year using the realized proportions relate to the sown proportion model outcome? Within year three, using realized proportions, the identity model was the best fit. This indicates changes from the second to third growing seasons are explained by the species present, but not their interactions. What needs to be determined is how to relate this result to the across year result using sown proportions to better understand the system. It may not be possible to compare the outcomes, as sown proportions are based on density and realized proportions are based on biomass production.

In the Diversity-Interaction modeling approach, diversity effects are based on potential interactions in a community, which is inherently determined by the number of individuals present. This information is difficult to ascertain over time within a system, so interactions may be based on species contributions to plot biomass production. Species may vary in their size-density relationship, so a change in biomass production of a species does not necessarily mean a change in the number of individuals of that species (density in the plot) (Marquard et al. 2009). Therefore, an increase in biomass of a species may not result in a change in density and subsequently species interactions. If contributions to plot biomass are not a good indicator of species interactions, no diversity effects would be present and the species identity model would be the best fit. This reasoning suggests that using sown proportions may provide more information about potential interaction frequency among species than realized proportions.

However, if species do increase in biomass by increasing the number of individuals, interaction frequencies will change over time. To accurately account for interaction changes in a diverse species pool over multiple growing seasons, a density measure for each species every growing season would be necessary. A density measurement was not recorded for this experiment, so a comparison cannot be made between the realized proportion of biomass and realized species density.

Possible effects of using plant species varying greatly in size and biomass production have been thoroughly discussed within the context of experimental designs that maintain plot level density of individuals and replace the proportion of one species for another (Huston 1997; reviewed in Joliffe 2000; Connolly et al. 2001). However, relating these effects to how the proportion used alters interpretation in biodiversity experiments may not be as apparent to ecologists who want to use these models. Results indicate that more emphasis should be placed on interpretation of results within the context of what proportions are used for calculation of diversity effects on productivity. For similar experiments with a diverse species pool, we recommend that realized proportions be used in the additive partitioning model, as expected biomass is based on biomass production in the previous year rather than an arbitrary relative abundance value. In Diversity-Interactions modeling, it seems that there are benefits to understanding each species contribution to plot biomass production within each year (realized proportions), but a species density measurement may also be needed to fully understand results. More discussion and analyses are needed to determine if model

outcomes using sown and realized proportions in the Diversity-Interaction approach can be compared.

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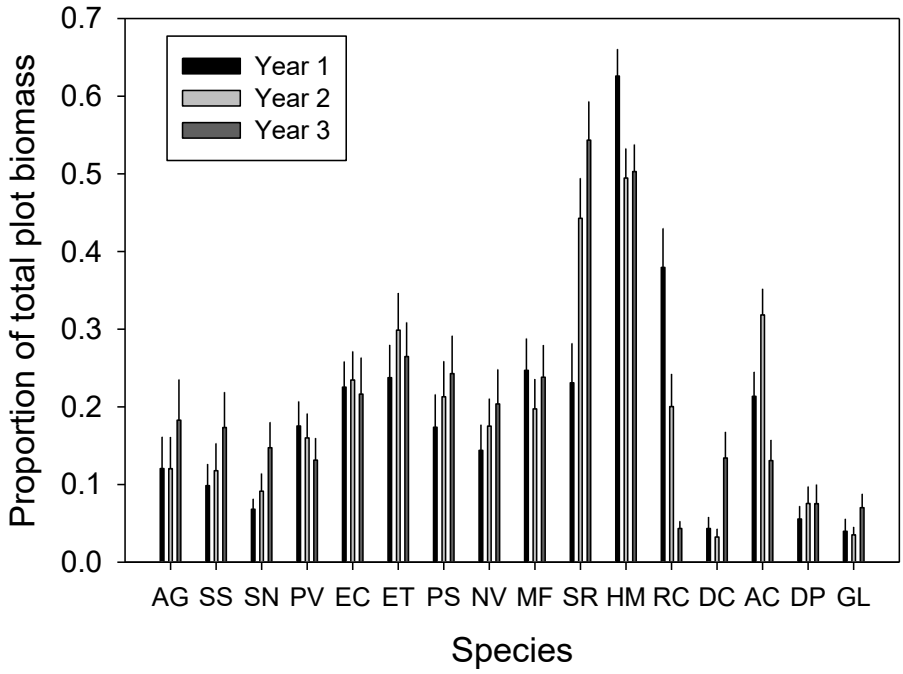

Figure 3.1. Proportion of total plot biomass (Mean ± SE) produced by each species across all treatments for all three years of the experiment. Species labeled with the first letter of their genus and specific epithet: *Andropogon gerardii* (AG), *Panicum virgatum* (PV), *Schizachyrium scoparium* (SS), *Sorghastrum nutans* (SN), *Elymus canadensis* (EC), *Elymus trachycaulus* (ET), *Pascopyrum smithii* (PS), *Nassella viridula* (NV), *Monarda fistulosa* (MF), *Solidago rigida* (SR), *Helianthus maximiliani* (HM), *Ratibida columnifera* (RC), *Desmodium canadense* (DC), *Astragalus Canadensis* (AC), *Dalea purpurea* (DP), *Glycyrrhiza lepidota* (GL).

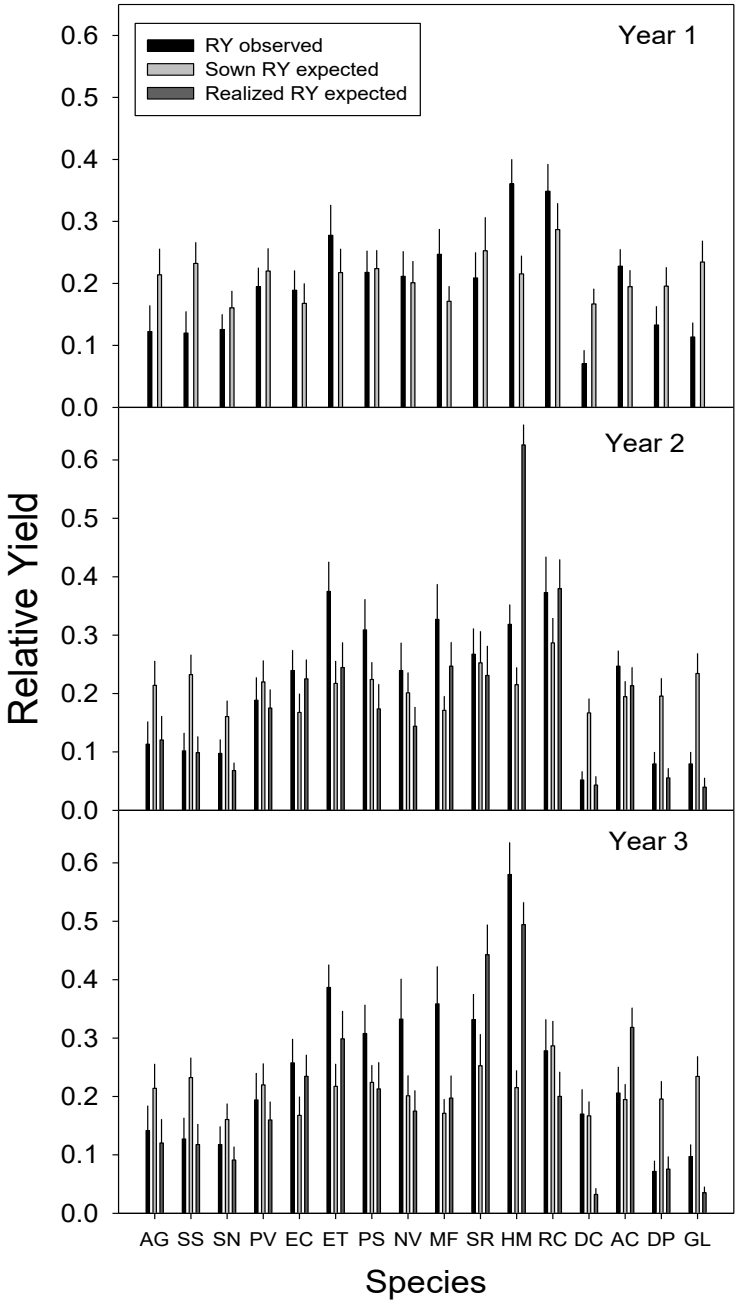

Figure 3.2. Relative yield (RY) observed, relative yield expected using sown proportions, and relative yield expected using realized proportions (mean ± SE) across all treatments for all three years of the experiment. Species labeled with the first letter of their genus and specific epithet.

Table 3.1. Results from Repeated Measures ANOVA of planted richness, evenness, and pattern effects on additive partitioning model selection effects and complementarity effects using sown and realized proportions. Values are F- statistics and degrees of freedom (df).

	Sown Selection		Realized Selection		Complementarity	
Effect	df	F	df	F	df	F
Block	4,68	2.52*	4,68	0.97	4,68	3.84**
Richness (R)	2,69.5	8.60**	2,68	2.09	2,65.6	0.75
Evenness (E)	2,69.5	3.22*	2,68	2.91†	2,65.6	1.16
Pattern (P)	1,69.5	0.98	1,68	0.00	1,65.6	1.32
R × E	4,69.5	1.74	4,68	0.56	4,65.6	1.52
R × P	2,69.5	0.74	2,68	0.40	2,65.6	0.16
E × P	2,69.5	2.38	2,68	0.30	2,65.6	0.94
R × E × P	4,69.5	1.11	4,68	0.31	4,65.6	1.13
Year (Y)	2,71	14.61**	2,71	78.64**	2,71	17.43**
Y × R	4,84.4	3.02*	4,84.4	9.10**	4,84.4	3.70**
Y × E	4,84.4	2.11†	4,84.4	1.39	4,84.4	0.92
Y × P	2,71	0.68	2,71	1.33	2,71	0.60
Y × R × E	8,98.5	1.06	8,98.5	0.93	8,98.5	1.08
Y × R × P	4,84.4	0.46	4,84.4	0.89	4,48.4	1.25
Y × E × P	4,84.4	0.47	4,84.4	1.16	4,48.4	0.33
Y × R × E × P	8,98.5	0.42	8,98.5	0.66	8,98.5	1.53

(* = $p < 0.05$, ** = $p < 0.01$, † = $p < 0.1$)

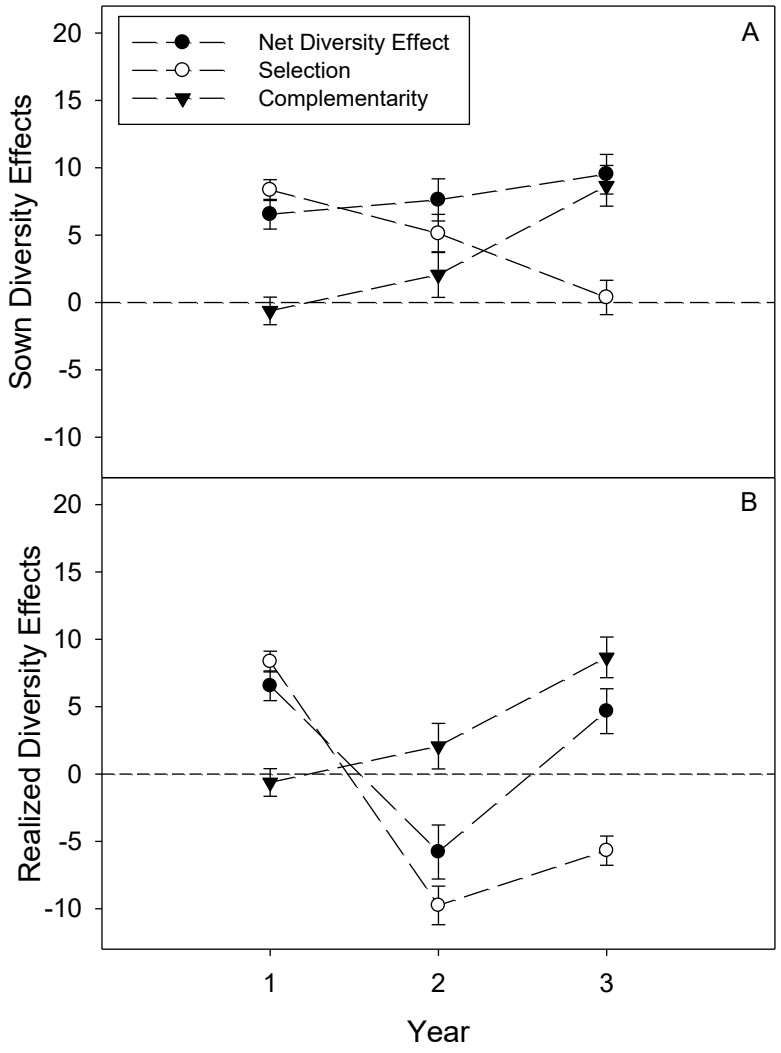

Figure 3.3. The net diversity effect, selection effect, and complementarity effect (LS transformed mean ± SE) for each year of the experiment using sown (A) and realized proportions (B) in the additive partitioning model of Loreau and Hector (2001).

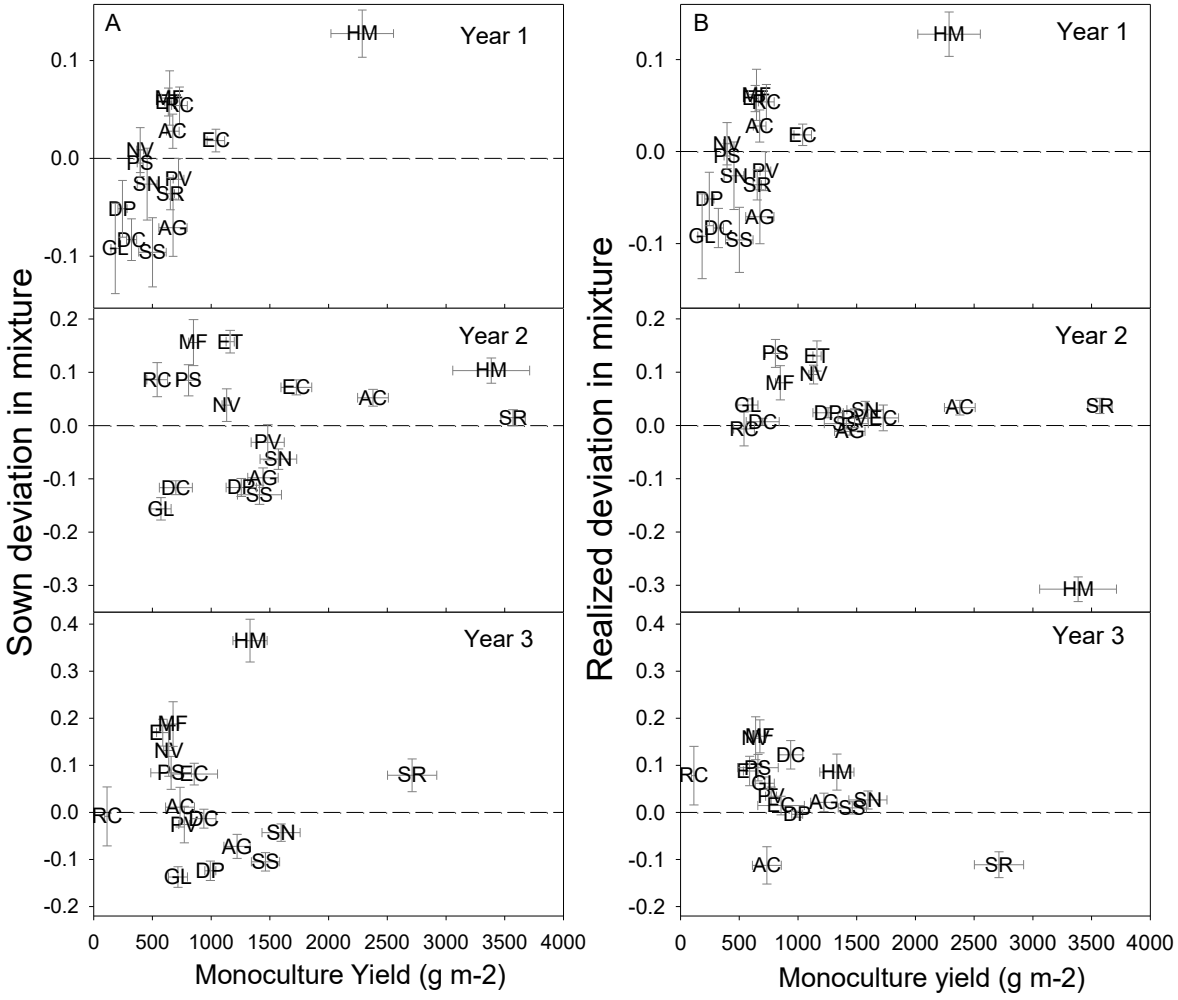

Figure 3.4. Difference in species relative yields ($\Delta RY = RY_{Observed} - RY_{Expected}$) using sown (A) and realized (B) proportions across all treatments (mean ± SE) and in relation to their monoculture yields (mean ± SE) in each growing season. Species labeled with the first letter of their genus and specific epithet: *Andropogon gerardii* (AG), *Panicum virgatum* (PV), *Schizachyrium scoparium* (SS), *Sorghastrum nutans* (SN), *Elymus canadensis* (EC), *Elymus trachycaulus* (ET), *Pascopyrum smithii* (PS), *Nassella viridula* (NV), *Monarda fistulosa* (MF), *Solidago rigida* (SR), *Helianthus maximiliani* (HM), *Ratibida columnifera* (RC), *Desmodium canadense* (DC), *Astragalus Canadensis* (AC), *Dalea purpurea* (DP), *Glycyrrhiza lepidota* (GL).

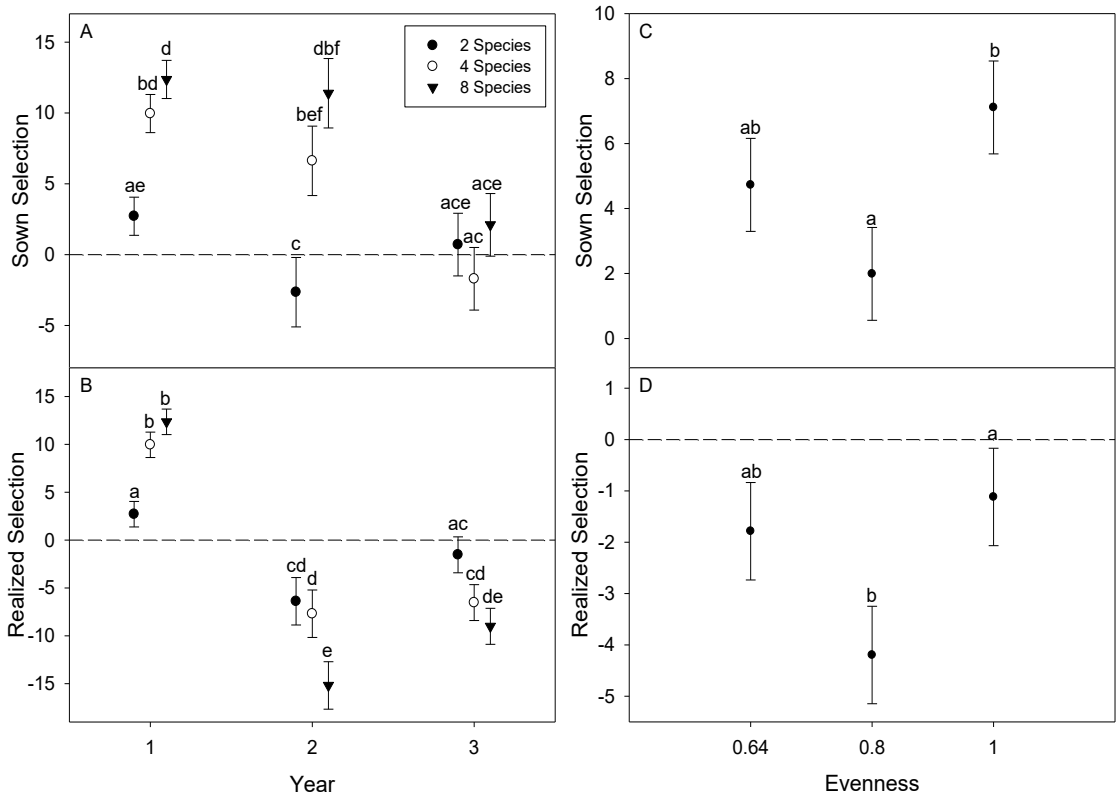

Figure 3.5. Selection effects (transformed ls mean ± SE) from the additive partitioning model for all years of the experiment at each richness level using sown (A) and realized proportions (B). The overall effect of evenness level on selection effects calculated with sown (C) and realized (D) proportions. Different letters within each graph indicate a significant difference (LSD test).

Table 3.2. Results (F-statistic and p value) of Diversity-Interaction model comparison for sown and realized proportions for all three years of the experiment.

	Year 1		Year 2				Year 3			
	Sown		Sown		Realized		Sown		Realized	
Model Comparison	F	p	F	p	F	p	F	p	F	p
Identity vs. Avg. pairwise	21.16	< 0.0001	19.45	< 0.0001	0.36	0.5491	22.52	<0.0001	2.22	0.1381
Identity vs. Functional group	2.69	0.0047	2.23	0.0191	0.95	0.4870	2.54	0.0075	0.62	0.7819
Identity vs. Add. species contribution	3.96	<0.0001	2.53	0.0020	1.46	0.1211	3.56	<0.0001	1.04	0.4206
Avg. pairwise vs. Functional group	0.69	0.7008	0.40	0.9197	1.01	0.4300	0.41	0.9117	0.44	0.8916
Avg. pairwise vs. Add. species contribution	2.58	0.0020	1.29	0.2190	1.54	0.0998	2.03	0.0173	0.96	0.5002

Table 3.3. Estimates of identity effects, diversity effects, and standard error (SE) using sown and realized proportions for all three years of the experiment.

	Year 1		Year 2				Year 3			
Species proportion	Sown		Sown		Realized		Sown		Realized	
Best fit model	Additive species		Average pairwise		Identity		Additive species		Identity with random term	
Variable	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Andropogon gerardii (AG)	**680.93**	80.09	**1454.62**	130.56	**1519.37**	152.99	**1193.39**	108.51	**1156.97**	116.30
Schizachyrium scoparium (SS)	**513.78**	84.86	**1389.77**	134.15	**1459.62**	165.80	**1465.83**	114.63	**1448.23**	119.56
Sorghastrum nutans (SN)	**473.78**	88.01	**1668.47**	146.82	**1674.83**	175.00	**1578.73**	118.43	**1574.04**	126.70
Panicum virgatum (PV)	**696.50**	84.32	**1614.38**	136.38	**1580.62**	161.42	**725.63**	114.50	**775.92**	123.97
Elymus canadensis (EC)	**1043.64**	84.79	**1893.81**	135.09	**1779.80**	148.17	**845.03**	114.70	**787.09**	114.84
Elymus trachycaulus (ET)	**666.39**	82.60	**1260.92**	130.47	**1258.57**	142.67	**617.32**	112.08	**588.67**	104.93
Pascopyrum smithii (ET)	**403.86**	87.97	**881.45**	137.72	**915.47**	154.62	**635.62**	118.46	**645.43**	115.10
Nassella viridula (NV)	**414.55**	85.87	**1234.30**	137.03	**1321.68**	163.94	**637.53**	117.03	**697.86**	121.61
Monarda fistulosa (MF)	**658.09**	87.64	**927.28**	143.09	**1031.21**	150.94	**666.34**	118.03	**700.99**	120.32
Solidago rigida (SR)	**653.45**	80.61	**3482.68**	128.06	**3557.47**	149.35	**2589.48**	113.59	**2452.88**	101.67
Helianthus maximiliani (HM)	**2281.04**	85.61	**3460.06**	133.13	**2724.23**	103.39	**1305.33**	116.11	**1362.15**	98.89
Ratibida columnifera (RC)	**709.43**	78.76	**631.35**	122.38	**764.20**	125.41	162.14	108.26	90.30	112.34
Desmodium canadense (DC)	**336.95**	87.62	**732.48**	139.22	**772.92**	172.44	**930.06**	118.24	**908.94**	128.19
Astragulus canadensis (AC)	**690.25**	87.83	**2513.50**	142.18	**2519.48**	157.31	**720.08**	118.21	**806.51**	116.80
Dalea purpurea (DP)	**251.02**	87.77	**1359.99**	141.47	**1333.74**	174.89	**965.68**	118.17	**944.8**	127.26
Glycyrrhiza lepidota (GL)	**187.52**	88.01	**756.50**	143.64	**683.29**	176.93	**702.48**	118.38	**685.93**	129.72
Block 1	-85.02	46.79	-101.95	79.36	-99.63	91.90	83.66	65.92	113.66	68.94
Block 2	-49.28	47.10	**-225.39**	79.84	†-177.44	92.06	-48.19	66.73	-30.33	69.66
Block 3	-11.89	47.73	-115.25	79.62	-140.27	91.64	23.13	66.84	34.29	69.72
Block 4	81.06	47.21	-50.80	79.84	-35.72	92.17	33.31	67.68	63.10	70.25
Block 5	0.00	.	0.00	.	0.00	.	0.00	.	0.00	.
Average pairwise interaction	.	.	**631.22**	143.12	.	.	.	.	.	.
AG interaction	305.32	407.58					-706.91	692.37		
SS interaction	196.55	344.13					382.20	626.05		
SN interaction	546.41	450.63					594.99	829.56		
PV interaction	183.92	409.34					**1495.91**	700.94		
EC interaction	-101.02	436.52					162.78	737.63		
ET interaction	6.68	348.56					91.421	622.55		

Table 2. cont.

	Year 1 Sown		Year 3 Sown	
Variable	Estimate	SE	Estimate	SE
PS interaction	269.70	339.19	-133.54	571.19
NV interaction	10.59	365.31	770.59	625.96
MF interaction	229.52	419.30	-896.88	711.01
SR interaction	-313.54	418.11	630.19	781.25
HM interaction	**1620.60**	357.12	713.32	614.50
RC interaction	135.03	331.47	-760.41	572.15
DC interaction	-81.64	379.78	-456.89	622.49
AC interaction	†-612.70	346.46	**1349.39**	546.57
DP interaction	122.23	400.66	†1193.87	647.85
GL interaction	463.59	385.05	595.95	603.70

Bold indicates a significant estimate ($p < 0.05$) and † indicates $p < 0.10$.

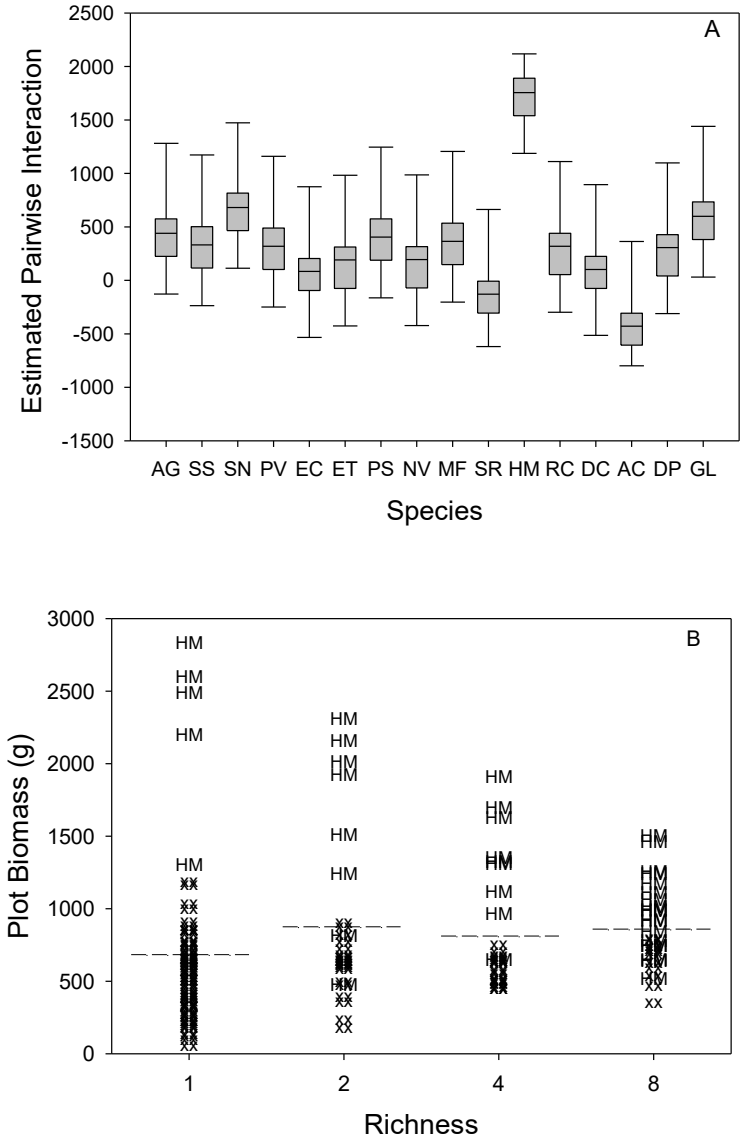

Figure 3.6. A) Boxplots of the estimated pairwise interaction strength (calculated by adding the interaction effect of each species in the pairwise interaction from Table 3.3) for all species in year one of the experiment. B) The total yield and average yield (----) for monoculture (richness = 1) and mixture (2, 4, and 8 species) plots with (HM) and without (xx) *H. maximiliani* in year one of the experiment.

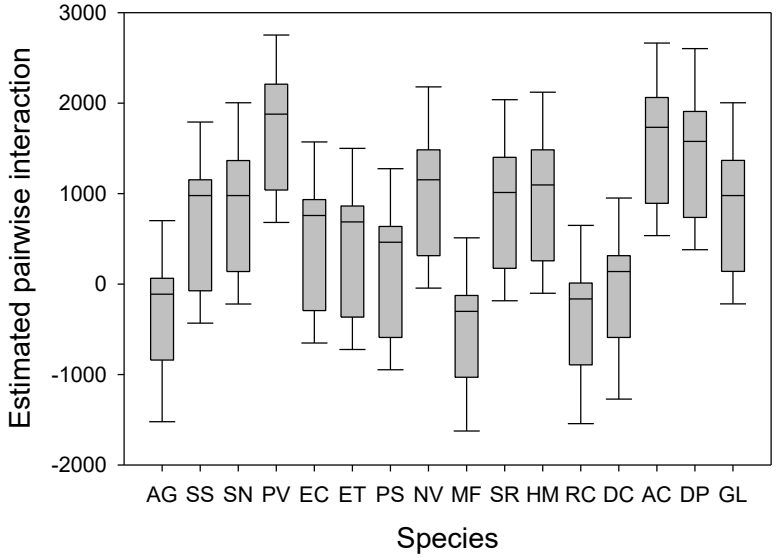

Figure 3.7. Boxplots of the estimated pairwise interaction strength (calculated by adding the interaction effect of each species from Table 3.3) for all species using sown proportions in year three of the experiment.

Table 3.4. Details of the likelihood ratio tests (LRT) for the comparison of the identity model in year three with and without the random interaction parameters included using realized proportions.

Model	Model description	No. parameters	2 Loglikelihood	LRT	LRT stat	P-value
D	Identity model	21	2175.80			
E	D with random PP1-120	22	2171.30	E v D	4.5	0.0339

CHAPTER IV:
EFFECTS OF SOIL COMPONENTS ON DECREASED PRODUCTIVITY IN MONOCULTURES OF GRASSLAND SPECIES

Abstract

Productivity of plant species grown in monocultures may decline over time due to decreased access to soil nutrients or an increase in soil pathogens. Relief from these detriments may contribute to increased species performance when planted in mixture. To determine whether abiotic or biotic soil feedbacks affect biomass production within a suite of tallgrass prairie species, we conducted a field experiment to confirm the presence of feedbacks and greenhouse experiments to determine the nature of the feedbacks. The focal species selected had the greatest decline within their respective functional groups (cool season grass, warm season grass, forb, and legume) within monoculture plots of a companion biodiversity-ecosystem function experiment. Seedling performance was assessed in field soils conditioned for two growing seasons by the same species, species within the same functional group, and by the remaining focal species. To determine the mechanism (abiotic vs. biotic) of the feedback, individuals were grown in conspecifically conditioned soil plots that removed soil biota by sterilization (autoclaving) and heating to 60 ° C. Performance of the legume *Astragulus canadensis* was reduced in field plots conditioned by conspecifics, and the performance of all three non-leguminous species was increased in field plots conditioned by *A.*

canadensis. In the greenhouse, removal of soil biota increased *Elymus canadensis* and *Panicum virgatum* growth. *A. canadensis* growth was decreased in sterile soil. Results suggest that soil biota may reduce growth of the grasses, but soils conditioned by non-leguminous heterospecifics may not reduce abundance to innocuous levels. Results reinforce our understanding of the beneficial role of legumes for heterospecific growth, and suggest that abiotic limitations may limit legume growth with increased abundance. Nutrient limitation played a larger role in declining monocultures than soil biota among species used in this experiment.

Introduction

Plant species can affect the chemical and biotic properties of the soil they occur in, and these effects in turn, can affect the growth of the host plant (Bever et al. 1997; Klinoronomos 2002) and surrounding individuals (Petermann et al. 2008) through soil feedbacks. These feedbacks influence overall plant community dynamics (reviewed in van der Putten et al. 2013) by contributing to species coexistence (Petermann et al. 2008; de Kroon et al. 2012) and driving succession (Kardol et al. 2006; Bauer et al. 2015). More recent studies have focused on the importance of negative feedbacks in the relationship between plant diversity and productivity and have shown that the inclusion of soil feedback effects in these experiments may give more insight into species-specific mechanisms driving the relationship (Schnitzer et al. 2011; Maron et al. 2011; Hendriks et al. 2013).

Over time species tend to perform better in mixture than monoculture, which leads to increased community productivity (Fargione et al. 2007; Marquad et al. 2009). These increases can result from abiotic and biotic mechanisms. Nitrogen-fixing legumes are well-known for their ability to increase local nutrient availability and subsequently mixture performance (Spehn et al. 2005; Temperton et al. 2006; Fargione et al. 2007). Species can also perform better in mixture because of the decreased abundance of species-specific soil biota due to lower density of the host (Maron et al. 2011; Schnitzer et al. 2011; Hendriks et al. 2013). The determination of the soil component (biotic vs. abiotic) contributing to decreased monoculture yields should lead to better understanding of the mechanisms of species-specific overyielding and the diversity-productivity relationship associated with species mixtures (Maron et al. 2011; Schnitzer et al. 2011; Hendriks et al. 2013; van der Putten et al. 2013).

Uncoupling abiotic versus biotic feedbacks can be challenging. Some techniques used to remove soil biota from soil samples can alter nutrient availability (reviewed in Brinkman et al. 2010) and may increase levels of phytotoxic elements (Wolf et al. 1989). Also, most studies are done in a greenhouse setting with different abiotic conditions than found in the field, which may influence results (Heinze et al. 2016). For these reasons, the use of multiple approaches (field and greenhouse experiments) may provide more information about the presence of soil feedbacks and the driver (abiotic vs. biotic) of the feedbacks observed.

The objectives of this experiment were to determine the presence and drivers of soil feedbacks in monocultures of common prairie species used in a long term plant community ecology field experiment at the University of North Dakota (SPaCE experiment) (McKenna & Yurkonis 2016). To do so, we supplemented an in-field soil conditioning experiment with a greenhouse soil biota removal experiment. Four focal species were selected based on decreasing monoculture productivity from year two to year three in the SPaCE experiment (see Chapter 2). Each focal species was from a different functional group: a cool-season grass, a warm-season grass, a non-leguminous forb, and a legume.

To determine the presence of negative feedbacks, the performance of the four focal species was assessed in field plots conditioned for two growing seasons with monocultures of the 16 grassland species used in the SPaCE experiment. We hypothesized that if nitrogen limitation is driving decreased monoculture productivity, then non-leguminous species should perform best in soils conditioned by nitrogen-fixing legumes. Soil biota in the soil can have more species-specific effects (van der Putten 2003; Hendriks et al. 2013). We hypothesized that if species-specific soil biota were causing negative feedbacks, then increased growth would occur in soils conditioned by species within the same functional group.

To determine the mechanism (abiotic vs. biotic) of the negative feedback, focal species were grown in soil cores collected from plots conditioned by conspecifics. Soil cores were heated treated to remove soil biota by two methods: sterilization in an

autoclave and heating in an oven to 60 ° C. To account for increased nutrient availability with soil heating, a soil core was heated to 60 ° C and reinoculated (rescued) with a soil slurry containing biota. We expected species with a negative feedback in the field would perform better when soil biota were removed.

The knowledge of species-specific feedbacks, along with insight into the feedback mechanism (biotic or abiotic), can lead to insights on the how feedbacks lead to community responses in diversity studies and lead to better predictions of plant performance.

Methods

Species selection

Species for this experiment were selected based on their performance in the Species Pattern and Community Ecology (SPaCE) experiment at the University of North Dakota. The SPaCE experiment consists of monocultures and mixtures of 16 native prairie species (four species from each functional group: warm-season grass, cool-season grass, non-leguminous forb, and legume) planted in spring 2012 (1 x 1 m plots) in a field that had been in continuous agriculture for 15 years (for additional experiment details see McKenna and Yurkonis 2016). We compared species productivity (aboveground biomass) in monocultures and mixtures (as described in Loreau and Hector 2001) across three growing seasons (2012 - 2014). The species in each functional group (warm-season grass, cool-season grass, forb, and legume) whose average monoculture yield (n = 5) decreased the most from 2013 to 2014 (year two to year three

of the experiment) was selected for use in these soil effects experiments (Fig. 4.1). These species were: the cool-season grass *Elymus canadensis* (Canada wild rye), the warm-season grass *Panicum virgatum* (switchgrass), the forb *Helianthus maximiliani* (Maximillian sunflower), and the legume *Astragulus canadensis* (Canada milkvetch).

Soil conditioning

Field soil was conditioned for the experiments described below by planting additional monoculture plots (1 x 1 m; 64 individuals m^{-2}) of the 16 SPACE experiment species in a randomized complete block design (n = 4) in spring 2013. Species were grown from seed (obtained from Prairie Restorations Inc., Princeton, MN) for 16 weeks in the University of North Dakota greenhouse in pots with only other conspecifics prior to transplanting. Legume seeds were inoculated with genus-specific inoculant (Prairie Moon Nursery, Winona, MN) prior to seeding. Plots were weeded and the aboveground biomass at the end of the growing season was left standing in the first and second year.

Experiment 1: Field home and away

This experiment was conducted in 2015 (the third growing season) with the field-established monocultures (n = 4). Conditioned field soil was isolated within five PVC pipes (10 cm diameter x 20 cm long; one individual per pipe) positioned in a quincunx (five on a dice) pattern in each monoculture plot. PVC pipes were used to prevent belowground competition for nutrients and space between the resident species and planted individuals. In each focal species plot, two of the PVC pipes were planted with a 14 week old conspecific seedling and one individual of each of the other three species

were planted separately into the remaining three pipes. To test whether feedbacks were related to species functional identity, each focal species was additionally planted, following the same isolation technique, within monocultures of the same functional group. *E.canadensis* was planted in *Pascopyrum smithii (*western wheatgrass), *Elymus trachycaulus* (slender wheatgrass), and *Nassella viridula* (green needle grass) monocultures. *P. virgatum* was planted in *Andropogon gerardii* (big bluestem), *Schizachyrium scoparium* (little bluestem), and *Sorghastrum nutans* (Indian grass) monocultures. *H. maximiliani* was planted in *Monarda fistulosa* (wild bergamot), *Ratibida columnifera* (yellow coneflower), and *Solidago rigida* (stiff goldenrod) monocultures. *Astragulus canadensis* was planted in *Desmodium canadense* (showy tick trefoil), *Dalea purpurea (*purple prairie clover), and *Glycyrrhiza lepidota* (American licorice) monocultures. Thus each focal species experienced seven soil treatments: home soil, soil from the remaining focal species (between functional group), and soil from three other species within the same functional group (within functional group). Resident plot aboveground biomass was trimmed to a height of 5 cm throughout the growing season to prevent light competition with focal plants.

Biomass of the individuals in each PVC pipe was harvested, dried, and weighed in August 2015. For each home treatment, the two observations were averaged, and the average was used for the analysis. Species biomass production across soil treatments was compared using ANOVA (proc glm; SAS v9.3) with soil treatment as a fixed effect and block as a random effect. Biomass values were natural log transformed to meet

ANOVA assumptions. Significant ANOVA results were followed by a Tukey's HSD test to determine differences among treatments.

Experiment 2: Greenhouse soil biota

In August 2015, we conducted a greenhouse experiment to test effects of soil biota on species growth within conspecifically conditioned soil. Soil conditioned by each of the focal species was collected from the monoculture plots with a bulk density hammer (5 x 16 cm cores). Samples (four cores per plot; 4 replicate plots per species) were kept in the hard plastic sleeves in order to maintain field conditions. Soil cores from each plot were randomly assigned to one of four treatments: 1) sterilized 2) oven-heated, 3) heated and re-inoculated (rescue), and 4) control. Because the soil sleeves could not withstand high temperatures, cores for the autoclave treatment were first heated to 60 ° C and removed from their plastic sleeves. These soil cores were then autoclaved (30 min at 120 ° C) and placed back into their original sleeves with care taken to minimize soil disturbance and maintain core orientation. Oven-heated cores were heated to 60 ° C for 48 hours. Compared to the sterilization treatments the oven treatment may allow bacterial spores (Trevors 1996) and fungal mutualists (Izzo et al. 2006) to survive, which may alter recolonization times of biota. Sterilization by autoclaving may also have stronger effects on the levels of harmful extractable elements than oven heating (Wolf et al. 1989). The heating treatments were compared to determine if differences in responses would occur. Rescue treatment cores were first oven-heated to 60 ° C for 48 hours and then a 60 mL soil slurry sieved (No. 20, 60, 100,

and 325 sieves) from a soil sample of the same volume from the same plot was added over a period of three days (20 mL increments). Control cores were stored at 2 ° C prior to planting. A single seedling (5 weeks old for *E. canadensis* and *P. virgatum* and 3 weeks old for *H. maximiliani* and *A. canadensis*) was planted into a soil core and watered as needed. After six weeks, above and belowground biomass was harvested, dried (60 ° C for 48 hours), and weighed. Due to poor establishment and survivorship, *H.* maximilian*i*, was not included in the analysis. Species biomass responses were compared among soil treatments with ANOVA (proc glm; SAS v9.3) with soil treatment as a fixed effect and block as a random effect. Aboveground biomass for *P. virgatum* and root biomass for *E. canadensis* were transformed (natural log) to meet assumptions. Significant ANOVA results were followed by a Tukey's HSD test to determine differences among treatment groups.

Supplemental Information:

Additional studies were conducted to test for soil nematode differences between focal species and to test for treatment effects on soil nitrogen availability (methods and results in the Supplement).

Results

Experiment 1: Field home and away

Soil conditioning affected biomass production of *E. canadensis* ($F_{6,18} = 5.43$, $p = 0.0023$), *H. maximiliani* ($F_{6,18} = 2.81$, $p = 0.0413$), and *A. canadensis* ($F_{6,18} = 7.25$, p =

0.0005) and marginally affected biomass of *P. virgatum* ($F_{6,18}$ =2.58, p = 0.0551). *E. canadensis* and *P. virgatum* were most productive when planted in plots conditioned by the legume *A. canadensis* (Fig. 4.2A, B). The trend was similar for *H. maximiliani*, but only *H. maximiliani* individuals grown in *A. canadensis* conditioned soils were significantly different from individuals grown in conspecifically conditioned (Home) soils (Fig. 4.2C). In contrast, *A. canadensis* was least productive when grown in conspecifically conditioned soil (Fig. 4.2D).

Experiment 2: Greenhouse soil biota

Heating treatments of conspecifically conditioned soil affected *E. canadensis* ($F_{3,9}$ = 8.33, p = 0.0058), *P. virgatum* ($F_{3,9}$ = 19.08, p = 0.0003), and *A. canadensis* ($F_{3,9}$ = 5.07, p = 0.0251) aboveground growth. Treating the soil increased *E. canadensis* (Fig. 4.3A) and *P. virgatum* (Fig. 4.3B) growth irrespective of the actual soil treatment. *A. canadensis* growth was reduced by sterilization, but was not affected by oven heating or the rescue treatment (Fig. 4.3C). Heating treatments marginally affected the root biomass of *E. canadensis* ($F_{3,9}$ = 3.34, p = 0.0697; Fig. 4.3D), but did not affect root biomass of *A. canadensis* ($F_{3,9}$ = 1.11, p = 0.3960), or *P. virgatum* ($F_{3,9}$ = 0.66, p = 0.5982).

Discussion

The objective of this study was to determine the presence and drivers of negative soil feedbacks in four grassland species in newly established communities. Although soil biota appeared to limit growth of the two grasses in a greenhouse setting, it appears that conditioning soil with other non-leguminous plant species does not

release the grasses from these effects. *A. canadensis* was the only species limited by conspecific soil in the field and it appears this effect arises as a result of an abiotic versus a biotic limitation. The only positive effect observed was that soils conditioned by the legume *A. canadensis* increased growth of the other focal species, likely resulting from a fertilization effect. Findings suggest that declines associated with these species within newly established diversity-productivity studies arise as a result of abiotic nutrient limitations rather than through the accumulation of deleterious soil biota.

Experiment 1: Field home and away

The performance of non-legume species increased in soil conditioned by the legume *A. canadensis* in the in-field home and away experiment, which suggests that the growth of the non-legume species are limited by nitrogen availability. All non-legume monocultures may be depleting nitrogen availability at close to the same rate, so performance of the focal species was similar to growth in home soil. The legume *A. canadensis* was least productive in soil primed by conspecifics, which suggests that an abiotic or biotic soil component was altered enough to limit growth.

Experiment 2: Greenhouse soil biota

The heating treatments in the greenhouse experiment led to increased growth in both grass species, which would suggest deleterious biota effects. This is consistent with other greenhouse studies that have observed negative feedbacks attributed to soil microbes in *P. virgatum* (Hawkes *et al.* 2012, Bauer *et al.* 2015*)* and *E. canadensis* (Bauer *et al.* 2015). However, in this study the reintroduction of soil biota in the rescue

treatment did not alter growth. This may have come about because the reintroduced communities did not establish or the increased nutrient availability from heating (Fig. S4.1) offset the negative effects of the biota.

Because there was no effect of the oven or rescue treatments on *A. canadensis* growth, it is likely that soil biota does not have an impact on growth in this experiment. The decreased growth in the sterilization treatment was most likely caused by an increase in a soil nutrient that is toxic at high levels, such as manganese (Mn^{2+}) (Wolf et al. 1989; Mahmood et al. 2014).

Synthesis:

Both soil biota and nutrient availability likely affect the growth of the two grass species. The grass species used in this experiment are very common, and therefore, the soil biota that reduced their growth may be ubiquitous generalists. For example, there was no difference in the presence (only the abundance) of plant parasitic nematodes among plant species (Table S1; Fig. S4.4), so planting in nutrient limited plots conditioned by other non-leguminous species would provide little relief. Planting these grasses in legume field plots would result in greater growth due to increased nutrients.

A nutrient limitation is most likely driving the decreased monoculture yield of *A. canadensis*. While growth was reduced in conspecific conditioned field soils, the greenhouse experiment suggests that this was not a result of soil biota. The USDA NRCS plant fact sheet recommends the addition of phosphorus and potassium to increase stand longevity and yield. Otherwise, the stands will only persist for three to four years.

While our study looked at the effects over the first three growing seasons, feedback effects may vary over longer time periods, and soil biota may become a more important factor as nutrients become even more limited (Van der Putten & Peters 1997; de Deyn et al. 2004; Bezemer et al. 2006). Results suggest nutrient limitation is more important for overyielding than species specific biota for the focal species within the time frame assessed.

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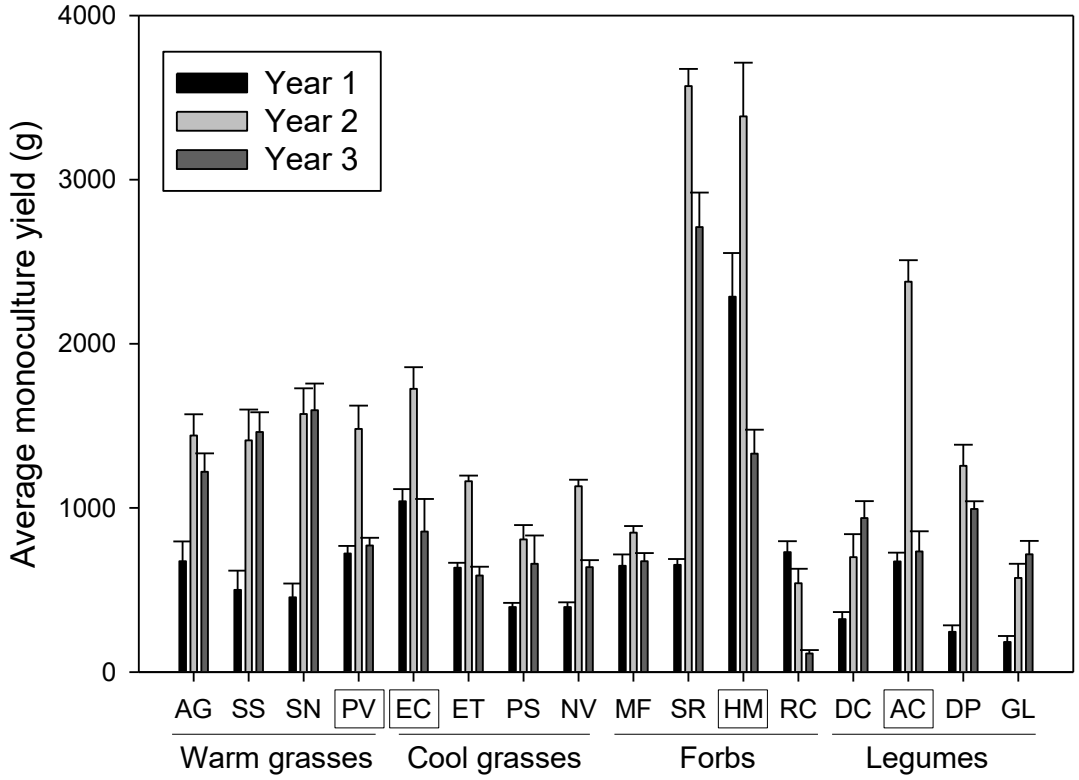

Figure 4.1. Monoculture yields (mean ± SE) of all species for all three years of the SPACE experiment. Species labeled with the first letter of their genus and specific epithet: *Andropogon gerardii* (AG), *Schizachyrium scoparium* (SS), *Sorghastrum nutans* (SN), *Panicum virgatum* (PV), *Elymus canadensis* (EC), *Elymus trachycaulus* (ET), *Pascopyrum smithii* (PS), *Nassella viridula* (NV), *Monarda fistulosa* (MF), *Solidago rigida* (SR), *Helianthus maximiliani* (HM), *Ratibida columnifera* (RC), *Desmodium canadense* (DC), *Astragalus canadensis* (AC), *Dalea purpurea* (DP), *Glycyrrhiza lepidota* (GL). The four squares (□) indicate the species from each functional group that were chosen for the soil experiments.

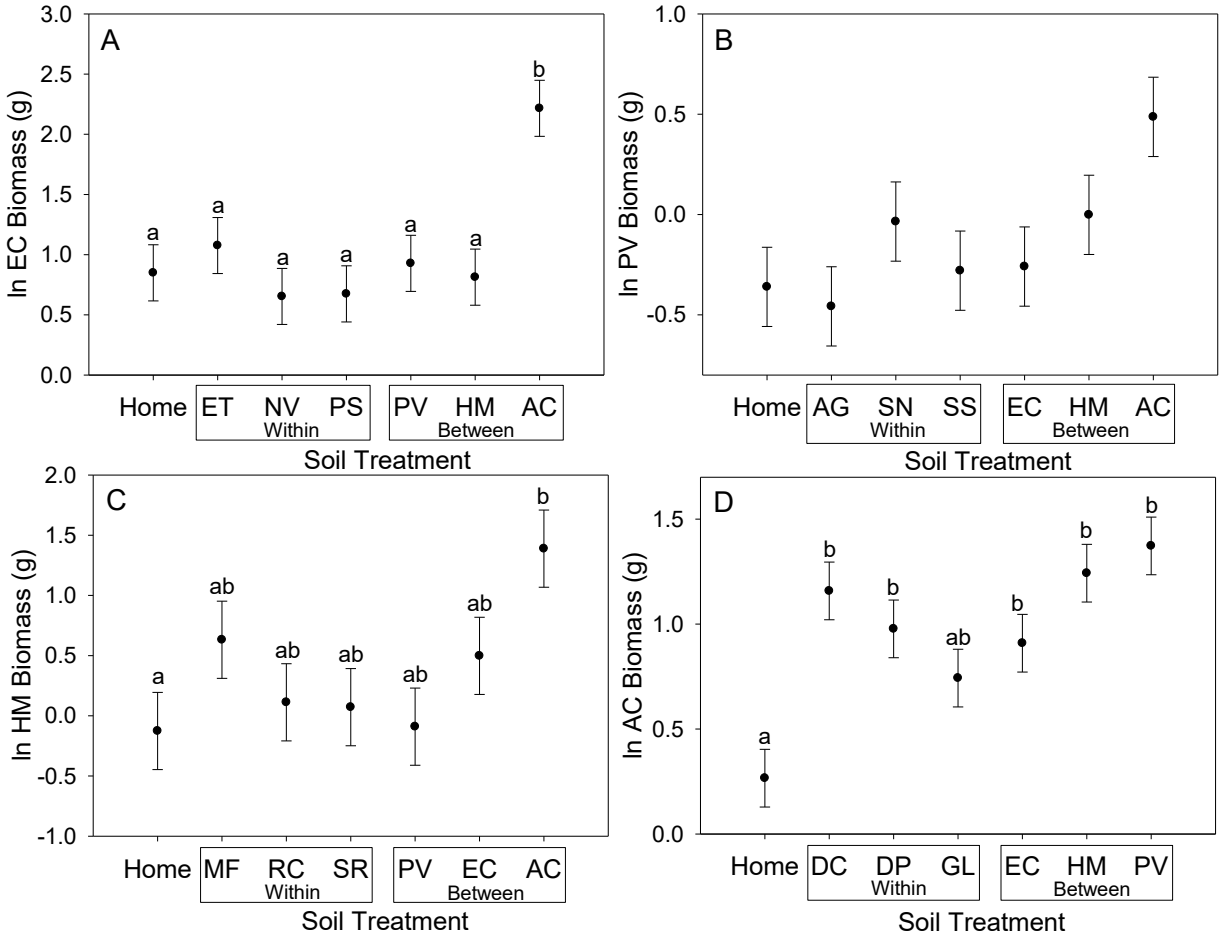

Figure 4.2. Aboveground biomass production (LS mean ± SE grams) of *E. canadensis* (A), *P. Virgatum* (B), *H. maximiliani* (C), and *A. canadensis* (D) in all soil treatments. The first bar in each graph is the home treatment (conspecifically conditioned soil), the next three are within functional group species, and the last three are the other three focal species (other functional groups). Bars within the same panel with different letters are significantly different (Tukey's HSD test). No multiple comparisons test was performed for panel B because the ANOVA was marginally significant.

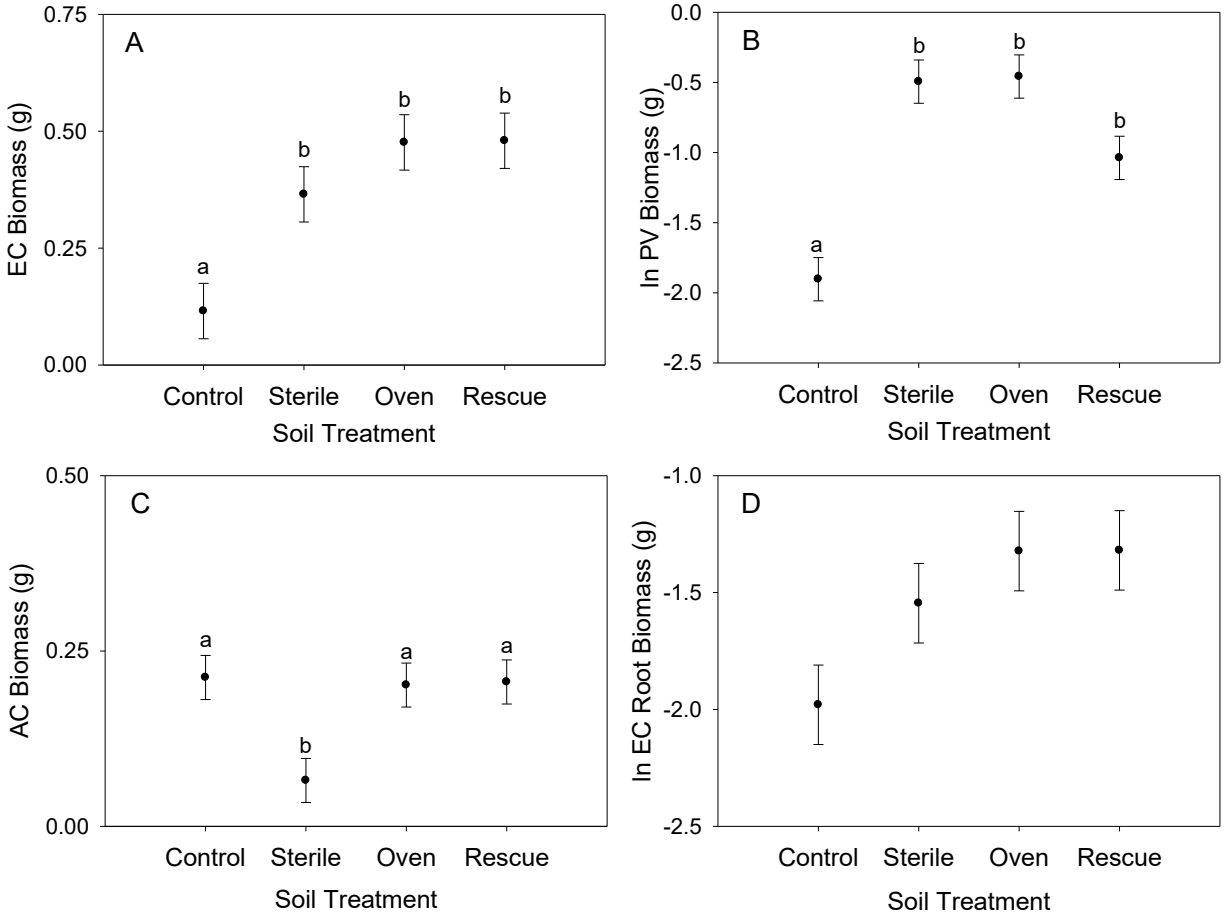

Figure 4.3. Aboveground biomass (LS mean ± SE grams) of *E. canadensis* (A), *P. virgatum* (B), *A. canadensis* (C) and root biomass (LS mean ± SE) of *E. canadensis* (D*)* after 6 weeks growth under greenhouse conditions in conspecifically primed field soil that had been sterilized (Sterile), heated to 60 ° C (Oven), heated to 60 ° C and then soil slurry added (Rescue), or no treatment applied (Control). Bars within each panel with different letters are significantly different (Tukey's HSD test). No multiple comparisons test was performed for Panel D because the ANOVA was marginally significant.

Supplemental Methods and Results

Heating effects on soil nutrients methods:

To determine the effect of the heating treatments on nitrogen (NH_4^+ and NO_3^-) availability, five additional soil cores (1.5 x 15 cm) were collected from the four monocultures of *E. canadensis* and *P. virgatum* in November 2015. The cores from each plot were homogenized and equal weights were either air dried, sterilized in an autoclave (120 ° C for 30 minutes), or oven-heated to 60 °C for 48 hours. Samples were sent to Kansas State University (Soils Lab, Manhattan, KS) for nitrogen analysis. Nitrogen availability among heating treatments was analyzed with ANOVA (proc glm; SAS v9.3) with block (plot) as a random factor and treatment as fixed factor. Significant ANOVA results were followed by a Tukey's HSD multiple comparison test to determine differences between treatments.

Additional bulk density cores were collected and soil cores were either left untreated (control), oven-heated, or autoclaved as in experiment two. Additionally, fertilizer (20% N, 18% P, and 18% K) was added to a fourth soil core to mimic a nutrient flush after heating. A single seedling (4 weeks old) was planted into each soil core and watered as needed. For the fertilized treatment, the granular fertilizer (1.5 g) was mixed with tap water (500 mL) and added to the top of soil core three times during the experiment (at planting, week 2, and week 5). The application rate (0.6 mg/mL N per application) was based on the nitrogen analysis results from Kansas State. The total

nitrogen added over the course of the experiment (9 mg) was triple the difference between the control and sterilized treatments.

After six weeks, above- and belowground biomass was harvested, dried, and weighed. Only *E. canadensis* was included in this analysis due to poor germination of *P. virgatum*. Root biomass among soil treatments was analyzed with ANOVA (proc glm; SAS v9.3) with soil treatment as a fixed effect and block as a random effect. Root biomass was ln transformed to meet ANOVA assumptions. Significant ANOVA results were followed by a Tukey's HSD multiple comparison test to distinguish differences among treatments. Because aboveground biomass did not meet ANOVA assumptions, these data were analyzed using the ANOVA (row means scores) CMH statistic (proc freq; SAS v9.3). To determine differences among treatments, an ANOVA was performed on ranked data and followed by Bonferroni adjusted multiple comparisons test using LS means.

Heating effects on soil nutrients results:

Sterilization and oven heating increased ammonium and nitrate in *E. canadensis* (NH4: $F_{2,9}$ = 35.96, p = 0.0005; NO3: $F_{2,9}$ = 10.07, p = 0.0121) and *P. virgatum* (NH4: $F_{2,6}$ = 96.36, p < 0.0001; NO3: $F_{2,6}$ = 58.06, p = 0.0001) soil (Fig. S4.1). Although heating (sterilization and oven) increased soil nitrogen, heating to remove soil biota affected *E. canadensis* in excess of this fertilization effect.

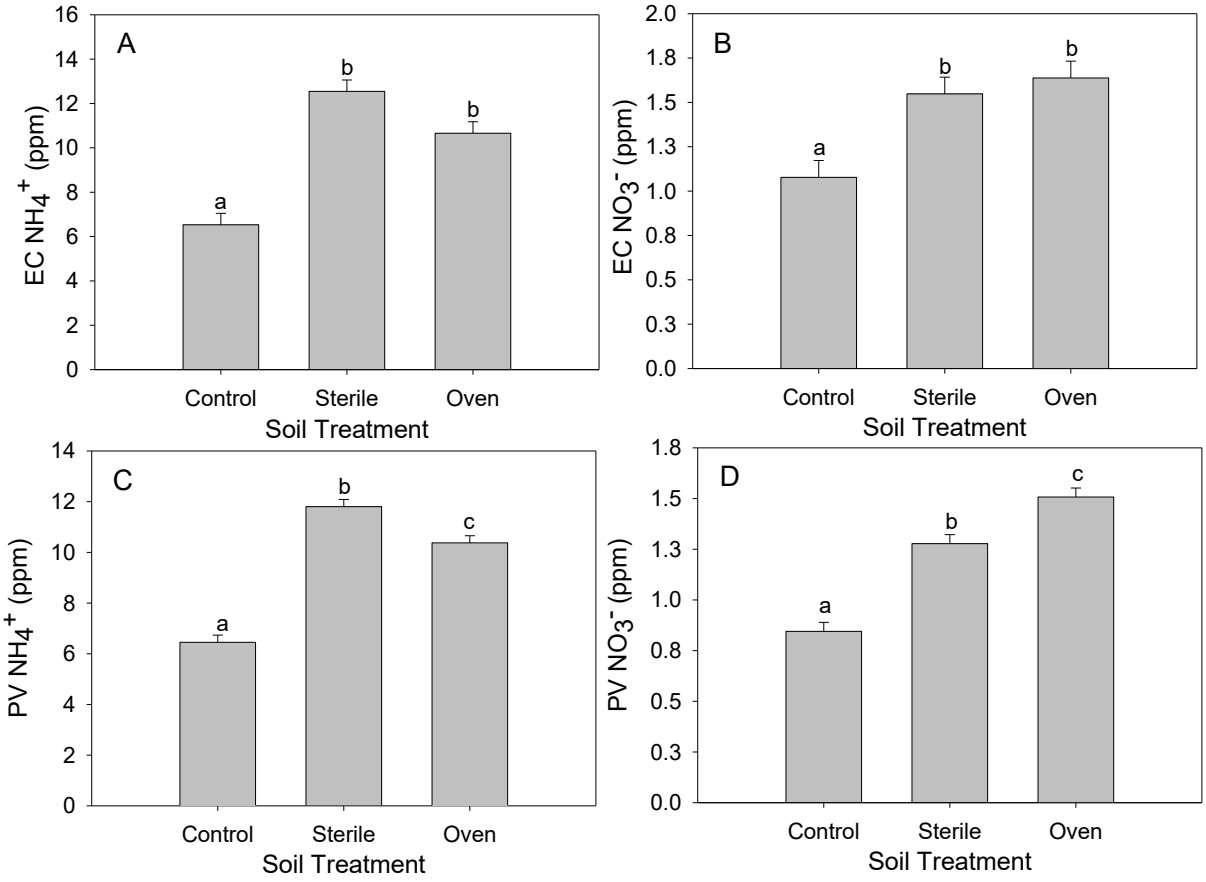

Figure S4.1. LS mean ammonium (NH_4^+) and nitrate (NO_3^-) for soils primed by *E. canadensis* (A,B) and *P. virgatum* (C, D) and either heated to 60 ° C (Oven), autoclaved (Sterile), or untreated (Control). Bars within each graph with different letters are significantly different (LSD test).

Both heating treatments increased above- (CMH statistic = 12.0, p = 0.0074) and belowground biomass ($F_{6,9}$ = 13.22, p = 0.0012) relative to the nitrogen added and control soils (Fig. S4.2).

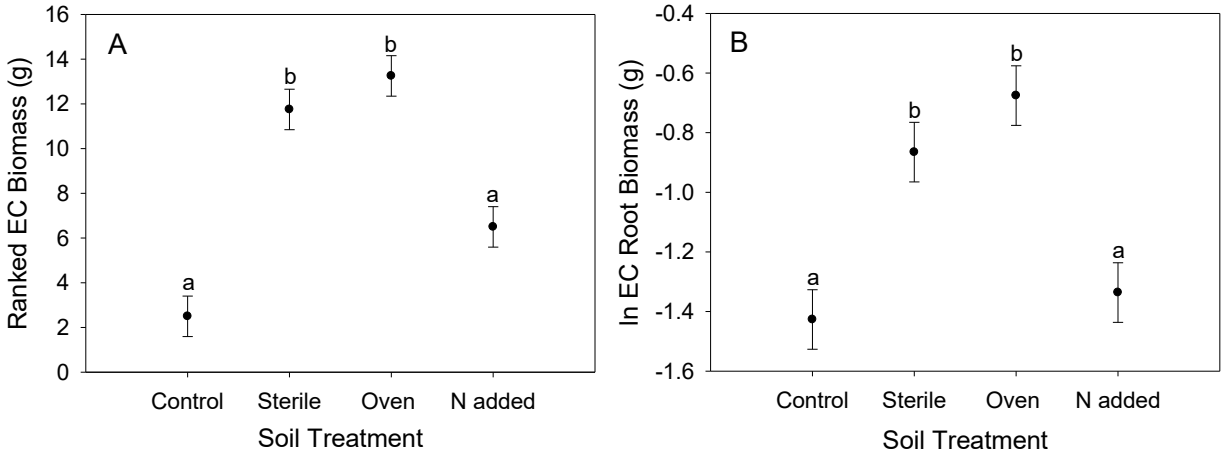

Figure S4.2. Aboveground biomass (LS ranked mean ± SE; A) and root biomass (ln transformed LS mean ± SE; B) of *E.* canadensis grown in conspecifically primed soil cores that were sterilized, heated to 60 ° C, or had nitrogen added. Different letters within each graph indicate treatments are significantly different (Bonferroni adjusted Mann-Whitney (A) and Tukey's HSD test (B)).

Nematode community methods:

In August 2015, three smaller soil cores (1.5 cm x 15 cm) were taken from the field plots of the four focal species (four plots per species) for soil nematode extraction to test for differences in soil nematode communities. Nematode extraction was done with 50-70 g of soil using soil sieves (No. 60 (0.250 mm opening and No. 325 (0.045 mm opening)) and a modification of the Baermann pan method (Viglierchio and Schmitt 1983). After extraction, 10% of each sample was enumerated using a counting dish and stereoscope. Samples were then heat relaxed and fixed in DESS (DMSO and EDTA) solution. After fixing, 120-150 nematodes placed onto slides. The first 100 specimens

were identified to family or genus on an upright microscope using 200x-400x magnification and classified by feeding type according to Yeates *et al.* 1993. Due to similarities in morphology, the genera *Tylenchus* and *Boleodorus* were lumped together and classified as algal and hyphal feeders.

To visualize differences in soil nematode communities among species treatments, Nonmetric Multidimensional Scaling (NMS), using the Sorensen distance measure, was used (PC-ORD 6.0, MjM Software Design, Gleneden Beach, OR, see Peck 2010 for description of analysis steps using the program). Autopilot mode was selected (250 runs) and a random number seed was used for starting configurations. Genera and families present in less than 3 samples were not included in the NMS analysis. To test for differences among species, Blocked Permutation based MANOVA (PerMANOVA), using the Sorensen distance measure, was used (PC-ORD 6.0, MjM Software Design, Gleneden Beach, OR, see Peck 2010 for description of analysis steps using the program).

To determine whether there were differences in parasitic nematodes (endo-, migratory endo-, and ecto-parasitic) among species, the abundance (number per gram of dry soil) of each plant parasitic genera was used as a response variable in a one way ANOVA (proc glm; SAS v9.3) with plant species as a fixed effect and block as a random effect. The abundances of plant parasitic nematodes were ln transformed to meet ANOVA assumptions. Significant ANOVA results were followed by Tukey's HSD multiple comparison test to determine differences among species.

Nematode community results:

Nematode community analysis with NMS gave a three-dimensional solution (Fig. S4.3). The nematode communities associated with both grasses appear to be distinct form those associated with *A. canadensis* and *H.* maximiliani, and the overall PerMANOVA was significant ($F_{3,9}$ = 2.19, p = 0.0024). However, the differences among species were not significant in pairwise tests. The overlay of the nematode abundance vectors suggests that presence of fungal hyphal feeders and root feeders (Table S4.1), except *Helicotylenchus sp*., in plots conditioned by the grasses were driving the separation.

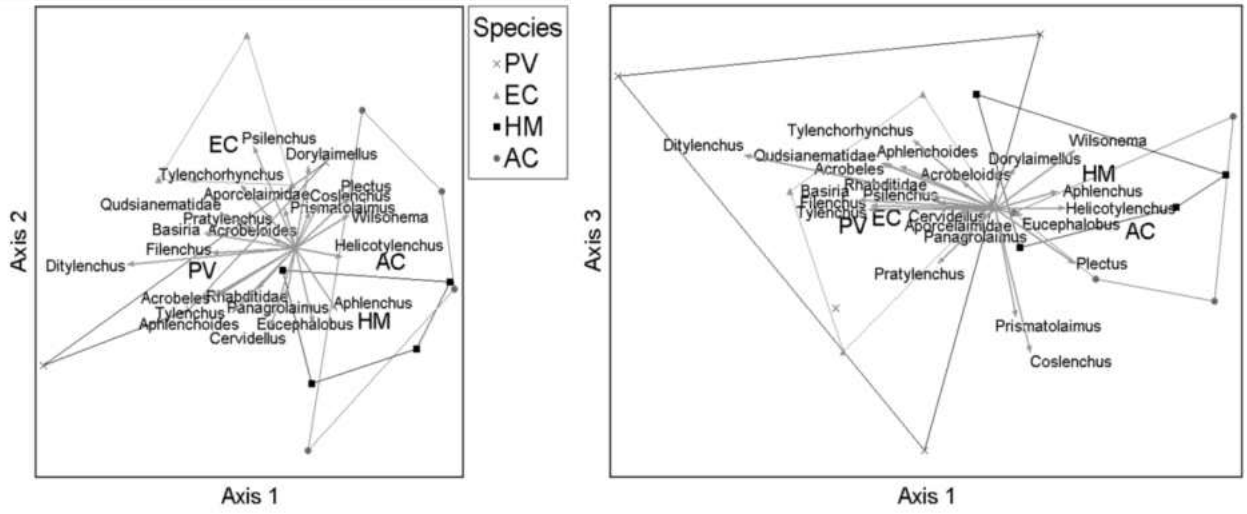

Figure S4.3. NMS ordination results of nematode community comparison among monoculture plots conditioned with *P. virgatum*, *E. canadensis* (EC), *H. maximilani* (HM), and *A. candensis* (AC). The two letter species code is located at the centroid for each set of plots. Vectors are family or genus of nematodes observed.

Of the five plant-parasitic genera present in the soil samples (Table S4.1), *Xiphenema sp.* were only found in two plots, and *Hoplolaimus sp.* were only found in one plot in very low abundance. Therefore, only three genera were included in the ANOVA analysis. There was no difference among plant species in the abundance of *Pratylenchus sp.* ($F_{3,9} = 0.66$, $p = 0.5966$) or *Helicotylenchus sp.* ($F_{3,9} = 2.59$, $p = 0.1175$), but *E. canadensis* had a greater abundance of *Tylenchorhynchus sp.* than *A. canadensis* plots ($F_{3,9} = 7.00$, $p = 0.0100$; Fig. S4.4).

Table S4.1. Feeding habits of the nematode genera and families collected from monoculture plots containing *E. canadensis*, *P. virgatum*, *H. maximiliani*, and *A. canadensis.*

Nematode Taxa	
Bacterial Feeders	Hyphal feeders
Acrobeles	*Aphlenchoides*
Acrobeloides	*Aphlenchus*
Alaimidae	*Ditylenchus*
Cephalobus	*Dorylaimellus*
Cervidellus	*Tylencholaimellus*
Chiloplacus	*Tylenchus/Boleodorus*
Eucephalobus	
Panagrolaimus	*Root Hair feeders*
Plectus	*Basiria*
Prismatolaimus	*Coslenchus*
Rhabditidae	*Filenchus*
Wilsonema	*Psilenchus*
Plant parasites	Omnivores
Helicotylenchus	Aporcelaimidae
Hoplolaimus	Quadsianemitadae
Pratylenchus	*Mesodorylaimus*
Tylenchorhynchus	
Xiphenema	

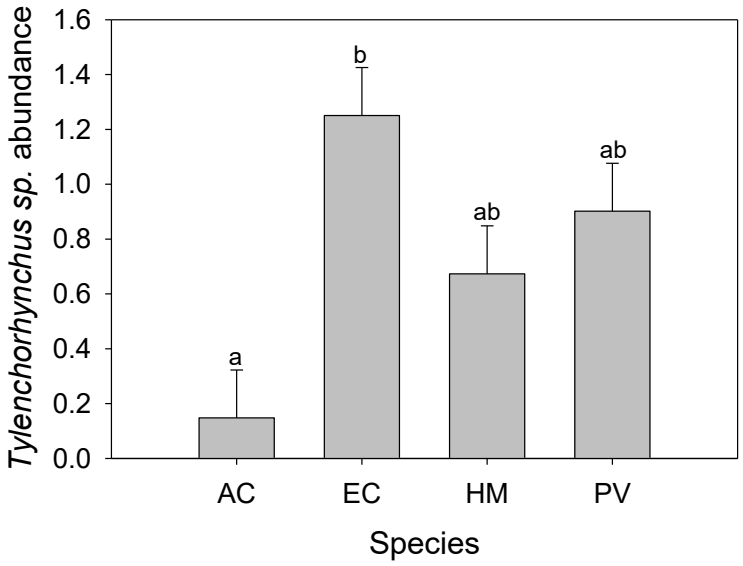

Figure S4.4. Abundance (LS mean number per gram dry soil ± SE) of *Tylenchorhynchus sp.* in soils collected from monoculture plots of *A.canadensis* (AC), *E. canadensis* (EC), *H.maxmiliani* (HM), and *P. virgatum* (PV). Different letters indicate a significant difference (Tukey's HSD test).

Supplement References

Peck, J.E. 2010. Multivariate analysis for community ecologists. MjM Software Design. Gleneden Beach, OR.

Viglierchio, D.R. and R.V. Schmitt. 1983. On the methodology of nematode extraction from field samples: Baermann funnel modifications. Journal of Nematology. 15: 438-444.

Yeates, G. W., T. Bongers, R. G. M. De Goede, D. W. Freckman, and S. S. Georgieva.1993. Feeding habits in soil nematode families and genera—an outline for soil ecologists. Journal of Nematology 25: 315.

CHAPTER V: ABOVEGROUND AND BELOWGROUND EFFECTS OF FUNGAL PRESENCE IN A NATIVE AND CULTIVATED WESTERN WHEATGRASS

Abstract

The presence of aboveground fungal endophytes in introduced cool-season grasses can have above- and belowground effects which may lead to changes in plant community dynamics. Very few studies have looked at effects of fungal endophytes in grasses native to the northern Great Plains. Knowing the role of fungal presence in native cool season grasses may assist land managers in restoration attempts and improve outcomes. The goal of this experiment was to determine fungal effects in a native grass western wheatgrass (*Pascopyrum smithii*) and one of its' cultivars, 'Rodan' western wheatgrass. Seedlings (n = 16) of each grass type were planted into a sub-plot (0.5 x 0.5 m; 4 x 4 grid pattern) of a larger plot (1 x 1 m) in the spring of 2012. The seedlings within each plot were either all native wheatgrass or all cultivar, and each individual consistently tested positive or consistently tested negative for fungal presence using an immunoblot assay before planting. The buffer (remainder of main plot without seedlings) was seeded with a native seed mix. Plots were weeded monthly during the growing season. At the end of two growing seasons, all plant material was cut 3-5 cm above the soil surface, separated to species, dried to constant mass (60 ° C), and weighed. Main plot biomass was analyzed separately from buffer biomass. Soil

cores were taken for root analysis, nematode community analysis, and soil chemistry analysis. Main plot biomass was greater in plots planted with the Rodan cultivar. This was expected as this was one of the characteristics selected for in this population. There were indicators of fungal presence effects on buffer biomass, abundance of *Tylenchorhynchus* spp., root length, and bacterial substrate use. However, the lack of specificity of the fungal testing method created difficulty in interpreting the results. We recommend the use of multiple methods to determine specific fungal presence to ensure consistent fungal treatments are applied in field experiments.

Introduction

Prairie restoration and reconstruction managers typically create a seed mix that encompasses all functional groups of a prairie plant community: C4 (warm-season) grasses, C3 (cool-season) grasses, forbs, and legumes (Smith et al. 2010). Cool-season grasses are a key component of the prairie plant community because they provide forage, habitat, and compete against non-native plants in the spring and the fall (Vinton et al. 2001). These attributes are of even greater importance in the northern plains region because plant communities in the northern latitudes are afforded a very short growing season. Besides the advantages these grasses provide due to their functional status, native cool-season grasses can be associated with fungal endophytes of the genus *Epichloe* (Faeth et al. 2004), which may alter aspects of plant community establishment (Malinowski & Belesky 2000; Cheplick & Faeth 2009) and soil processes (Omacini et al. 2012).

Endophytic fungi of the genus *Epichoe* live in the above-ground intercellular space of leaf sheaths, stems, inflorescences, and seeds of the grass (Kuldau & Bacon 2008). The endophytes rely on nutrients from the grass for cell processes, and the grass provides shelter and a mode of reproductive transmission. *Epichloe spp*. have been studied extensively in non-native forage grasses, and the symbiosis has most often been categorized as mutualistic (Saikkonen et al. 2006; Cheplick & Faeth 2009). The very few studies done in grasses native to North America have shown that the symbiosis can vary from parasitic to mutualistic, and relationship between endophyte and host may be context dependent upon abiotic and genetic factors (Saikkonen *et. al* 2006; Davitt et al. 2011).

Presence of the endophytes (E+) in introduced forage grasses may lead to increased competitive ability of the host through tolerance to abiotic and biotic factors. For example, E+ grasses produce alkaloids that reduce above-ground herbivory by ungulates (Clay & Schardl 2002) and insects (Richmond et al. 2004). The lack of herbivory could lead to an increase in competitive ability of E+ grasses, which could decrease the species richness of the plant community (Cheplick & Faeth 2009). Also, the presence of an endophyte may increase the drought tolerance and growth of the host by altering the host's physiology (reviewed in Malinowksi & Belesky 2000), and allow E+ grasses to invade diverse plant communities (Rudgers et al. 2005). The summation of these advantages could lead to changes in the above-ground plant community structure.

Even though *Epichloe* are only present in above-ground tissue, several studies indicate impacts on soil nematodes. Non-native E+ Tall Fescue (*Festuca arundinacea*) plots had lower numbers of two species of plant-parasitic nematodes (*Pratylenchus scribneri* and *Tylenchorhunchus acutus*) than plots containing endophtye free (E-) grasses in a field study (West et al. 1988). Kimmons et al. (1990) found lower levels of *P. scribneri* on E+ tall fescue, and Elmi et al. (2000) found that reproduction and populations of the root-knot nematode *(Meloidogyne marylandi*) were reduced on E+ tall fescue in laboratory studies. The lower numbers of nematodes could be due to the circulation of inhibitory compounds to the roots or the creation of a mechanical barrier by changing the morphology of the roots (Neher 2010). Malinowski et al. (1999) found that root biomass decreased, but the number and length of root hairs was greater in E+ plots than E- plots. No matter the mechanism, grasses with lower herbivorous nematode abundance may have greater biomass and fitness (de Deyn et al. 2003).

E+ grasses could also affect the populations of bacteria and fungi in the soil through alteration of the composition and quantity of root exudates. Organic carbon and nitrogen pools have been found to differ between E+ and E- tall fescue, which could indicate a change in microbial communities (Franzluebbers et al. 1999; Franzluebbers & Stuedemann 2005). In a laboratory study Van Hecke et al. (2005) found an increase in carbon root exudates in E+ Isogenic tall fescue (*Festuca arundinacea* cv. Jesup), and higher microbial activity in soils receiving the E+ exudates. Also, arbuscular mycorrhizal fungi (AMF) colonization of roots may be decreased in E+ grasses compared to E-

grasses (Mack & Rudgers 2008), and decreased colonization of E+ roots may increase the colonization of E- conspecifics (Omacini et al. 2006).

Non-native forage grasses hosting these endophytes have been suggested to alter aspects of plant and soil communities, but the impacts on plant and soil communities by native grass hosts of the northern prairie are unknown. The impacts of using E+ cool season prairie grasses in restorations and reconstructions needs to be studied because the endophyte can have such a far reaching effects on plant competition and soil processes. The use of these grasses, without the knowledge of the benefits or detrimental effects of endophytes, could lead to unsuccessful restoration attempts or unexpected results.

The objective of this study was to determine whether *Epichloe* endophytes have the same impacts in the native cool-season western wheatgrass (*Pascopyrum smithii*) and one of its' culitvars, Rodan western wheatgrass. Both are used in prairie restorations, reconstructions, and for forage production in the northern plains region. The Bismark plant material center in North Dakota developed the Rodan cultivar by selecting and crossing populations for improved leafiness, stand development, winter hardiness, drought tolerance, and disease resistance (USDA and NRCS). Specifically, this experiment addresses whether the presence of *Epichloe* in native western wheatgrass or the Rodan cultivar increases aboveground biomass and tillering/rhizomatus growth, and whether fungal effects reach belowground by altering the community structure of

soil nematodes (with emphasis on root herbivores), root morphology, or nutrient pools in the soil.

Methods

Plant material selection

Native *Pascopyrum smithii* seed was obtained from Milborn Seed (Brookings, SD, USA), and Rodan seed was obtained from the Bismark Plant Material Center (Bismark, ND, USA). One-hundred individuals from each seed source were grown in the University of North Dakota's greenhouse in early winter 2012. Once an individual reached a 3 tiller stage, endophyte presence was assessed with an immunoblot assay (Phytoscreen endophyte detection kit; Agrinostics Ltd. Co., Watkinsville, GA, 162 USA), which tests for the presence of the *Epichloe coenophiala* (formerly *Neotyphodium coenophiala*; Leuchtmann et al. 2014) cell wall proteins. A single tiller from each individual was harvested at the soil surface and placed in a freezer (-10 ° C) inside of a small plastic bag (one tiller per bag). For testing, tillers were removed from the freezer and allowed to thaw. A razor was used to cut a fresh section of each tiller, and the section was pressed onto the assay's membrane in two separate locations, using each end of the tiller section. The membrane was then processed according to the kit directions. All tillers were processed within two months of collection. Processed membranes were scored (deemed positive, negative, or questionable) by at least three observers to ensure accuracy. Tillers that were consistently scored as positive and consistently scored as negative across all observers were used as parent plants to create

more individuals by separating and replanting tillers. After separation, all individuals were tested for endophyte presence again once they reach a three tiller stage.

Experimental design

The field experiment was conducted at the University of North Dakota's Mekinock Field Station in Grand Forks County in spring 2012. The site was planted with wheat the year before the study, and had been in continuous agriculture for 15 years. Soils at the site are moderately well drained LaDelle silt loam with 0 to 2 % slopes. Experimental plots (1 x 1 m) were arranged in a randomized complete block design with 5 blocks. Experimental plots had two parts: the main plot (0.5 x 0.5 m) centered within each plot and the buffer (0.25 m on all sides). The main plot was planted with 16 individuals of either native or Rodan western wheatgrass arranged in a 4 x 4 grid (Fig. 5.1), and all of the individuals in each main plot had either consistently tested positive for fungal presence (E+) or negative for fungal presence (E-). This resulted in a 2 x 2 factorial design with grass type (native and Rodan) and fungal presence (E+ and E-) as treatments.

The buffer area (0.25 m on all sides; Fig. 5.1) in each plot was seeded at 600 seeds/m^2 with a native prairie mix composed of three warm season grasses (*Sorghastrum nutans, Andropogan gerardii, Schizachyrium scoparium*) three forb species (*Helianthus maximiliani, Monarda fistulosa, Ratibida columnifera*) and three legumes (*Dalea purpurea, Desmodium canadensis, Glycyrrhiza lepidota*).

Plant Sampling Methods

Throughout the growing season, weeds were removed from each monthly. Very few natives seeded in the buffer established in the main plot over the two growing seasons. These were removed were removed to avoid alteration of soil responses. Fungal presence was monitored in plots by randomly harvesting 10 tillers/rhizomes per plot three weeks after planting in the first growing season and the first week of June in the second growing season. Harvested tillers were tested for fungal presence using an immunoblot assay (same method as used in *Plant material selection*).

In late summer (end of August) of 2012 and 2013, an average tiller/rhizome count was calculated by taking the average tiller/rhizome count of five grid squares in the main plot. The same five grid squares were used for each count, and the pattern of squares chosen was the same for all plots. All plant material was then cut 3-5 cm above soil surface, separated to species, dried to constant mass (60 C), and weighed. Main plot biomass was analyzed separately from buffer biomass.

In Rodan plots, *Elymus trachyaulus* was misplanted in place of the Rodan cultivar (0-2 individuals per plot). The biomass was analyzed separately in fall of 2013, but the biomass was combined in the first year. ANCOVA analysis with the proportion of *Elymus trachyaulus* planted as the covariate showed no influence on the main plot biomass and buffer biomass (results not presented). In the fall of 2013, the biomass of each Rodan plot was adjusted for the number of misplants. The main plot biomass was divided by the number of Rodan individuals. This average biomass per individual of Rodan was

multiplied by the number of *Elymus trachyaulus* misplants and added to the main plot biomass. *Elymus trachyaulus* was not included in the analysis of main plot biomass.

Belowground sampling

Three soil cores (2 cm wide x 20 cm depth) were taken from each main plot in the fall of 2012, spring of 2013, and fall of 2013 to gauge change in soil responses over time. Samples for each plot were homogenized and allocated for: nematode extraction, soil chemical analysis, root length and biomass analysis, and soil metabolic profile analysis. For each sample, ~10 g of wet field soil was placed into a soil tin, dried at 100 ° C for at least 24 hours, and then weighed to calculate gravimetric soil moisture and determine the dry weight of each sample. This dry weight was calculated to relativize weight across samples varying in moisture content.

Nematodes

Nematode extraction was done with 50-70 g of soil using soil sieves (No. 60 (0.250 mm opening and No. 325 (0.045 mm opening)) and a modification of the Baermann pan method (Viglierchio and Schmitt 1983). After extraction, 10% of each sample was enumerated using a counting dish and stereoscope. Samples were then heat relaxed and fixed in DESS (DMSO and EDTA) solution. After fixing, 120-150 nematodes were picked onto slides using a stereoscope at low magnification. The first 100 specimens were identified to family or genus and on an upright microscope using 200x-400x magnification and classified by feeding type (Yeates et al. 1993).

Roots

In the first growing season, roots were collected from 50 g of each homogenized sample. Root samples were placed on a stack of soil sieves (No. 10 (2 mm opening) and No. 20 (0.841 mm opening)), and soil was washed away from the roots using a hose with a spray nozzle. All visible roots from each sieve were collected. Roots were scanned with an STD4800 Scanner (400 dpi) and analyzed using WINRHIZO^{TM} software (scanner and software from Regent Instruments Inc., Quebec, QC, Canada). The roots were then dried at 60 C and weighed. In spring and fall 2013, roots were collected from each nematode soil sample, rather than a separate 50 g of soil, for comparison of nematode abundance and root morphology.

Soil metabolic profile

BIOLOG LOG ECO plates (Hayward, CA, USA) were used to compare the physiological profile of the bacterial communities for each treatment. A plate contains 31 different carbon substrates utilized by certain bacteria as well as a control well where water is added. Utilization of a substrate is indicated by a color change in the well. A soil slurry was prepared by adding 2.0 g dry weight equivalent of a soil sample to 200 ml of deionized water, then 100 µl of the soil slurry was applied to each well on the plate. The plates were incubated at 25˚C, and color change (absorbance values) was measured at 24, 48, and 72 hours with an EPOCH plate reader (Winooski, VT, USA) at 595 nm. There were two plate replicates per soil sample. The absorbency readings were

prepared for analysis by subtracting the control value from each well and averaging across plate replicates.

Soil Chemistry

Soil samples were sent to Kansas State University soil laboratory (Manhattan, KS) for NO_3^- and NH_4^+ analysis (KCl extraction). Also, PRS probes (Plant Root Simulator probes; Western Ag Innovations Inc., Saskatoon, SK Canada) were used to assess nutrient supply rates and presence (NO_3^-, NH_4^+, Ca^{2+}, Mg^{2+}, K^+, P, Fe, Mn, Cu, Zn, B, S, Pb, Al, Cd) for the second growing season. The probes contain an ion exchange membrane that continuously absorbs charged ionic species during the burial period. To avoid competition with roots for ions, a PVC pipe (20 cm long x 10 cm diameter) was driven into the ground in the center of each main plot. The probes were installed inside the PVC in May 2013, and all plants were removed from inside the PVC during the burial period. In August 2013, the probes were removed, rinsed with deionized water, and shipped to Western Ag. innovations for analysis.

Data Analysis

Repeated Measures ANOVA with Kenwood-Rogers degrees of freedom adjustment (Proc mixed; SAS 9.3, SAS Institute Inc., Cary NC, also see Littell et al. 1998 and Littell et al. 2002) was used to test for grass population and fungal presence effects on tiller/rhizome number, plant biomass in the main plot and buffer, herbivorous nematode abundance, root morphology, and soil nitrogen. Sampling time, grass population, fungal presence, and block were considered fixed effects. Main plot

biomass, buffer biomass, root length, ammonium, nitrate, and abundance of herbivorous nematodes were natural log transformed to ANOVA assumptions. Significant ANOVA interaction results were followed by a Least Significant Difference (LSD) multiple comparison test to distinguish differences between groups. Nonmetric Multidimensional Scaling (NMS), using the Sorenson distance measure, was used to visualize differences in bacterial substrate use, nematode communities, and PRS probe results. Blocked Multi-Response Permutation Procedure (BMRPP), using the Euclidean distance measure, was used to test for differences in groups. Both NMS and BRMPP were done in PC-ORD 6.0 (MjM Software Design, Gleneden Beach, OR, see Peck 2010 for description of analysis steps using the program).

Results

Fungal presence

The average percentage of positive testing rhizomes (n = 10 rhizomes/plot) increased in plots with and without fungal presence within grass population from year 1 to year 2, and the disparity between positive and negative plots was less in year 2 (Fig. 5.2).

Aboveground Biomass

Average tiller/rhizome number per grid square in the main plot decreased ($F_{1,16}$ = 8.63, p = 0.0097) from year one (LS mean ± Standard Error (SE) = 34.88 ± 1.278 g) to

year two (LS mean ± SE = 28.51 ± 1.726 g), but grass population ($F_{1,11}$ = 1.26, p = 0.2849) and fungal presence ($F_{1,11}$ = 0.01, p = 0.9110) had no effect.

Main plot biomass increased from year one (LS transformed mean ± SE = 4.73 ± 0.028 g) to year two (LS transformed mean ± SE = 5.36 ± 0.0525 g; Table 5.1). Rodan plots (LS transformed mean ± SE = 5.12 ± 0.051 g) produced marginally more biomass in the main plot than native plots (LS transformed mean ± SE 4.97 ± 0.051 g; Table 5.1). Fungal presence had no effect on main plot biomass (Table 5.1).

Buffer biomass significantly increased from year one (LS transformed mean ± SE = 2.11 ± 0.161) to year two (LS transformed mean ± SE = 5.87 ± 0.043; Table 5.1). Plots with fungal presence (LS transformed mean ± SE = 4.19 ± 0.136 g) had marginally more biomass in the buffer than plots without fungal presence (LS transformed mean ± SE 3.79 ± 0.136 g; Table 5.1). Rodan plots with fungal presence had marginally greater buffer biomass than Rodan plots without fungal presence (Grass population x Fungal presence; Table 5.1, Fig. 5.3).

Nematodes

The nematode community data from fall 2012 produced a one dimensional solution with NMS, and the BRMPP found no difference between treatments (A = 0.0124, p = 0.2967). In spring 2013, a two dimensional solution was found with NMS, but the BRMPP found not difference between treatments (A = 0.0054, p = 0.3890). In the fall of 2013, the data was too weakly structured to perform NMS, and the BRMPP showed no difference between treatments (A = -0.0164, p = 0.6528).

The two most prominent migratory plant parasitic nematodes in this experiment were *Pratylenchus* spp. and *Tylenchorhynchus* spp. (Table 2). There was no effect of grass population ($F_{1,11.8}$ = 1.21, p = 0.2940) or fungal presence ($F_{1,11.8}$ = 0.01, p = 0.9322) on the abundance of *Pratylenchus* spp. *Tylenchorhynchus* spp. increased overtime ($F_{2,15}$ = 38.90, p < 0.001), and grass population marginally effected abundance ($F_{1,11.7}$ = 4.20, p = 0.0633). Rodan plots (LS transformed mean ± SE = 1.33 ± 0.079 nematodes/gram dry soil) had marginally greater abundance than native plots (LS transformed mean ± SE = 1.10 ± 0.079 nematodes/gram dry soil). There was also a sampling time by fungal presence interaction ($F_{2,15}$ = 6.21, p = 0.0109). Fungal presence had no effect on abundance at sampling points two or three, but plots with fungal presence had greater abundance of *Tylenchorhynchus* spp. than plots without fungal presence at sampling point one (Fig. 5.4).

Roots

Root biomass increased over time, but there was no effect of grass population or fungal presence (Table 5.1). Root length increased over time, and plots without fungal presence (LS transformed mean ± SE = 1.53 ± 0.067 cm/g dry soil) had greater root length than plots with fungal presence (LS transformed mean ± SE = 1.28 ± 0.067 cm/g dry soil). There was no difference in root length between grass populations.

Soil Metabolic Profile

In fall 2012, the overall BMRPP for the BIOLOG ECO plates at 24 hours (A = -0.0312, p = 0.8974), 48 hours (A = 0.0378, p = 0.1417), and 72 hours after the soil solutions were placed on the plates were not significant (A = 0.0146, p = 0.2577).

In spring 2013 at 24 hours, the overall BMRPP (A = 0.0001, p = 0.4572) and pairwise comparisons were not significant. At 48 hours, the overall BMRPP was significant (A = 0.0561, and p = 0.0314), and Rodan plots with and without fungal presence were significantly different in the pairwise comparisons (A = 0.1367, p = 0.0234). A two dimensional solution was found with the NMS (Fig. 5.5). At 72 hours, the overall BMRPP was significant (A = 0.0629, and p = 0.0076), and a two dimensional solution was found with the NMS (Fig. 5.6). In the pairwise comparisons, Rodan plots with and without fungal presence (A = 0.1511, p = 0.0177), Rodan plots with fungal presence and native plots without fungal presence (A = 0.1308, p = 0.0184), and Rodan plots without fungal presence and native plots without fungal presence (A = 0.0654, p = 0.0382) were significantly different.

In fall of 2013 the overall BMRPP and pairwise comparisons at 24 hours (A = -0.0453, p = 0.9601) 48 hours (A = -0.0363, p = 0.8551) and 72 hours (A = -0.0592, p = 0.9977) were not significant.

Soil Chemistry

Ammonium in soil samples decreased overtime ($F_{2,15} = 58.85$, $p < 0.001$) , but there was no effect of grass population ($F_{1,11.4} = 1.11$, $p = 0.3149$) or fungal presence ($F_{1,11.4} = 2.47$, $p = 0.1437$). Nitrate in soil samples decreased overtime ($F_{2,15} = 24.45$, $p < 0.001$), but there was no effect of grass population ($F_{1,11.4} = 0.08$, $p = 0.7831$) or fungal presence ($F_{1,11.4} = 0.01$, $p = 0.9403$). The overall BRMPP of the PRS probe data was not significant ($A = 0.0026$, $p = 0.4359$).

Discussion

The objective of this experiment was to determine whether the seed source population or fungal presence had aboveground or belowground effects in western wheatgrass. As would be expected, the Rodan cultivar had greater biomass production in the main plot than the native population. This is one of the properties selected for in the Rodan population and it is substantiated by USDA NRCS studies (Rodan release brochure 1988 and revised 2012). Grass population also had an effect belowground, as Rodan plots had a greater abundance of *Tylenchorhynchus* nematodes. Fungal presence increased buffer biomass, increased the abundance of *Tylenchorhynchus* spp., decreased root length, and altered bacterial substrate use. However, interpretation of fungal presence effects was complicated by the lack of specificity of the testing kit used.

While the increased biomass production was expected in Rodan plots, it is more difficult to interpret why Rodan plots had a greater abundance of *Tylenchorhynchus* spp. Regardless of the grass seed source, root length, root biomass, and abundance of

Tylenchorhynchus spp. increased over time. This suggests that the population of *Tylenchorhynchus* spp. was limited by root abundance, and root growth was not effected by *Tylenchorhynchus* spp. abundance. *Tylenchorhynchus rosbustoides* has been shown to reduce aboveground biomass of western wheatgrass (Smolick 1982), but in this experiment the plots with greater nematode abundance had greater aboveground biomass. It may be that the roots were not scanned at a fine enough resolution to examine root hair length. Individuals from the Rodan population may have more root hairs, which could possibly support a greater number of *Tylenchorhynchus* spp. Another possibility is that there is a difference in the quality of the roots, such as nutritional value or texture, which allows for greater reproduction of *Tylenchorhynchus* spp. in Rodan plots.

The results also suggest that fungal presence altered buffer biomass, abundance of *Tylenchorhynchus* spp., root length, and bacterial substrate use. However, due to insights gained during the experiment, interpretation of the results is extremely difficult. The kit used to test for endophyte presence is specifically designed for use with *Epichloe coenophiala* in *Festuca arundinacea* (Tall Fescue). There have been some instances where the kits have been used for native grasses present in the northern Great Plains, but they were used in conjunction with genetic confirmation or visual inspection for fungal hyphae (Vinton et al. 2001; Saha et al. 2009). Only the kit was used in this experiment. This fact along with the subjectivity involved in determining a positive or negative result based on color change and Jensen et al. 2011 finding false positives and

false negatives when using this type of kit in tundra grasses, led to the questioning of the test results.

During the second growing season of the experiment, several cuttings were made of stems and leaves of tillers in plots with and without fungal presence. The material was brought into lab, surface sterilized, and then placed on plates with potato dextrose agar. Once a culture began to grow, it was separated onto another plate. Sections of these cultures were then blotted onto the endophyte testing kits and analyzed. Several of the cultures returned a positive result or a result that could be misinterpreted as a positive result based on color change (pink color appeared). ITS DNA sequencing revealed the cultures to be fungal rusts and other saprophytic fungi, but not of the genera *Epichloe* (data not presented). Rust presence on leaves and stems was apparent in some plots during the growing season, and there was visual evidence of what appeared to be *Claviceps purpurea* (Ergot) on some of the seed heads. These results led to the conclusion that the identity of the actual treatment of plots with and without aboveground fungal presence was not known and interpretation of results should be conservative or not attempted.

The tests of cultures grown in the lab were not replicated in any fashion, and therefore cannot be used to scrutinize the results of the kits when used for *Festuca arundinacea.* These results also do not mean that a *Neotyphodium* or *Epichloe* endophyte is not present in Rodan or native western wheatgrass. The tests could very

well be picking up their presence, but it may be that some species of the fungi are difficult to culture in the lab, and therefore were not identified through ITS sequencing.

In conclusion, there is a need to understand the role of endophytes in native grasses, but the use of multiple techniques, molecular techniques always being one of them, is necessary to ensure treatments are properly identified and taken into account.

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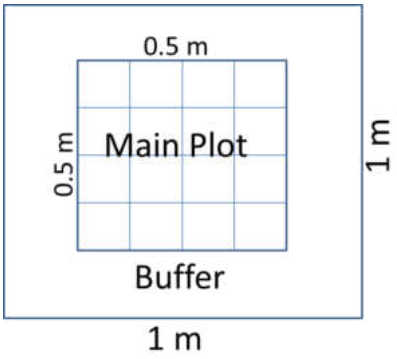

Figure 5.1. Diagram of experimental plots used in the experiment. The main plot was planted with 16 transplants of a single grass population with or without fungal presence, and the buffer was seeded with native plant species.

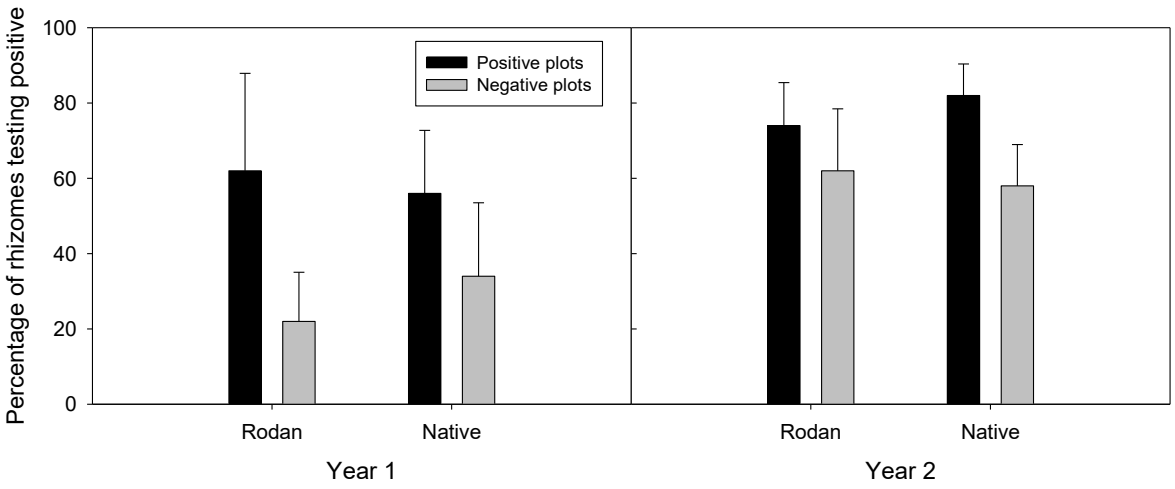

Figure 5.2. The mean percentage of rhizomes (n = 10 rhizomes/plot) that had a positive fungal test for plots planted with individuals of each grass population (Rodan and Native) and had (positive) or did not have (negative) fungal presence. Each bar is the average across 5 replicates. Error bars are standard deviation.

Table 5.1. The ANOVA results (degrees of freedom (df) and F statistic) for grass population (native or Rodan) and fungal presence (E+ or E-) effects on aboveground biomass in the main plot and buffer, root biomass, and root length over the three sampling periods.

	Main plot		Buffer biomass		Root biomass		Root length	
Variable	df	F	df	F	df	F	df	F
Block	4,12	0.45	4,12	0.86	4,12	1.09	4,12	1.83
Fungal presence (FP)	1,11.8	0.34	1,13.3	4.43†	1,12.2	0.61	1,13.5	7.35*
Grass population (GP)	1,11.8	4.17†	1,13.3	0.15	1,12.2	0.00	1,13.5	1.16
FP x GP	1,11.8	0.13	1,13.3	3.17†	1,12.2	1.22	1,13.5	0.99
Time	1,16	55.50**	1,16	756.64**	2,15	53.44**	2,15	119.89**
Time x FP	1,16	0.68	1,16	1.51	2,15	1.09	2,15	0.15
Time x GP	1,16	0.40	1,16	0.00	2,15	0.09	2,15	2.33
Time x FP x GP	1,16	0.24	1,16	2.87	2,15	0.25	2,15	0.24

(† = $p < 0.10$, * = $p < 0.05$, ** = $p < 0.01$)

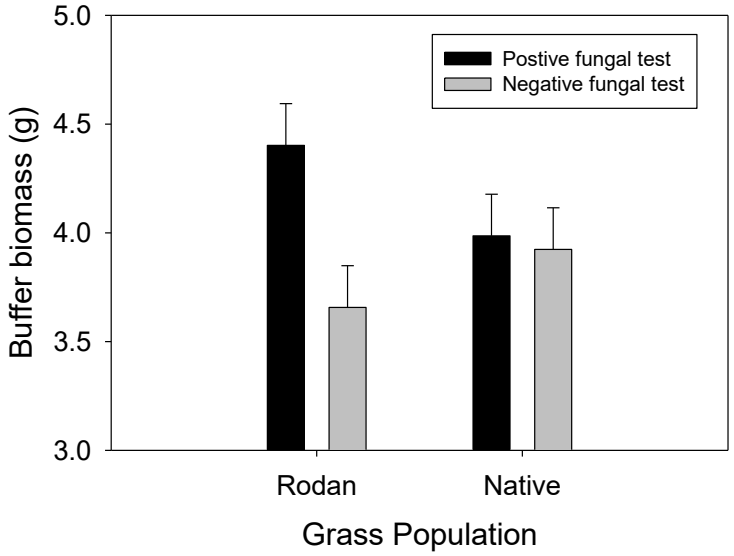

Figure 5.3. Buffer biomass (LS ln transformed mean ± SE) for plots of each grass population with (positive) and without (negative) fungal presence. No multiple comparisons test was performed because the ANOVA was marginally significant.

Table 5.2. Nematode genera and families observed in soil samples at the three sampling times and the percent of samples with each type present (n =20 samples).

	Fall 2012	Spring 2013	Fall 2013
Bacterial feeders			
Acrobeloides	95	100	100
Acrobeles	50	40	50
Panagrolaimus	50	65	70
Panagrellus	0	10	10
Eucephalobus	90	70	50
Cephalobus	25	0	0
Plectus	90	95	100
Plectus II	50	40	10
Prismatolaimus	65	70	20
Rhabditidae	100	100	100
Alaimidae	35	5	25
Chiloplacus	35	55	35
Cervidellus	45	60	15
Wilsonema	15	15	15
Algal and fungal feeders			
Aphlenchoides	80	85	75
Aphlenchus	70	95	95
Boleodorus	15	60	70
Ditylenchus	45	75	60
Dorylaimellus	50	40	35
Tylencholaimus	5	0	5
Tylencholaimellus	5	15	30
Tylenchus	55	55	65
Root hair feeders			
Basiria	25	50	65
Coslenchus	70	35	70
Filenchus	95	100	100
Psilenchus	15	15	50
Neopsilenchus	35	20	5
Clavilenchus	15	0	5
Plant parasites			
Helicotylenchus	45	85	65
Meliodygne	45	35	25
Pratylenchus	100	95	90
Paratylenchus	35	25	0
Tylenchorhynchus	100	100	100
Xiphenema	20	5	30
Omnivores			
Aporcelaimidae	60	50	30
Eudorylaimus	5	5	5
Microdorylaimus	10	10	50
Thonus	0	0	35
Predators			
Pristionchus	0	0	10

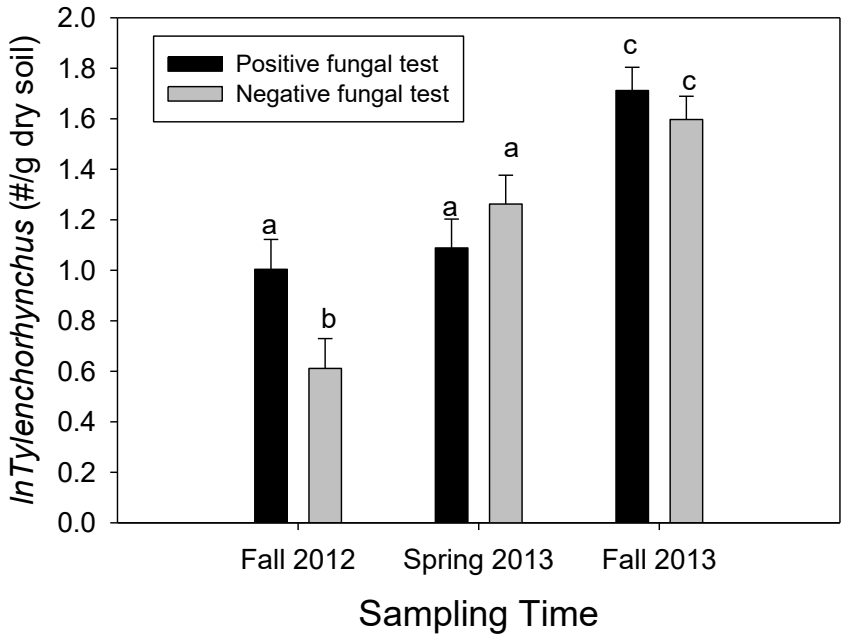

Figure 5.4. Ln transformed LS mean (± SE) abundance of *Tylenchorhynchus* spp. (#/g dry soil) in plots with (positive) and without (negative) fungal presence at the three sampling points. Bars with different letters are significantly different (LSD test).

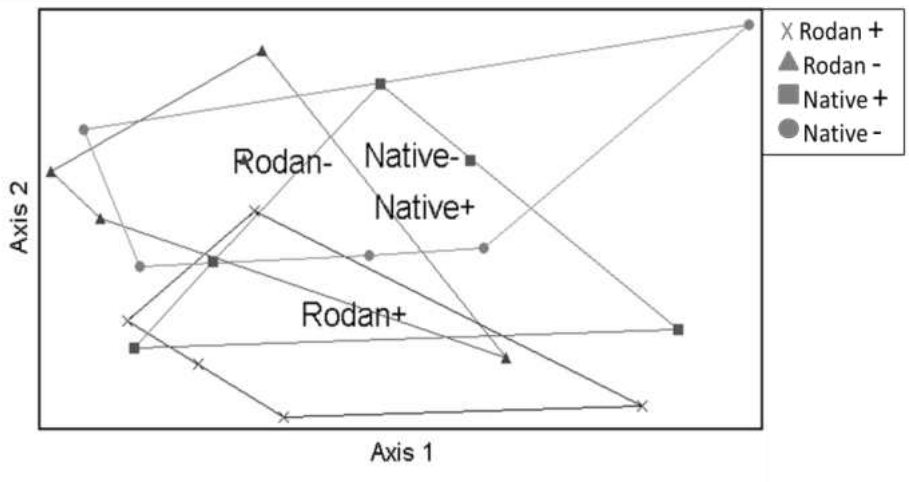

Figure 5.5. NMS ordination of spring 2013 BIOLOG ECO plate results at 48 hours for Native and Rodan plots with (+) and without (-) fungal presence. Plot type labels (Rodan+, Rodan-, Native+, Native-) are arranged at the centroid for each group.

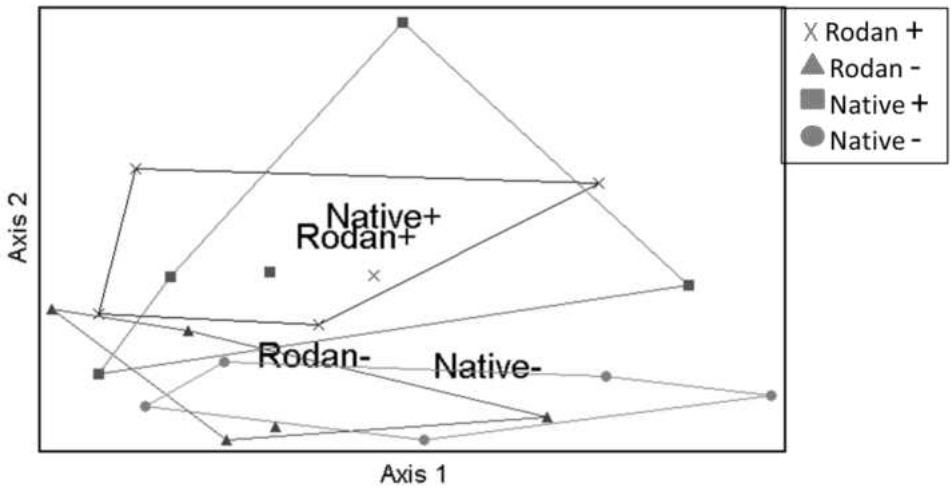

Figure 5.6. NMS ordination of spring 2013 BIOLOG ECO plate results at 72 hours for Native and Rodan plots with (+) and without (-) fungal presence. Plot type labels (Rodan+, Rodan-, Native+, Native-) are arranged at the centroid for each group.

CHAPTER VI: CONCLUSIONS

The objective of the work in the preceding chapters of this dissertation was to examine effects of plant community structure, soil components, and aboveground fungal presence on plant productivity. The knowledge and insights gained from each experiment are summarized in the following paragraphs.

In Chapter Two, the goal was to ascertain how changes in neighborhood interspecific relationships in plant communities affect diversity and productivity responses along perennial grassland richness and evenness gradients. Aggregation decreased productivity and increased diversity, which reinforce previous findings. The lack of an aggregation effect on complementarity suggests that productivity was decreased by increasing species coexistence and not by decreasing facilitation and niche-partitioning. Due to the minimal effects on species productivity, the scale of aggregation was likely not large enough to substantially isolate individuals from heterospecific interactions. This experiment improved upon previous studies by planting communities in the field, using a larger plot size (1 x 1 m), and by using a large diverse species pool (16 species). In future studies, holding species and arrangement within plots constant across treatments may decrease variability in species responses and give better insight on aggregation effects.

To gain insight on the mechanisms of the diversity-productivity relationship, ecologists may use the additive partitioning model and Diversity-Interactions modeling. While the utility of using each modeling approach has been discussed, little has been said about the difference in interpretation using sown and realized proportions. In Chapter Three, varying proportions in the modeling approaches altered model outcomes and interpretations of diversity effects on productivity. In the additive partitioning model, the magnitude of selection effects was greater using sown proportions than realized proportions due to changes in the relationship between the treatments and selection effects. In the Diversity Interaction modeling approach, diversity effects were present in years two and three using sown proportions and absent using realized proportions. In both modeling approaches, the difference in outcomes was due to the difference in reference point in time and may be related to the size-density relationship of species in the pool. Realized proportions are based on within year variation in biomass production, and sown proportions are based on the planted density of individuals at the beginning of the experiment. Because expected yields are based on biomass production in the additive partitioning model, it seems more appropriate to use realized proportions to avoid a size bias in the analysis. In Diversity-Interactions modeling, an increase in contribution to biomass would have to equal an increase in species interactions in order to use realized proportions for calculating potential interactions in diversity effects. This seems unlikely to be true when using a diverse species pool, so it may better to use initial proportions to calculate potential interactions. More discussion and analysis are needed to determine how to interpret

diversity effects within the context of sown and realized proportions and whether it is possible to relate the outcomes using the two proportions within the modeling approaches.

In both modeling approaches used in Chapter Three, the productivity of species in mixture and monoculture and was used for analysis. In monotypic stands of plant species, the abiotic and biotic components of the soil can substantially decrease productivity. Planting species in mixtures may result in increased performance due to increased access to soil nutrients through niche-partitioning and facilitation or through a decreased abundance of species-specific soil pathogens. The knowledge of the presence of soil effects and the driver of soil effects (abiotic or biotic) should give more insight into species performance in biodiversity experiments. In Chapter Four, the growth of four focal species was assessed in soils conditioned for two growing seasons by conspecifics and heterospecifics in the field and in conspecifically conditioned soils with and without soil biota in the greenhouse. In the field the three non-leguminous species only increased in increased biomass in plots conditioned by a legume, which suggest nitrogen limitation was restricting growth in monoculture. The focal legume had the least growth in plots conditioned by conspecifics, which suggests a negative soil feedback. In the greenhouse, the two grasses had an increase in growth in soils with biota removed. This suggests soil biota limit their growth. The legume was only affected in soils that were sterilized, which suggests that the soil sterilization caused an increase in a phytotoxic element. The summation of the field and greenhouse results suggests that soil biota may influence growth of the two grasses, but conditioning of

soils by other species may not reduce their numbers enough to limit effects. Nutrient limitations were likely the cause of the decreased growth in the legume. The results reinforce the role of legumes in facilitative effects. In future studies, focal species should be planted in more plots conditioned by species that are not in the same functional group as the focal species. This would allow for the determination of the variation in growth among more soil biota environments. In greenhouse experiments, monitoring of the soils should be done throughout the experiment to ensure the effectiveness of removal treatments and the rates of recolonization.

Productivity responses may also vary for a particular species with the presence of aboveground symbionts. Fungal endophytes that live in the intercellular space of introduced cool-season grasses may increase competitive ability of the host and alter plant community dynamics. However, little is known about the presence and effects of aboveground fungal presence in grasses of the northern Great Plains. In Chapter Five, the effects of aboveground fungal presence was assessed in native western wheatgrass (*Pascopyrum smithii*) and one of its' cultivars, 'Rodan'. Fungal presence increased rhizome spread in monoculture plots of 'Rodan', but not in the native plots. Fungal presence also increased the abundance of parasitic nematodes (*Tylenchorhynchus* spp.) during the first growing season, decreased root length, and altered the physiological profile of the bacterial communities. However, due to the lack of specificity of the test for the presence of fungi in the aboveground plant tissue, these results cannot be interpreted with confidence. There is definitely a need to understand the role of endophytes in native grasses, but in future studies multiple techniques, molecular

techniques always being one of them, should be used to ensure treatments are properly identified and taken into account.

Hopefully this knowledge contributes to conversations on ecological theory and can be used for applications such as improving restoration and reconstruction techniques and creating diverse multifunctional communities.