UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

ASSEMBLY THROUGH INVASION AND RESTORATION: EFFECTS ON BIODIVERSITY AND ECOSYSTEM FUNCTION

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

Degree of

MASTER OF SCIENCE

By

JOSHUA D. KOURI
Norman, Oklahoma
2020

ASSEMBLY THROUGH INVASION AND RESTORATION: EFFECTS ON BIODIVERSITY AND ECOSYSTEM FUNCTION

A THESIS APPROVED FOR THE
DEPARTMENT OF MICROBIOLOGY AND PLANT BIOLOGY

BY THE COMMITTEE CONSISTING OF

Dr. Lara Souza, Chair

Dr. Abigail Moore

Dr. Katharine Marske

Acknowledgements

We thank Karen Castillioni, Greg Newman, Juliana Teixeira, Erica Nadolski, Dorsa Hajimirsadeghi, and Bradley Sanchez for help with field and lab work. We are deeply grateful for the collaboration of Sara Baer and Katie Eckhoff of the University of Kansas and Kansas Biological Survey. Sara and Katie established the restoration experiment and conducted all associated fieldwork, as well as provided us data on species composition. Amy Buthod assisted with the identification of plants in our plots. Greg Newman and Michael Patten provided helpful feedback planning and interpreting statistical analyses. Katharine Marske and Abigail Moore helped with the conceptual development of this project and gave feedback on our manuscript. Greg Newman, Karen Castillioni, and Juliana Teixeira also provided valuable comments on our manuscript. The work was partially supported by grants from NSF EAGER (NSF Award #1745404), the University of Oklahoma Graduate Student Senate, and UOBS Graduate Summer Research Fellowship.

Table of Contents

List of Chapter 1: Tables and Figures

List of Chapter 2: Tables and Figures

List of Chapter 1: Supplementary Materials

List of Chapter 2: Supplementary Materials

Abstract

It is well established that human activities have damaged many natural systems. Two critical aspects of mitigating this damage are identifying pathways to impacts and identifying the characteristics of successful interventions. We address both topics with a pair of independent studies linked by their focus on plant conservation. First, we investigated the ecological impacts of an invasive warm season grass on plant populations and communities in a temperate mixed-grass prairie ecosystem. Invasive plants are a well-known threat to native plant and animal communities and understanding their impacts is critical to developing management strategies. We investigated how impacts vary across a gradient of invasion and examined the impact of the invader on the intra- and inter-specific diversity of native plants. We found that most (but not all) impacts scale linearly as invader abundance increases. Increasing invasion reduced the height and abundance of the dominant native grass and shifted its functional trait composition (within-species effects). Increasing invasion also decreased total plant cover as well as the abundance of specific plant functional groups (inter-specific effects). We show that the direct ecological impacts of invasive species may be compounded by shifts in the functional traits of dominant native species. Second, we studied whether deterministic (initial seed mix of prairie restoration) or stochastic (establishment year precipitation) processes exert stronger influence on community function. It is critical to planning effective restoration actions to understand whether stochastic climate variation can influence the trajectory of restored communities. We calculated abundance-based community-weighted means (CWMs) of four functional traits across nine years post-restoration in two communities. We found that while climate differences led to dissimilar species compositions among restored communities, the CWMs of three of the four functional traits converged over time. This suggests that the function of restored communities is robust to variation in climate variables. The results of both studies provide valuable information for conservation practitioners.

Chapter 1: The impacts of an invasive grass (*Bothriochloa ischaemum*) vary across a gradient of abundance in a mixed-grass prairie

Chapter 1: Abstract

Managing biological invasions is one of the top priorities of biodiversity conservation. Invasive plants are a well-known threat to native plant and animal communities and understanding their ecological impacts is critical to developing individualized management strategies. While much is known about the impacts of invasive plants, there are still questions about the roles of functional traits and abundance gradients in determining invader impacts. In this study we investigate how the impacts of the invasive grass *Bothriochloa ischaemum* vary across a gradient of invasion and examine the impact of this species on the functional traits of the dominant native grass, *Schizachyrium scoparium*. We found that most of the ecological impacts of *B. ischaemum* scale linearly with its abundance. Increasing invasion reduces the height and abundance of the dominant native *S. scoparium* individuals and shifts their functional trait composition. Increasing invasion also reduces cover of native C_3 and C_4 grasses, total foliar cover, and subdominant foliar cover and leads to shifts in species and functional group composition. However, the impact on legume abundance saturated at low invader abundance (1–15% cover) and remained constant as invader abundance increased. We show that the direct ecological impacts of invasive species may be compounded by shifts in the functional traits of dominant native species and that while invader abundance determines the severity of most impacts, some impacts do not scale linearly with invader abundance.

Introduction

Biological invasions are one of the most pressing threats to global biodiversity (Mack et al. 2000, Ricciardi et al. 2013) and many studies and reviews have focused on the community and ecosystem impacts of invasions (Mack & D'Antonio, 1998; McKinney, 2004; Liao et al., 2008; Gaertner et al., 2009; Powell et al., 2011). Our understanding of invasions has grown considerably, but important questions remain, such as how the impacts of invasion scale with invader abundance (Vilá et al., 2011). Understanding the impacts of invasive species on key native species (such as dominant plants) can help clarify issues of scale and inform management strategies (Drenovsky et al., 2012).

Two areas of interest in the scaling of invasive plant impacts are the roles of functional traits in invasions and the importance of invader abundance. Although few invasion impact studies focus on functional traits (i.e., impacts on functional trait variation and composition in native populations are relatively overlooked), doing so can improve our understanding of both invasion mechanisms and impacts (Sodhi et al., 2019). Functional traits are the measurable attributes of a plant that influence its interactions with its biotic and abiotic environment (Drenovsky et al., 2012). Plant functional traits can have significant effects on the surrounding community and its resulting ecosystem services and functioning (de Bello et al., 2010). The traits of the dominant plant species tend to exert the greatest influence on ecosystem processes (Grime, 1998; Diaz and Cabido, 2001; Mokany et al., 2008; Roscher et al., 2012; Finegan et al., 2015). The important role of functional traits leads to two pathways for invasive plants to impact communities and ecosystems. First, when invaders come to dominate native communities, differences between native and invasive functional traits can directly alter ecosystem functioning (Daehler, 2003; Funk & Vitousek 2007; Ehrenfeld, 2010; Drenovsky et al., 2012). But if the presence of an invader can trigger changes in the functional trait expression of the dominant native species as well, that could provide a second pathway to alter ecosystem functioning. Many plant species are known to exhibit high levels of phenotypic plasticity in response to their surroundings (Sultan, 2000) and invasive plants are known to alter environmental conditions (Vilá et al., 2011), possibly triggering a phenotypic response from native species. The impacts arising directly from the functional traits of invasive plants could be compounded by changes to the traits of the dominant native plants that scale up to indirect impacts on communities and ecosystems.

Additionally, there is a need for quantitative assessment of ecological impacts across a gradient of invader abundance levels (Ehrenfeld, 2010; Vilá et al., 2011; Hulme et al., 2013: Scasta et al., 2015). Incorporating these gradients into invasion studies can provide more clarity on the relationship between impacts and abundance than comparisons between uninvaded and highly invaded sites (Hulme et al., 2013). It is important to understand if impacts scale linearly with abundance or if there are thresholds below which invaders have little impact or above which impacts begin to saturate (Vilá et al., 2011). Furthermore, employing a gradient design allows for the utilization of more powerful regression analysis (rather than ANOVA) and offers a quantitative description of the relationship between invader abundance and impact (Cottingham et al., 2005). This study contributes to filling both of the above knowledge gaps by incorporating functional trait analysis into an investigation of community impacts across a gradient of invader abundance.

The prairie ecosystems of the central United States provide an excellent opportunity for studying the impacts of invasive plants, given the well-documented introductions and establishment of a variety of non-native species (D'Antonio and Vitousek, 1992; Williams and Baruch, 2000). The goal of this study is to investigate how the invasive grass *Bothriochloa ischaemum* impacts the native plant community across a gradient of invasion, considering both within-species impacts (on the functional traits of the native dominant grass, *Schizachyrium scoparium*) and across-species impacts (on species and functional group composition). We specifically asked 1) Is there a negative relationship between the abundance of the invader and the abundance of the dominant native grass? 2) Do changes in the abundance of the invasive grass lead to changes in the mean, variance, or composition of functional trait values for the dominant native grass? 3) Does increasing abundance of the invasive grass change the richness or composition of the native plant community? We hypothesized that as the invasive species increased in abundance the native dominant grass would decrease in abundance and shift its functional trait composition, compounding changes in the community and ecosystem.

Methods

Study Site

We conducted our study in a temperate mixed-grass prairie at the Kessler Atmospheric and Ecological Field Station (KAEFS, http://kaefs.ou.edu/), located in central Oklahoma, USA (34°59'N, 97°31'W). The area was farmed until 1973 and has since been subject to light grazing in designated areas (Xu *et al.*, 2013). We conducted our study in two sections of KAEFS that have not been farmed or grazed for at least 40 years prior to the start of the experiment (Shi et al., 2015; Castillioni et al., 2020) and are dominated by warm-season C_4 bunch grasses with a diverse mixture of subdominant forbs (Shi et al., 2015). Mean annual precipitation was 885mm for the period of 1994 to 2018 and the mean annual temperature was 16.2°C from 1997 to 2018 (Oklahoma Climatological Survey, Norman, OK, USA). The monthly mean temperature ranges from 4.4°C in January to 27.7°C in July (Oklahoma Climatological Survey, Norman, OK, USA).

The soil at KAEFS is a Nash-Lucien complex with neutral pH, a high water holding capacity (approximately 37%), and a moderately penetrable root zone, and extends to a depth of about 70cm (Xu *et al.*, 2013).

Focal Species

Our focal native species is *Schizachyrium scoparium*, a warm-season C_4 bunch grass. This drought-tolerant species is typically dominant in mixed-grass prairies and provides food and habitat for a variety of native wildlife (Schmidt et al., 2008). Our focal invasive species is *Bothriochloa ischaemum,* a C_4 grass originating in Eurasia that has become a problematic invader in the Great Plains region of the United States (Gabbard and Fowler, 2007). *Bothriochloa ischaemum* has been intentionally seeded on both private lands and along public roadsides through several state and federal agencies for use as cattle fodder and erosion control (Gabbard and Fowler, 2007; Wilson et al., 2012). Where it has escaped cultivation and established persistent populations, *B. ischaemum* has a variety of negative impacts on the native community. This species has been shown to reduce the abundance and diversity of native plant communities (Hickman et al., 2006; Gabbard and Fowler, 2007; Robertson and Hickman, 2012), inhibit the growth of native plants (Schmidt et al., 2008), reduce arthropod abundance and bird diversity (Hickman et al., 2006), and replace the dominant native C_4 grasses in the habitat (Robertson and Hickman, 2012). The ecosystem impacts of *B. ischaemum* invasion are poorly known, but it has been shown to directly reduce plant-available soil nitrogen (Basham, 2013) and may indirectly alter nutrient cycling and pollination services through its impacts on insect communities (Litt and Steidl, 2010). The wide breadth of negative impacts caused by *B. ischaemum* invasion makes this grass one of the most detrimental exotic forage species (Scasta et al., 2015).

Experimental Design

We located study plots across two locations (sub-sites) consisting of mixed grass prairie surrounded by encroaching eastern red cedar (*Juniperus virginiana*) woodlands. These sites are dominated by native C_4 grasses such as little bluestem (*Schizachyrium scoparium*), Indian grass (*Sorghastrum nutans*), and tall dropseed (*Sporobolus compositus*), as well as forbs such as *Lespedeza cuneata*, *Symphyotrichum ericoides*, and *Desmanthus illinoensis* (Shi et al. 2015, Castillioni et al., 2020). The two sub-sites represent an invasion gradient of *B. ischaemum*. The northwestern sub-site is lightly invaded and the southern sub-site is more heavily invaded, but invasion levels are variable within the sub-sites. In July 2019 we established 40 2m × 2m experimental plots, divided between the two sub-sites based on their size (plots 1–25 in the northwestern sub-site and plots 26–40 in the southern sub-site, **Figs. S1-2**). Plots were established along short transects (ranging from 11m to 23m) of four to eight plots, with 1m between adjacent plots and at least 5m between transects. Plot-level *B. ischaemum* cover ranged from 0% to 80%, with a mean of 23% cover.

Plot-Level Plant Composition and Abundance

We recorded plant species composition (i.e., species identity and presence/absence) for each plot in July 2019. Species composition was recorded as binary presence/absence values for each species in each plot and species richness was recorded as the total number of species in each plot, excluding *B. ischaemum*. Abundance (percent foliar cover) was measured at the functional group level (C_3 grasses, C_4 grasses, herbaceous forbs, woody forbs, and legumes, as well as bare ground and litter) using cover class values adapted from Braun-Blanquet (1932): Class 0 = 0% cover, Class 1 = 0–1%, Class 2 = 1–5%, Class 3 = 5–25%, Class 4 = 25–50%, Class 5 = 50–75%, Class 6 = 75–95%, Class 7 = 95–100%. Since the interval between classes is unequal, we used the median value of each cover class in our analyses. Additionally, we calculated total foliar cover (the sum of all functional group cover values), subdominant foliar cover (the sum of all functional groups except C_4 grasses), and a native C_4 grass cover value (by subtracting the *B. ischaemum* percent-cover value from the total C_4 grass cover value) for each plot. Since the foliage of multiple functional groups often overlapped, the total coverage values of all functional groups in a plot often exceeded 100%. We recorded the cover of the invasive *B. ischaemum* at two levels of precision: a continuous estimate of *B. ischaemum* percent foliar cover for each plot (to take advantage of a regression approach) and a categorical assessment based on four cover "treatments," which were used in compositional analyses. The four "treatments" of *B. ischaemum* cover result from natural (i.e., unmanipulated) variation in the foliar cover of the invader across the invasion gradient. Fourteen plots had no *B. ischaemum* cover (Zero), while nine plots had Low (1% to 15% cover), nine plots had Medium (16% to 49% cover), and eight plots had High (50% cover and above) *B. ischaemum* abundance. Following this two-tiered classification scheme, a plot that was observed to have 65% *B. ischaemum* cover, for example, would also be given a categorical value of "High."

Assessing Within-Species Impacts on S. scoparium

We assessed the impact of the invasive *B. ischaemum* on the abundance and functional traits of individuals of the native C_4 grass *Schizachyrium scoparium*. The abundance of *S. scoparium* was recorded at the same two levels of precision as *B. ischaemum* abundance. Aboveground individual- and leaf-level functional traits of *S. scoparium* were measured to determine how the magnitude of invasion impacted intra-specific trait variation and composition. We randomly selected three individuals of *S. scoparium* from each plot and tagged them so that repeated measures could be made when necessary. For each of these individuals, we measured their height as distance from the ground to the tallest portion of each plant and individual foliar cover as the percent of the plot covered by the foliage of each individual plant. Three mature leaves from each individual were collected to calculate specific leaf area (SLA) and leaf dry matter content (LDMC). Fresh mass of each leaf was determined to 0.01 g, scanned at 300 dpi using WINfolia software (Regent Inc., Canada) to determine area, and finally dried at 80°C for at least 48 hours before the dry mass was recorded. SLA was calculated as leaf area / dry mass and is reported as cm^2 of leaf area per gram of dry leaf matter. LDMC was calculated as leaf dry mass / fresh mass and represents the fraction of total leaf mass accounted for by dry material. We used the plot-level average of each trait in our analyses and also calculated plot-level variance for each trait. These traits were selected because they can all be considered "effect traits" (Funk et al., 2017), which can directly influence neighboring species and the surrounding ecosystem.

Statistical Analyses

Since plots were divided between two-sub-sites we first ran an ANOVA in R 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria, 2019) to determine if the sub-sites differed in *B. ischaemum* cover. The sub-sites were different ($p < 0.001$, $F = 39.54$), so we used mixed effects models in our analyses, with sub-site as a random effect. Linear mixed effects models were performed using the function "lmer" in the package "lme4" (Bates et al., 2015) in R. In several cases, the variance accounted for by sub-site was equal to zero, so linear fixed effects models were constructed with the function "lm" from the "stats" package in R. The mixed effects models were used to test the impact of invasion on the abundance of *S. scoparium*, the abundance of several functional groups (C_3 grasses, herbaceous forbs, woody plants, bare ground, and litter), species richness, and the value of two *S. scoparium* functional traits (LDMC and individual cover). The fixed effects models were used to test the impact of invasion on the total abundance of C_4 grasses, the abundance of native C_4 grasses and legumes; the amount of total and subdominant functional group cover; the values of two *S. scoparium* functional traits (SLA and height); and the variance of all four *S. scoparium* functional traits. Because preliminary results suggested that the impact on *S. scoparium* and legume cover might be driven by the presence/absence of *B. ischaemum* we constructed additional linear fixed effects models to examine the impact on these variables only in plots with *B. ischaemum,* and one-way ANOVAs when there was no linear relationship within invaded plots. We also performed a one-way ANOVA to determine if variance in *S. scoparium* functional trait means was impacted by the cover category of *B. ischaemum*.

To determine how varying degrees of *B. ischaemum* invasion impact the composition of the plant community, we performed PERMANOVAs (Anderson, 2001; Anderson et al., 2006) in PRIMER (Plymouth Marine Laboratory, UK), running each with 999 permutations. We tested the effect of *B. ischaemum* cover category (Zero, Low, Medium, High) on species composition (recorded as presence/absence), functional group composition (C_4 grasses, C_3 grasses, forbs, woody plants, and legumes), and *S. scoparium* trait composition (height, cover, SLA, LDMC). The *S. scoparium* trait values were standardized (trait value / maximum of the trait) before analysis. For significant PERMANOVAs, we then performed pairwise permutation tests to determine which *B. ischaemum* cover categories differed from one another and ran SIMPER analyses to determine which species, functional groups, or functional traits drive the dissimilarity between cover categories. Since a significant PERMANOVA result can indicate compositional differences due to both shifts in location and shifts in dispersion (Anderson, 2001), we used permutational analysis of multivariate dispersions (PERMDISPs) to assess which results may be due to differences in dispersion within cover categories.

Results

Impacts of Invasion on S. scoparium

The invasive grass *Bothriochloa ischaemum* negatively impacted the abundance of the native grass, *Schizachyrium scoparium* (**Fig. 1**), and changed the composition of its functional traits (**Fig. 2**). As the foliar cover of the invasive grass increased, the foliar cover of native *S. scoparium* decreased linearly (p = 0.023, F = 35.17). In the plots without the invasive species, the mean foliar cover of the native grass was 50% (range = 15–70%), while in plots where the invader was present, the mean foliar cover of *S. scoparium* was only 20% (range = 0–40%). There was a 59% reduction in mean foliar cover of *S. scoparium* from the uninvaded to the invaded plots. While it appeared that this effect might be driven simply by the presence/absence of *B. ischaemum*, an additional linear model focused only on plots where *B. ischaemum* was present still indicated a linear reduction in *S. scoparium* cover as *B. ischaemum* cover increases (p = 0.002, R^2 = 0.34).

Increased *B. ischaemum* cover also negatively impacted the average height of *S. scoparium* and contributed towards the dissimilarity in overall functional trait composition. The average height of *S. scoparium* decreased (p = 0.031, R^2 = 0.12) as *B. ischaemum* cover increased, but the variance of this functional trait did not change significantly along the invasion gradient (p = 0.994). The mean and variance of the other functional traits did not change significantly across the invasion gradient either: individual foliar cover (mean, p = 0.139; variance, p = 0.183), SLA (mean, p = 0.411; variance, p = 0.285), LDMC (mean, p = 0.536; variance, p = 0.380). Increasing *B. ischaemum* cover significantly impacted trait composition of *S. scoparium* individuals (p = 0.031). Pairwise tests showed that plots with High *B. ischaemum* cover (above 50% foliar cover) were significantly different from plots with Zero *B. ischaemum* (p = 0.031) and Low (1–15%) *B. ischaemum* cover (p = 0.014); plots in the Medium category did not differ from any other category. The individual height and foliar cover accounted for 67% of the dissimilarity between Zero and High *B. ischaemum* cover, while individual foliar cover and SLA accounted for 71% of the dissimilarity between Low and High *B. ischaemum* cover. The one-way ANOVAs on trait variance confirmed that there was no impact of invasion category on trait variance (all p-values above 0.15).

Impacts of invasion on the native plant community

Invasion by *B. ischaemum* negatively impacted several diversity characteristics of the native plant community and altered the compositional similarity of both species and functional groups. As *B. ischaemum* foliar cover increased, the total species richness declined ($p < 0.001$, $F = 13.26$, **Fig. S3**). Plots with Zero or Low *B. ischaemum* cover had an average of 16.43 ± 1.87 and 18.33 ± 4.69 species, respectively. Plots with Medium or High *B. ischaemum* cover had an average of 14.67 ± 2.92 and 14.25 ± 5.15 species. There was also a significant effect of *B. ischaemum* cover on the species composition (**Fig. 3**) of these plots ($p = 0.013$). Pairwise tests indicated that only the plots in the Zero and High categories of *B. ischaemum* cover differed significantly ($p = 0.0003$). **Table 1** indicates the species that contribute the most to the dissimilarity between these two categories. Legumes such as *Dalea purpurea* and *Psoralidium tenuiflorum*, and C_3 grasses such as *Dichanthelium oligosanthes* were found more frequently in plots with Zero *B. ischaemum* cover compared to plots with High *B. ischaemum*, while species such as *Chamaecrista fasciculata* (legume) and *Croton monanthogynus* (forb) were found more often in plots with High *B. ischaemum* compared to plots with Zero cover. Finally, the PERMDISP results indicated that there was greater within-treatment dispersion for plots with Medium invasion (mean = 44.4) compared to Zero (mean = 34.1; $p = 0.004$) and Low (mean = 39.7; $p = 0.025$) invasion and greater within-treatment dispersion for plots with High invasion (mean = 46.1) compared to Zero invasion ($p = 0.015$).

As *B. ischaemum* cover increased, several plant functional groups decreased in their abundance (**Fig. S4**). Unsurprisingly, native C_4 grasses decreased dramatically as *B. ischaemum* cover increased. About 84% of the total variation in native C_4 grass cover was explained by the invader increase ($p < 0.001$, $R^2 = 0.84$). The slope of this relationship was -1.01, indicating a 1:1 replacement of native C_4 grasses with the invasive C_4 grass. There was also a significant decline in C_3 grass cover, although this effect was relatively weak ($p = 0.039$, $F = 5.26$). There appeared to be a decline in legume foliar cover as *B. ischaemum* cover increased ($p < 0.001$, $R^2 = 0.32$), but this relationship did not hold up when we only looked at invaded plots ($p = 0.209$). A one-way ANOVA showed there was a difference in legume cover based on *B. ischaemum* invasion category, and a Tukey HSD test in R indicated that plots with Zero invasion differed from all invasion levels (all p-values < 0.001), but none of the invasion categories differed from one another (all p-values > 0.74). Legumes were subdominant in all plots, but account for a substantially greater proportion of total foliar cover in uninvaded plots compared to invaded plots. Legumes had a mean foliar cover of 33% (range = 15–63%) in uninvaded plots but only 9% (range = 0–15%) in plots with *B. ischaemum*, a 74% reduction in cover. The other functional groups (total C_4 grass cover, herbaceous forbs, and woody plants), as well as the amount of bare ground and litter in each plot, did not vary in cover across the invasion gradient (all p-values > 0.062). However, increasing invasion by *B. ischaemum* (**Fig. 4**) reduced the total foliar cover of each plot ($p = 0.002$, $R^2 = 0.23$) and the total foliar cover of subdominants (all functional groups except C_4 grasses; $p < 0.001$, $R^2 = 0.36$). This reduction in total foliar cover did not lead to an increase in bare ground because the foliage of the different plants overlapped and led total foliar cover to often exceed 100%.

There was a significant effect of *B. ischaemum* cover category on the relative abundance of plant functional groups (p = 0.0001). Pairwise tests indicated that all categories of *B. ischaemum* cover differed from one another except for Medium and High invasion plots (**Fig. 5**). Plots with Zero *B. ischaemum* differed from all other levels of *B. ischaemum* cover (Low, p = 0.0007; Medium, p = 0.0001; High, p = 0.0001). Plots with Low *B. ischaemum* cover also differed from plots with Medium (p = 0.0054) and High (p = 0.0001) cover of the invasive species. Decreases in native C_4 grass and legume cover as invasion increased accounted for most of the dissimilarity between invasion categories.

There were also significant differences in the dissimilarity of functional group abundance for plots *within* the different treatment levels. There was significantly more dispersion within the Medium (mean = 20.1) and High (mean = 27.2) invasion plots than in the Zero (mean = 10.6) or Low (mean = 8.1) invasion plots. Dispersion within plots with Zero invasion differed from the dispersion within plots with Medium (p = 0.002) and High (p = 0.002) invasion; plots with Low invasion also differed from plots with Medium (p = 0.002) and High (p = 0.001) invasion. This indicates that functional group composition is more consistent within plots with Zero-Low *B. ischaemum* invasion and more variable within plots with Medium-High invasion.

Discussion

Invasive species are a well-known threat to diversity and function *across* a variety of ecosystems (Mack et al. 2000; Ricciardi et al. 2013), but proper assessment of their ecological impacts *within* individual systems is key to developing individualized management strategies (Drenovsky et al., 2012). Our study shows that the invasive C_4 grass *Bothriochloa ischaemum* has significant within-species (on a population of native *Schizachyrium scoparium*) and across-species (on community metrics of a mixed-grass prairie) impacts. Invasion by *B. ischaemum* significantly reduced both the abundance and height of the native *S. scoparium*. Individuals of *S. scoparium* growing in high-invasion plots had a dissimilar suite of functional trait values from individuals growing in uninvaded plots, with decreasing trait values leading towards smaller plants in highly invaded plots. Further, *B. ischaemum* invasion reduced the species richness of the native plant community and caused declines in native C_4 grass cover, C_3 grass cover, legume cover, total foliar cover, and subdominant species cover. Invasion also led to significant differences in the species composition of uninvaded and highly invaded plots, as well as dissimilarity in functional group composition across most levels of invasion. In general, the *within-species* impacts were stronger than across-species impacts, which has interesting implications for how impact scales across levels of organization.

The finding that *B. ischaemum* reduces the size and abundance of *S. scoparium* supports our prediction of a negative relationship between exotic and native grass abundance and confirms the harmful impacts of this invader on co-occurring dominant native grasses (Schmidt et al., 2008; Robertson and Hickman, 2012; Duell et al., 2016). We also found support for our prediction that invasion would lead to shifts in trait composition of *S. scoparium*. Our study is the first to show that high invasion by *B. ischaemum* alters the functional trait composition of a native species, building on previous work that showed invasion could alter trait composition at the community level (Sodhi et al., 2019). In assessing the effects of functional traits on ecosystem processes it is important to consider changes in trait syndromes rather than just single traits (Kichenen et al., 2013). While the mean of three of the four traits we measured (SLA, LDMC, and individual cover), and intraspecific variance of all traits, were not significantly impacted when analyzed individually, the shifts in these traits compounded one another, resulting in a dissimilar suite of traits in high-invasion patches compared to uninvaded patches. The *S. scoparium* individuals that persist under High invasion are smaller (lower height and individual cover) and exhibit more signs of resource stress (lower SLA and higher LDMC; Cornelissen et al., 2003) than individuals growing under Zero or Low invasion. Additionally, although the impacts on height and trait composition are relatively weak, they are likely amplified by the simultaneous strong impact on abundance, potentially causing serious consequences for *S. scoparium* growing in invaded systems. Plant height is correlated with aboveground biomass, fecundity, and competitive vigor (Cornelissen et al., 2003) and can provide an estimate of reproductive fitness (Younginger et al., 2017). This could lead to a positive feedback loop promoting further invasion of *B. ischaemum* if *S. scoparium* individuals growing in heavily invaded habitat have lower fitness. This cycle could be exacerbated by the ability of *B. ischaemum* to directly and indirectly inhibit *S. scoparium* growth through potential allelopathic compounds. Leachate from *B. ischaemum* reduced germination of *S. scoparium* seeds by 91% as well as significantly reducing the survival and biomass accumulation of *S. scoparium* seedlings (Greer et al., 2014b). *Bothriochloa ischaemum* invasion indirectly inhibits the growth of native warm-season C_4 grasses by altering the arbuscular mycorrhiza fungal community on which they depend (Duell et al., 2016), an effect that can continue even after the invader has been removed (Wilson et al., 2012).

The replacement of the native *S. scoparium* with the exotic *B. ischaemum* is likely to have negative impacts on the broader community and ecosystem as well. The direct ecological influences of these two species are poorly studied but some general predictions can be made. *Bothriochloa ischaemum* has lower-quality litter and a shorter, but more productive, growing season than *S. scoparium*, which can lower plant-available soil nitrogen and increase aboveground net primary productivity of invaded sites (Basham, 2013). The decreased species richness and lower abundance of subdominant C_4 grasses and legumes that we observed suggests that *B. ischaemum* creates a less favorable microhabitat for subdominant native plant species, reducing the available nitrogen and increasing canopy cover relative to *S. scoparium*. This "changing of the guard" will also likely have indirect ecological consequences. Grasslands dominated by *B. ischaemum* and closely related species provide reduced habitat quality compared to native grasslands for a variety of wildlife. Both arthropod abundance and diversity are reduced in invaded sites, altering nutrient cycling and reducing pollination services (Litt and

Steidl, 2010). Many songbirds avoid invaded grasslands (Hickman et al., 2006), as do rodents (Greer et al., 2014a) and the foxes that feed upon them (Kamler et al., 2003; Pavur, 2016).

Although *B. ischaemum* has clear impacts on *S. scoparium*, invasion does not impact all aspects of the rest of the plant community. Our results showed that *B. ischaemum* invasion reduces plant species richness, the abundance of native C_3 and C_4 grasses, the abundance of legumes, and both total foliar cover and subdominant foliar cover, but that invasion does not impact herbaceous forb or woody plant cover. This suggests that the impact of *B. ischaemum* invasion on the community is variable but generally negative. Interestingly, although total C_4 grass cover remained constant across all plots, areas dominated by the *invasive* C_4 grass had significantly lower species richness, total cover, and subdominant cover than areas with the equivalent abundance of the *native* C_4 grass. This highlights that the 1:1 replacement of native grasses with exotic grasses does not correspond to an equivalent role in the community.

Invasion not only reduced the abundance of native species but also led to shifts in species and functional group composition of the community. Fargione et al. (2003) showed that native species were most successful at hindering invasion by exotic species from the same functional group, and that native C_4 grasses are the functional group that has the strongest negative impact on exotic species from other functional groups. Our results suggest that if the invader is a strong enough competitor, the opposite is true as well: *B. ischaemum* has the strongest negative impact within its functional group (native C_4 grasses), but also exerts negative impacts on other functional groups such as legumes. Although the reductions in C_3 grass cover were relatively weak, they combined with reductions in native C_4 grass and legume cover to produce significantly different species and functional group compositions. These changes in composition can have significant impacts on nitrogen cycling (Mack et al., 2001). The loss of legumes could be a particularly detrimental effect of *B. ischaemum* invasion, potentially altering the nutrient cycling of invaded areas that can no longer support these nitrogen fixers (Knops et al., 2002). Nitrogen accumulation rates are positively related to legume cover but negatively related to C_3 grass and forb cover (Knops and Tilman, 2000), so the loss of legumes is likely to compound the decrease in available nitrogen caused directly by *B. ischaemum* (Basham, 2013). Another intriguing result of our study is that there is greater dispersion within the Medium and High invasion plots compared to Zero and Low invasion plots, indicating greater heterogeneity of species and functional group composition at increased levels of invasion. In other words, the composition of all uninvaded plots was essentially the same, but highly invaded plots differed greatly from one another. Although plant invasions have decreased variation *across* sites (McKinney, 2004; Baiser et al., 2012; Sodhi et al., 2019) our results suggest that they can increase variation *within* individual sites. Due to the nature of our study as a natural experiment taking advantage of natural variation in exotic species abundance, our results, taken in isolation, cannot conclusively state that invasive species abundance caused the observed differences in community and intraspecific composition (rather than differences in the community causing variations in invader abundance). However, in light of previous field studies that confirm the patterns of impact from *B. ischaemum* (Gabbard and Fowler, 2007; Robertson and Hickman, 2012) and greenhouse studies that provide mechanisms for impact (Schmidt et al., 2008; Greer et al., 2014b) it is ecologically justifiable to conclude that the observed impacts are caused by *B. ischaemum* invasion.

There have been repeated calls for invasion impact studies to assess impacts over a gradient of invader abundance (Ehrenfeld, 2010; Vilá et al., 2011; Hulme et al., 2013; Scasta et al., 2015) and our study contributes to filling this knowledge gap by demonstrating that the role of invader abundance varies depending on the impact of interest. The majority of the impacts we found (on *S. scoparium* abundance and height, species richness, native C_3 and C_4 grass cover, total cover, and subdominant cover) increase linearly as the abundance of the invader increases. The impact on legume abundance, however, was not related to invader abundance. Rather, the threshold for impact is very low (1–15% cover of the invader), but the magnitude of this impact is constant across the abundance gradient. This pattern has important implications for the management of *B. ischaemum*. If the abundance of this invader can be kept low, its impacts may be negligible for most native species even if it cannot be completely eradicated. However, complete eradication of the invader may be the only way to protect some native species. Furthermore, our results expand on the pattern observed by Vilá et al. (2011). They found that impacts on communities are more severe than impacts on ecosystem processes, which suggests that by the time ecosystem impacts are detected, communities have likely already sustained severe impacts. In this study, impacts on the native community such as declines in species richness and changes in species or functional group composition are less severe (in terms of F-value) than the impact on the native dominant species, *S. scoparium*, suggesting a progression of impact severity from species > community > ecosystem. This could have important implications for invasive species management and monitoring if impacts on communities and ecosystems are consistently preceded by strong impacts on the dominant native plants.

Conclusions

Biological invasions and their associated ecological impacts will continue to be a pressing challenge to biodiversity conservation. We contribute to addressing this challenge by demonstrating the importance of several characteristics of invasion impacts. First, as the abundance of an invasive grass increased, the abundance of a dominant native grass (along with the other co-occurring native grasses) linearly declined. Secondly, increasing the abundance of an invasive grass altered the functional traits of dominant native plants, compounding any direct impacts of the invader on native grass abundance. Finally, in most cases the abundance of the invasive species determines the severity of its impact, but not all impacts scale linearly with abundance. Understanding the implications of these results can help guide informed management strategies.

Chapter 1: References

Anderson M.J., Ellingson K.E., McArdle B.H. (2006). Multivariate dispersion as a measure of beta diversity. *Ecology Letters* 9: 683–693. doi: 10.1111/j.1461-0248.2006.00926.x

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26(1): 32–46. doi: 10.1046/j.1442-9993.2001.01070.x

Baiser, B., Olden, J.D., Record, S., Lockwood, J.L., and McKinney, M. L. (2012). Pattern and Process of biotic homogenization in the New Pangaea. *Proceedings of the Royal Society B.* 279: 4772 - 4777 doi:10.1098/rspb.2012.1651

Bartha, S., Szentes, S., Horváth, A., Házi, J., Zimmermann, Z., Molnár, C., Dancza, I., Margóczi, K., Pál, R.W., Purger, D., Schmidt, D., Óvári, M., Komoly, C., Sutyinszki, Z., Szabó, G., Csathó, A.I., Juhász, M., Penksza, K. and Molnár, Z. (2014), Impact of mid‐successional dominant species on the diversity and progress of succession in regenerating temperate grasslands. *Applied Vegetation Science* 17: 201-213, doi:10.1111/avsc.12066

Basham, T.S. (2013). Ecophysiology and Ecosystem-level Impacts of an Invasive C_4 Perennial Grass, Bothriochloa ischaemum. Dissertation, University of Texas, Austin.

Braun-Blanquet, J. (1932). Plant Sociology: the study of plant communities. *McGraw-Hill*, New York

Castillioni, K., Wilcox, K., Jiang, L., Luo, Y., Jung. C.G., and Souza, L. (2020). Drought mildly reduces plant dominance in a temperate prairie ecosystem across years. *Ecology and Evolution* 10: 6702– 6713, https://doi.org/10.1002/ece3.6400

Cornelissen J. H. C. , Lavorel S. , Garnier E. , Díaz S. , Buchmann N. , Gurvich D. E. , Reich P. B. , Steege H. ter , Morgan H. D. , Heijden M. G. A. van der , Pausas J. G. Poorter H. (2003) A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *Australian Journal of Botany* 51: 335-380, https://doi.org/10.1071/BT02124

Cottingham, K.L., Lennon, J.T. and Brown, B.L. (2005). Knowing when to draw the line: designing more informative ecological experiments. *Frontiers in Ecology and the Environment* 3: 145-152, doi:10.1890/1540-9295(2005)003[0145:KWTDTL]2.0.CO;2

D'Antonio, C.M. and Vitousek, P.M. (1992). Biological Invasions by Exotic Grasses, the Grass/Fire Cycle, and Global Change. *Annual Review of Ecology and Systematics* 23(1): 63-87, https://doi.org/10.1146/annurev.es.23.110192.000431

Daehler, C.C. (2003). Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. *Annual Review of Ecology, Evolution, and Systematics* 34: 183-211 https://doi.org/10.1146/annurev.ecolsys.34.011802.132403

de Bello, F., Lavorel, S., Díaz, S. et al. (2010). Towards an assessment of multiple ecosystem processes and services via functional traits. *Biodiversity Conservation* 19, 2873–2893 https://doi.org/10.1007/s10531-010-9850-9

Diaz, S. and Cabido, M. (2001). Vive la difference: plant functional diversity matters to ecosystem processes. *Trends in Ecology and Evolution* 16(11): 646-655, https://doi.org/10.1016/S0169-5347(01)02283-2

Drenovsky, R.E., Grewell, B.J., D'Antonio, C.M., Funk, J.L., James, J.J., Molinari, N., Parker, I.M. and Richards, C.L. (2012). A functional trait perspective on plant invasion. *Annals of Botany* 110(1): 141–153, https://doi.org/10.1093/aob/mcs100

Duell, E.B., Wilson, G.W.T., Hickman, K. R. (2016). Above- and below-ground responses of native and invasive prairie grasses to future climate scenarios. *Botany* 94(6): 471- 479, https://doi.org/10.1139/cjb-2015-0238

Ehrenfeld, J.G. (2012). Ecosystem consequences of biological invasions. *Annual Review of Ecology, Evolution, and Systematics* 41: 59-80 https://doi.org/10.1146/annurev-ecolsys-102209-144650

Fargione, J., Brown, C.S., and Tilman, D. (2003). Community assembly and invasion: An experimental test of neutral versus niche processes. *PNAS* 100 (15) 8916-8920; https://doi.org/10.1073/pnas.1033107100

Finegan, B., Peña‐Claros, M., de Oliveira, A., Ascarrunz, N., Bret‐Harte, M.S., Carreño‐Rocabado, G., Casanoves, F., Díaz, S., Eguiguren Velepucha, P., Fernandez, F., Licona, J.C., Lorenzo, L., Salgado Negret, B., Vaz, M. and Poorter, L. (2015). Does functional trait diversity predict above‐ground biomass and productivity of tropical forests? Testing three alternative hypotheses. Journal of *Ecology* 103: 191-201. doi:10.1111/1365-2745.12346

Fowler, N.L. (2002), The joint effects of grazing, competition, and topographic position on six savanna grasses. *Ecology* 83: 2477-2488. doi:10.1890/0012-9658(2002)083[2477:TJEOGC]2.0.CO;2

Funk, J.L. and Vitousek, P.M. (2007). Resource-use efficiency and plant invasion in low-resource systems. *Nature* 446: 1079–1081 https://doi.org/10.1038/nature05719

Funk, J.L., Larson, J.E., Ames, G.M., Butterfield, B.J., Cavender-Bares, J., Firn, J., Laughlin, D.C., Sutton‐Grier, A.E., Williams, L. and Wright, J. (2017), Revisiting the Holy Grail: using plant functional traits to understand ecological processes. *Biological Reviews* 92: 1156-1173. doi:10.1111/brv.12275

Gabbard, B.L. and Fowler, N.L. (2007). Wide Ecological Amplitude of a Diversity-Reducing Invasive Grass. *Biological Invasions* 9: 149–160 https://doi.org/10.1007/s10530-006-9012-x

Gaertner, M., Den Breeyen, A., Hui, C,. Richardson, D.M. (2009). Impacts of alien plant invasions on species richness in Mediterranean-type ecosystems: a meta-analysis. *Progress in Physical Geography: Earth and Environment* 33(3): 319-338, doi:10.1177/0309133309341607

Greer M., Noland M.A., Hickman K.R., Wilson G.W.T. (2014a). Old World bluestem and its effects on the small mammal communities of North Central Oklahoma, USA: an ecological game changer. pp 109–110 in *Proceedings of the 2nd Biennial Conference on the Conservation of America's Grasslands* pp. 109-110, Manhattan, KS; Kansas State University

Greer, M.J., Wilson, G.W.T., Hickman, K.R. and Wilson, S.M. (2014b). Experimental evidence that invasive grasses use allelopathic biochemicals as a potential mechanism for invasion: chemical warfare in nature. *Plant and Soil* 385: 165–179 https://doi.org/10.1007/s11104-014-2209-3

Hickman, K.R., Farley, G.H., Channell, R., and Steier, J.E. (2006). Effects of Old World Bluestem (Bothriochloa ischaemum) on food availability and avian community composition within the mixed-grass prairie. *The Southwestern Naturalist* 51(4): 524-530, https://doi.org/10.1894/0038-4909(2006)51[524:EOOWBB]2.0.CO;2

Hulme, P.E., Pyšek, P., Jarošik, V., Pergl, J., Schaffner, U., and Vilá, M. (2013). Bias and error in understanding plant invasion impacts. *Trends in Ecology and Evolution* 28(4): 212-218, https://doi.org/10.1016/j.tree.2012.10.010

Kamler J.F., Ballard W.B., Fish E.B., Lemons P.R., Mote K., and Perchellet C.C. (2003) Habitat use, home ranges, and survival of swift foxes in a fragmented landscape: conservation implications. *Journal of Mammalogy* 84:989–995, https://doi.org/10.1644/BJK-033

Kichenin, E., Wardle, D.A., Peltzer, D.A., Morse, C.W. and Freschet, G.T. (2013). Contrasting effects of plant inter‐ and intraspecific variation on community‐level trait measures along an environmental gradient. *Functional Ecology* 27: 1254-1261, doi:10.1111/1365-2435.12116

Knops, J.M.H. and Tilman, D. (2000), Dynamics of soil nitrogen and carbon accumulation for 61 years after agricultural abandonment. *Ecology*, 81: 88-98. doi:10.1890/0012-9658(2000)081[0088:DOSNAC]2.0.CO;2

Knops, J.M.H.., Bradley, K.L.. and Wedin, D.A.. (2002), Mechanisms of plant species impacts on ecosystem nitrogen cycling. *Ecology Letters*, 5: 454-466. doi:10.1046/j.1461-0248.2002.00332.x

La Pierre, K.J. and Smith, M.D. (2015). Functional trait expression of grassland species shift with short- and long-term nutrient additions. *Plant Ecology* 216: 307–318 https://doi.org/10.1007/s11258-014-0438-4

Liao, C., Peng, R., Luo, Y., Zhou, X., Wu, X., Fang, C., Chen, J. and Li, B. (2008). Altered ecosystem carbon and nitrogen cycles by plant invasion: a meta ‐ analysis. *New Phytologist* 177: 706-714, doi:10.1111/j.1469-8137.2007.02290.x

Litt, A.R. and Steidl, R.J.(2010). Insect assemblages change along a gradient of invasion by a nonnative grass. *Biological Invasions* 12: 3449–3463 https://doi.org/10.1007/s10530-010-9743-6

Mack, M.C. and D'Antonio, C.M. (1998). Impacts of biological invasions on disturbance regimes. *Trends in Ecology and Evolution* 13(5): 195-198, https://doi.org/10.1016/S0169-5347(97)01286-X

Mack, M.C., D'Antonio, C.M. and Ley, R.E. (2001). Alteration of ecosystem nitrogen dynamics by exotic plants: a case study of C_4 grasses in Hawaii. *Ecological Applications* 11: 1323-1335. doi:10.1890/1051-0761(2001)011[1323:AOENDB]2.0.CO;2

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M. and Bazzaz, F.A. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* 10: 689-710. doi:10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2

McKinney, M.L. (2004), Measuring floristic homogenization by non‐native plants in North America. *Global Ecology and Biogeography* 13: 47-53. doi:10.1111/j.1466-882X.2004.00059.x

Mokany, K., Ash, J. and Roxburgh, S. (2008). Functional identity is more important than diversity in influencing ecosystem processes in a temperate native grassland. *Journal of Ecology* 96: 884-893, doi:10.1111/j.1365-2745.2008.01395.x

Pavur GC (2016) Status of Mesocarnivores in the Last Area Known to be Occupied by Swift Foxes, Vulpes velox, in Texas. MS thesis. Lubbock: Texas Tech University. 73 p

Powell, K.I., Chase, J.M. and Knight, T.M. (2011). A synthesis of plant invasion effects on biodiversity across spatial scales. *American Journal of Botany* 98: 539-548, doi:10.3732/ajb.1000402

Pyšek, P., Jarošík, V., Hulme, P.E., Pergl, J., Hejda, M., Schaffner, U. and Vilà, M. (2012), A global assessment of invasive plant impacts on resident species, communities and ecosystems: the interaction of impact measures, invading species' traits and environment. *Global Change Biology* 18: 1725-1737. doi:10.1111/j.1365-2486.2011.02636.x

Ricciardi, A., Hoopes, M.F., Marchetti, M.P. and Lockwood, J.L. (2013). Progress toward understanding the ecological impacts of nonnative species. *Ecological Monographs* 83: 263-282, doi:10.1890/13-0183.1

Robertson, S.G. and Hickman, K.R.(2012). Aboveground plant community and seed bank composition along an invasion gradient. *Plant Ecology* 213: 1461–1475, https://doi.org/10.1007/s11258-012-0104-7

Roscher, C., Schumacher, J., Gubsch, M., Lipowsky, A., Weigelt, A., Buchmann, N., Schmid, B., and Schulze, E. (2012). Using Plant Functional Traits to Explain Diversity–Productivity Relationships. *PLOS ONE* 7(5): e36760, https://doi.org/10.1371/journal.pone.0036760

Scasta, J., Engle, D., Fuhlendorf, S., Redfearn, D., and Bidwell, T. (2015). Meta-Analysis of Exotic Forages as Invasive Plants in Complex Multi-Functioning Landscapes. *Invasive Plant Science and Management* 8(3): 292-306. doi:10.1614/IPSM-D-14-00076.1

Schmidt, C.D., Hickman, K.R., Channell, R., Harmoney, K., and Stark, W. (2008). Competitive abilities of native grasses and non-native (Bothriochloa spp.) grasses. *Plant Ecology* 197, 69–80, https://doi.org/10.1007/s11258-007-9361-2

Shi, Z., Sherry, R., Xu, X., Hararuk, O., Souza, L., Jiang, L., Xia, J., Liang, J., and Luo, Y. (2015). Evidence for long-term shift in plant community composition under decadal experimental warming. *Journal of Ecology* 103: 1131-1140. doi: 10.1111/1365-2745.12449

Smith, M.D., Wilcox, J.C., Kelly, T. and Knapp, A.K. (2004), Dominance not richness determines invasibility of tallgrass prairie. *Oikos* 106: 253-262. doi:10.1111/j.0030-1299.2004.13057.x

Sodhi, D.S., Livingstone, S.W., Carboni, M., and Cadotte, M.W. (2019). Plant invasion alters trait composition and diversity across habitats. *Ecology and Evolution* 9: 6199– 6210. https://doi.org/10.1002/ece3.5130

Sultan, S.E. (2000). Phenotypic plasticity for plant development, function, and life history. Trends in Plant Science 5(12): 537-542 https://doi.org/10.1016/S1360-1385(00)01797-0

Van Kleunen, M., Weber, E. and Fischer, M. (2010). A meta‐analysis of trait differences between invasive and non‐invasive plant species. *Ecology Letters* 13: 235-245. doi:10.1111/j.1461-0248.2009.01418.x

Vilà, M., Espinar, J.L., Hejda, M., Hulme, P.E., Jarošík, V., Maron, J.L., Pergl, J., Schaffner, U., Sun, Y. and Pyšek, P. (2011). Ecological impacts of invasive alien plants: a meta‐analysis of their effects on species, communities and ecosystems. *Ecology Letters*, 14: 702-708. doi:10.1111/j.1461-0248.2011.01628.x

Williams, D.G. and Baruch, Z. (2000). African Grass Invasion in the Americas: Ecosystem Consequences and the Role of Ecophysiology. *Biological Invasions* 2: 123–140 https://doi-org.ezproxy.lib.ou.edu/10.1023/A:1010040524588

Wilson, G.W.T., Hickman, K.R., and Williamson, M.M. (2012). Invasive warm-season grasses reduce mycorrhizal root colonization and biomass production of native prairie grasses. *Mycorrhiza* 22:327-336. DOI 10.1007/s00572-011-0407-x

Xu, X., Sherry, R.A., Niu, S., Li, D., & Luo, Y. (2013). Net primary productivity and rain-use efficiency as affected by warming, altered precipitation, and clipping in a mixed-grass prairie. *Global Change Biology* 19(9): 2753–2764. doi: 10.1111/gcb.12248

Younginger, B.S., Sirová, D., Cruzan, M.B. and Ballhorn, D.J. (2017). Is biomass a reliable estimate of plant fitness? *Applications in Plant Sciences* 5: 1600094. doi:10.3732/apps.1600094

Chapter 2: The function of restored communities converges over time despite dissimilar species compositions due to variable climate conditions in the year of establishment

Chapter 2: Abstract

It is well established that human activities have damaged many natural systems, prompting the development of restoration ecology. As this field has grown, questions have arisen as to what processes most strongly influence the trajectories of restored systems. It is critical to planning effective restoration actions to understand whether stochastic climate variation can influence the trajectory of restored communities. We studied two replicated sets of restored communities which differed in establishment year conditions (primarily precipitation) but were otherwise the same, to determine if the trajectory of community function is more heavily influenced by deterministic processes (initial seed mix of prairie restoration) or stochastic processes (establishment year conditions). We asked whether establishment year conditions impacted community function, and if so, whether function eventually converged. To answer these questions we calculated abundance-based community-weighted means (CWMs) of four functional traits (SLA, LDMC, leaf nitrogen content, and carbon : nitrogen ratio) across nine years post-restoration in two communities. We used profile analyses to investigate how the function of the communities changed over time. We found that while establishment year conditions led to dissimilar species compositions among restored communities and impacted the values of SLA and LDMC, the CWMs of three of the four functional traits converged over time. This pattern is likely driven by changes in the grass : forb ratio of the two communities, which converged despite the dissimilar species compositions and indicates that deterministic processes have the most influence at this level of organization. This suggests that the function of restored

communities is robust to variation in climate variables and provides valuable information for restoration practitioners.

Introduction

Elucidating the rules of community assembly to further understand the determinants of the richness and relative abundance of interacting species has been a major topic of interest for ecologists (Heil, 2004), particularly those interested in the factors determining the success of restored communities (Suding et al., 2004; Fukami et al., 2005). Human activities have severely degraded natural systems around the world (Steffen et al., 2004; Funk et al., 2008), including many systems critical to a variety of ecosystem functions and services (Díaz et al., 2019), leading to the initiation of ecological restoration projects (Matthews and Spyreas, 2010). These projects often aim to restore a specific target habitat type, species composition, or ecosystem function (Matthews and Spyreas, 2010). Since restoration projects can be costly in terms of resources, time, and opportunity it is important to understand what processes direct community assembly in order to achieve the target community or function (Stuble et al., 2017; Manning and Baer, 2018; Maccherini et al., 2018).

Two types of processes influence community assembly: deterministic processes, in which a given set of environmental and biotic conditions will lead to a predictable community, and stochastic processes, in which random events influence the ability of species to disperse and establish in the community and the order in which they do so (Chase, 2003; 2010; Heil, 2004). Many restoration projects are initiated under the assumption that deterministic processes have the strongest influence on community trajectory: sites restored using similar techniques and seed mixes should proceed along similar trajectories. However, in practice there are many stochastic processes that could lead to divergent restoration trajectories (Palmer et al., 1997). Climatic conditions, while predictable in the long-term, can give rise to random events such as droughts, which exert stochastic influence on communities by changing which species can establish at the restored site (Polley et al., 2013; Manning and Baer, 2018; Maccherini et al., 2018).

The relative influence of deterministic and stochastic processes on restoration trajectories can be assessed by following the development of communities with the same starting species pool across sites with varying environmental conditions (Bakker et al., 2003; Trowbridge, 2007), tracking the convergence or divergence of species composition over time. Several studies have demonstrated persistent compositional differences due to stochastic events in the year in which restored communities are established. Stuble et al. (2017) found that both mean annual temperature and number of precipitation events can drive dissimilarity in restored communities and found that these differences persisted at least four years post-restoration. Manning and Baer (2018) found that stochastic processes such as drought resulted in the assembly of tallgrass prairie communities with dissimilar species compositions after the first three years post-restoration. These communities continued along divergent trajectories through six years post-restoration (Baer, 2020, personal correspondence), supporting the idea that variable environmental conditions during establishment years lead to long-term divergence in the trajectories of species composition (Stuble et al., 2017). Another study, approaching the question from the opposite angle, found that even if environmental conditions are held constant, initial differences in species composition will persist, likely due to priority effects (Fukami et al., 2005). However, they also found evidence for convergence at the functional group level, suggesting that stochastic and deterministic processes may simultaneously act on different levels of organization (Fukami et al., 2005). One knowledge gap that remains, then, is whether stochastic or deterministic processes drive community function and whether communities that differ compositionally also differ functionally.

There is a growing awareness that in light of changing climate conditions (Suding et al., 2008) and the continued threat from invasive species (Funk et al., 2008) it will be increasingly necessary to restore communities to a target function rather than a reference species composition. The traits of the most abundant species in a community drive effects on ecosystem, landscape, and global scale processes (Funk et al., 2017) so indices that weight traits by species' abundance, such as community-weighted means (CWM), can reveal patterns in community function and explain variation in trait-environment relationships (Villeger et al., 2008; Funk et al. 2017). Variation in CWMs can identify shifts in assembly filters along ecological gradients and is most likely the strongest determinant of biotic effects on ecosystem function (Ackerly & Cornwell, 2007; Laliberte & Tylianakis, 2012; Funk et al. 2017). Thus, the function of a community can be assessed through the lens of community-weighted means of functional traits. Functional traits are the measurable attributes of a plant that interact with both the surrounding environment and other organisms (Drenovsky et al., 2012). The functional traits of plants can be classified non-exclusively as effect traits, which influence ecosystem function, and response traits, which respond to environmental conditions (Suding et al., 2008; Funk et al., 2017). Even single traits, such as specific leaf area (SLA), can explain a large amount of variation in ecosystem function, including primary productivity, litter decomposition rates, and plant-available soil nitrogen (De Deyn, Cornelissen & Bardgett, 2008; Lavorel & Grigulis, 2012; Loranger et al., 2012; Grigulis et al., 2013; Funk et al. 2017). By assessing community function over time through the lens of community-weighted means of important effect traits we can address the knowledge gap of the relative influence of deterministic vs. stochastic processes to determine community function.

In this study we investigate whether the differences in species composition caused by stochastic processes in a restoration experiment lead to ecologically significant shifts in the function of those communities via differences in functional trait CWMs. We analyzed the function of two restored communities (hereafter referred to as "sequences"), which were restored using the same seed mixes and establishment protocols, but experienced different environmental conditions (i.e., drastically different levels of precipitation) in the year of establishment before subsequently experiencing many years of shared conditions. This will allow us to elucidate whether community function is more heavily influenced by deterministic processes (initial seed mix, restoration procedures, long-term climate patterns) or stochastic processes (precipitation in establishment year). We assessed community function in terms of the community-weighted means (CWMs) of four effect traits: specific leaf area (SLA), leaf nitrogen content (leaf N), leaf carbon : nitrogen ratio (C:N ratio), and leaf dry matter content (LDMC). We compared the function of plots established during a year of normal precipitation (Sequence I) with that of plots established during a severe drought (Sequence II) in terms of CWMs of the four functional traits across the first nine years of each restoration. We asked 1) do the sequences differ in overall function across this time series? and 2) if they differ, is there a time at which their function converges? We hypothesized that the stochastic processes leading to divergent species compositions would also lead to divergent community functions. We predicted that the intense drought in the initial year of Sequence II would restrict the community to the establishment of species with a more conservative resource-use suite of functional traits (i.e., lower SLA and leaf N, higher LDMC and C:N ratio), altering the function of the community in comparison to Sequence I.

Methods

Study Site

The Sequential Restoration Project (SRP) was initiated at Konza Prairie Biological Station (KPBS), 9km south of Manhattan, KS, in 2010 to determine the relative influence of deterministic and stochastic processes on the assembly of restored tallgrass prairie plant communities (Manning and Baer, 2018). The site of the restoration experiment was in conventional agriculture for over 70 years before the experiment began. This site was originally covered by native tallgrass prairie dominated by warm-season C_4 grasses such as *Andropogon gerardii* and *Schizachyrium scoparium*, as well as a diverse mixture of subdominant forbs and grasses. The soil at this site is a Reading silt loam, the mean annual temperature is 12.7 ºC, and the site receives an average of 835mm of precipitation per year.

Experimental Design

Starting in 2010, and then every two years thereafter, adjacent sections of agricultural land were restored to native tallgrass prairie using the same initial seed mixes and following the same restoration procedures (Manning and Baer, 2018). A single field was used for all restoration sequences (the two in this study, as well as subsequent sequences that are not included in our analyses). Each sequence consists of four 20m x 20m plots (the last plot in Sequence I is only 10m x 20m due to a bordering forest), separated by a 5m buffer. Each of these four plots was restored independently following the same procedures so that we have four replicates for each establishment year. The prior agricultural community was cleared and the soil in each plot was shallowly disturbed with a field cultivator, then hand-raked before sowing seed by hand-broadcasting and lightly walking over the soil to compact it. Seeds were sown in a 60:40 ratio of forbs to dominant grasses and included native forbs such as *Baptisia australis, Helianthus pauciflorus,* and *Lespedeza capitata* as well as native grasses such as *Andropogon gerardii, Panicum virgatum, Schizachyrium scoparium*, and *Sorghastrum nutans* (see Table 1 in Manning and Baer, 2018, for a complete species list and live seed rate). Seeds were hand-collected from Konza Prairie and other nearby native prairie and a subsample of the seeds was sent to the Illinois Crop Association to determine the percent of live seed. To control for year-to-year variation in seed quality, the live seeding rate for the samples collected in 2009 (for use on Sequence I) was used for all subsequent sequences as well. The locally-collected *A. gerardii* had a low live seed rate and was supplemented with a cultivar (var. KAW), and the same ratio of locally-collected to purchased seeds was used for all subsequent sequences (Manning and Baer, 2018). The sequences received no management during the first three years but were subsequently burned annually in the spring to assist with establishment.

While the biotic and most abiotic factors remained the same for each restoration year, establishment year precipitation varied. Sequence I was restored in 2010, a year with precipitation near the long-term average of 835mm. Sequence II was restored in 2012, a year with 39% lower overall precipitation and 60% lower growing season precipitation than Sequence I. The species compositions were significantly different between these restoration sequences after their third growing season (Manning and Baer, 2018) and these differences persisted through at least six years (Baer, 2020, personal correspondence). Manning and Baer (2018) attributed this difference to the influence of drought hindering the establishment of late-successional (i.e. intentionally sown) species in Sequence II, which allowed for the natural colonization of weedy species.

Species Composition

The species composition of each sequence was recorded in August in the first year (2010 and 2012 for Sequence I and II, respectively) and in both June and August in the following years. Each of the four plots in each sequence is divided into four subplots, and there is a $10m^2$ circular sampling area within each subplot. The percent foliar cover of each species was recorded in these subplots using modified Daubenmire categories (Carter and Blair, 2012) and converted to the median value of the category. The maximum cover value between the June and August sampling dates was used for each species. We first calculated both the total foliar cover of each sequence in each of its first six years and the relative contribution of each species to that total cover value. We next sorted species by descending relative cover values in each year, then sequentially added species' cover values until we reached 80% of the total foliar cover. We selected 80% as the cutoff value primarily because it is the abundant species that most heavily influence community function (Ackerly & Cornwell, 2007). Relatively few species made up the top 80% (4–15 species per plot) and many rare species (<1% cover) made up the remaining 20% of total cover. This resulted in a list of 33 species that contributed to the top 80% of total foliar cover in at least one sequence in at least one sampling year (**Table S1**).

Functional Traits Database

We accessed trait values from the TRY plant trait database (Kattge et al., 2020) and calculated an average trait value for each functional trait for each species. Since the resulting trait values are therefore species-level average values, rather than direct measurements that account for intraspecific response to environmental conditions, we focused on effect traits rather than response traits. Three of the taxa in our species list were only identified to genus (*Eragrostis sp*., *Galium sp*., and *Lepidium sp*.) and one species (*Setaria faberi*) had no trait data in TRY, so the traits used for these taxa were the genus-level averages (average trait values from all species found at KPBS in each of these genera), an approach taken in similar trait-based studies (Magnago et al., 2013). We limited our study to four traits for which trait data were available for the majority of species in our study: specific leaf area (SLA), leaf dry matter content (LDMC), leaf nitrogen (leaf N), and carbon : nitrogen ratio (C:N ratio). Trait data were available for all species for SLA, but the other traits were not available for some species: LDMC (missing for 10 species), leaf N (missing for two species, *Bromus japonicus* and *Solidago altissima*) and C:N ratio (missing for one species, *B. japonicus*). These exceptions are unfortunate, but in most cases should not substantially alter the results. This data deficiency was most likely overcome by the fact that most missing species occurred roughly equally across the two treatments or only ever occurred at low cover values (an exception is *Helianthus pauciflorus*, which was at low abundance in Sequence I but medium abundance in Sequence II).

Once the trait data were downloaded from TRY, we performed a quality check to remove duplicate entries and trait records that came from manipulative experiments (e.g. nutrient addition treatments). Before calculating species-level average trait values, we created two separate trait datasets: a "Complete" set that included all TRY records that passed the initial quality check and a "Refined" set that only used a rarified subset of the data. To create the Refined trait set, we removed any entry with a TRY error risk score greater than 4 (unless it was the only entry a species had for a given trait). We then selected a random subset of the remaining traits for each species such that for each species, each of the four traits was calculated from the same number of entries (for example, if a species had eight entries for SLA but only six for LDMC, we would calculate the average SLA value from a random selection of six of the eight entries).

Calculating CWMs

We calculated community-weighted means (CWMs) in R version 3.6.1 (R Core Team, 2019) using the function "functcomp" in the package "FD" (Laliberté et al., 2014). This function returns a single value of each trait for each community (i.e. each plot in each year), which is the average trait value of all species in the community, weighted by their relative abundance in the community (Laliberté et al., 2014). We calculated CWMs of all four functional traits for the years 2010–2020 in all four plots of each sequence, based on the abundance values of our selected list of 33 species (**Table S2**). We calculated two CWM matrices, one using the Complete trait set and one using the Refined trait set. We then performed two-sample t-tests to compare the Complete and Refined CWMs for each trait. There was no difference in the CWMs for SLA, LDMC, or leaf N (all p-values > 0.6), so we used the CWMs from the Complete trait set in subsequent analyses. However, there were significant differences in the Complete and Refined C:N ratio datasets ($p < 0.001$). This is likely driven by several abnormal C:N ratio values that were included in the Complete trait set but excluded (based on error risk score) from the

Refined trait set. These values were upwards of 10x greater than the normal range of C:N ratios. Thus, we used the Refined CWMs of C:N ratio in subsequent analyses.

Statistical Analyses

We first analyzed several aspects of community composition between the two sequences. We used the R package "vegan" (Oksanen, et al., 2019) to confirm that species composition differed between the two sequences across our study. We used the function "vegdist" to calculate Bray-Curtis dissimilarity matrices for the species compositions of each plot and used the function "adonis" to perform PERMANOVAs (999 permutations) to determine if species composition differed between the sequences at ages 1, 3, 6, and 9. Additionally, since we knew which species were intentionally sown into the sequences and which colonized them naturally ("volunteers"), we performed t-tests in R to check for differences in the foliar cover ratios of sown species to volunteer species, introduced to native species, sown grasses to sown forbs, all grasses to all forbs, and annual grasses to perennial grasses between the two sequences. Finally, we performed t-tests to determine if there were differences in trait values between the grasses and the forbs included in this study.

Next, we utilized profile analysis to determine if the two sequences differed in function over time. Although profile analysis is an underutilized statistical technique in ecology (one of the few examples is Van Buskirk and Smith, 1991) it is a straightforward procedure that provides robust, intuitive results and is particularly well suited for analyzing data measured in the same plots over time (Tabachnick and Fidell, 2013). As a multivariate statistical technique, profile analysis can answer questions that would otherwise require using several univariate analyses, yet without increasing the Type I error rate. In this case, profile analysis is essentially an application of MANOVA in which the repeated measures of one dependent variable (e.g. SLA measured over time) are analyzed as multiple dependent variables (e.g. SLA in each sampling year is treated as a separate variable). This avoids pitfalls such as pseudoreplication that would occur by treating the non-independent sequential trait measurements as independent values. The profile analysis then answers the question, "do the two treatments (Sequences I and II) have different profiles for each functional trait?" by examining difference scores between consecutive time periods. There are three components of each profile: levels, flatness, and parallelism.

The first component, levels, is analogous to the test of group differences in ANOVA and asks if one sequence has a higher average trait value than the other (Tabachnick and Fidell, 2013). The null hypothesis of this test is that the levels are equal between the sequences, so if we reject this null it means that there is a significant main effect of sequence on the functional trait in question. The second component, flatness, tests for main effects of time (Tabachnick and Fidell, 2013). Flatness is evaluated against the null hypothesis that the average slope between time periods is zero. If the null is rejected, this indicates that the functional trait changes as the sequences age. The final component is parallelism, which asks whether the two sequences have parallel profiles and tests for sequence-by-age interactions (Tabachnick and Fidell, 2013). Parallelism is tested against the null hypothesis that the sequences have parallel profiles. If parallelism is rejected, then there is a significant sequence-by-age interaction (i.e. the functional traits respond differently over time based on establishment year conditions).

We conducted separate profile analyses on the CWMs of each of the four functional traits across age 1yr to 9yrs of each sequence. Since profile analysis requires equal intervals between measurements but species cover (and therefore CWMs) were not measured in Sequence I at age 7yrs (2016), we used the average value of each trait from age 6yrs (2015) to age 8yrs (2017) to fill in this gap. We used the CWMs from every other year (i.e. ages 1yr, 3yrs, 5yrs, 7yrs, and 9yrs) to analyze the decadal patterns in community function. We then performed additional post-hoc profile analyses of each trait to investigate fine-scale variation in function over the early years post-restoration (every year, age 1yr to 7yrs). We used the function "pbg" (profile by groups) from the R package "profileR" (Bulut and Desjardins, 2018) for all profile analyses. Post-hoc ANOVAs were performed in R to see at which ages the sequences differed in values of each trait.

Results

PERMANOVAs indicated that species composition was persistently divergent between the two sequences: age 1 ($p = 0.032$, $F = 9.03$), age 3 ($p = 0.031$, $F = 16.24$), age 6 ($p = 0.031$, $F = 18.67$), and age 9 ($p = 0.034$, $F = 9.91$). Although t-tests indicated there was no difference in the cover ratio of annual to perennial grasses ($p = 0.231$), introduced to native species ($p = 0.243$), or of volunteer to sown species ($p = 0.303$, **Fig. 1**) between the sequences, the ratio of grass to forb foliar cover ($p = 0.021$, **Fig. 2**) was significantly higher in Sequence I compared to Sequence II, as was the ratio of sown grasses to sown forbs ($p = 0.009$). The grasses included in our study had significantly higher trait values for SLA ($p = 0.020$) and LDMC ($p = 0.041$) than the forbs included in our study. Leaf N ($p = 0.063$) and C:N ratio ($p = 0.544$) did not differ between grasses and forbs.

Profile analyses demonstrated that each of the four traits we studied was significantly impacted by at least one of establishment year, age, or an interaction between the two. We found main effects (difference in "Levels") of establishment year in the CWMs of two traits. Both SLA (**Fig. 3A**) and LDMC (**Fig. 3B**) were higher in the community established under normal precipitation conditions (Sequence I) than in the community established during a drought ($p = 0.016$, $F = 10.98$; and $p = 0.001$, $F = 32.38$, respectively). Although post-hoc ANOVAs indicated some significant differences in individual years, there was no effect of treatment on leaf N ($p = 0.297$; **Fig. 3C**) or C:N ratio ($p = 0.054$; **Fig. 3D**) across the full time period of our study. Additionally, post-hoc ANOVAs indicated that by age 9, three of the four traits had statistically similar values between the sequences (SLA, leaf N, and C:N ratio).

The profiles of SLA ($p = 0.007$, $F = 38.25$), LDMC ($p = 0.021$, $F = 17.27$), leaf N ($p = 0.004$, $F = 55.50$) and C:N ratio ($p < 0.001$, $F = 394.19$) were not flat. This indicates that there is a significant change in all four traits over time. Both SLA and leaf N decrease as the communities age while LDMC and C:N ratio increase. We also found an interaction between restoration sequence and age for the CWMs of two traits, LDMC and C:N ratio (i.e. we rejected the null hypothesis of "Parallelism"), indicating that environmental conditions during the establishment year influenced how the traits changed over time. The trajectory of LDMC was different between the two sequences ($p = 0.019$ $F = 18.05$). The LDMC of each sequence increased over the first three to five years post-restoration before eventually leveling off. However, the increase was more gradual in the drought community (Sequence II) and took longer to level off. There was also a sharp decline in LDMC from age five to six in Sequence II, which did not occur in

Sequence I. The trajectory of C:N ratio was also different between the two sequences (p = 0.019, F = 18.26). The C:N ratios were initially low, then increased before leveling off. Again, this change was more gradual in Sequence II and did not level off until approximately five years of age.

Post-hoc profile analyses of the fine-scale variation in CWMs over the early years of restoration were largely consistent with the decadal patterns observed in the main analyses, but several interesting differences were apparent. For instance, there was a main effect of establishment year on C:N ratio (p = 0.007, F = 16.18) in the early years of restoration (Sequence I initially had a higher C:N ratio), but the C:N ratio of the two sequences converges in later years. Similarly, there was a sequence-by-age interaction in leaf N (p = 0.027, F = 827.56) response in the early years (a more gradual decline in leaf N for Sequence II) that disappeared at the decadal scale. Conversely, there was no effect of time on SLA (p = 0.157, F = 23.45) or LDMC (p = 0.174, F = 19.00) in the early years of the restoration (i.e. no significant change in trait values over time), but a significant effect of time over the full nine years of the study.

Discussion

Both deterministic and stochastic processes play a role in community assembly (Heil, 2004) and should therefore be considered in the planning of ecological restoration projects (Manning and Baer, 2018). While several studies have found that stochastic events such as droughts and extreme precipitation events can significantly alter the species compositions of restored communities (Stuble et al., 2017; Manning and Baer, 2018; Maccherini et al., 2018) it was uncertain whether such communities also differ functionally. We addressed this question by analyzing the change in community-weighted means of four important functional traits across two restoration sequences known to have different species compositions due in part to stochastic processes in the year of establishment. We hypothesized that the drought in 2012 would have stochastic influence on the function of the community in Sequence II, restricting establishment to species with functional trait values reflecting a conservative resource-use strategy. We found at least one significant effect of restoration sequence, age, or an interaction for each trait we examined and these effects sometimes persisted for many years after the establishment of the restored habitats; yet there was also evidence for convergence in three of the four traits (and uncertainty about the fourth, LDMC). Contrary to our predictions, drought in the year of establishment appeared to favor the establishment of species with high leaf nitrogen content and low C:N ratios and LDMC. All three of these functional traits differed over the first three years of the restoration (except leaf N in the first year). However, overall there were no significant differences in leaf N or C:N ratio across the full nine years of each restoration. Both of these traits had converged by their fourth year and remained relatively constant thereafter. Similarly, the CWMs of specific leaf area defied our initial predictions. While there was an overall treatment effect across the full nine years of each sequence, SLA did not differ in the first two years. This trait diverged during the middle years (ages three through eight) but appears to have converged again by the ninth year of each sequence. Only LDMC differed in each year and showed no signs of convergence. Overall our results suggest that deterministic processes control community function even when species compositions remain divergent.

The patterns we observed appear to be primarily driven by deterministic processes, such as the shared seed mixes, restoration procedures, long-term climate conditions, and local species pool, which led to convergence of the function of the two communities. In contrast, the stochastic processes associated with establishment year precipitation resulted in the development of dissimilar communities, but did not extend to differences in function. One of the most striking initial differences between the sequences is the much higher ratio of sown grasses to sown forbs in Sequence I compared to Sequence II (**Fig. 2B**). Intentionally-sown, late-successional grasses such as *Andropogon gerardii* and *Sorghastrum nutans* established much more successfully in Sequence I while weedy forbs such as *Erigeron canadensis*, *Abutilon theophrasti*, and *Mollugo verticillata* dominated the early years of Sequence II (drought), and these differences in species composition were maintained through the ninth year of the study. This led to a higher grass to forb ratio in most years for Sequence I, while Sequence II had more forbs than grasses in each of the nine years we examined. Other studies have also shown that wetter years favor the establishment of grasses and drier years the establishment of forbs (Pitt and Heady, 1978; Stuble et al., 2017). The higher dominance of grasses in Sequence I likely explains the higher SLA and LDMC observed in this sequence, as the grass species included in our study had significantly higher values for both traits than the forbs included in our study. But despite persistant differences in species composition and initial differences in functional group dominance, the ratio of grasses to forbs converged in later years. Functional differences subsequently tended to decrease over time as the sown species (both grasses and forbs) began to dominate both communities in later years and led to the convergence in the ratio of grasses to forbs, and thus the convergence of community function.

Several caveats should be addressed. First, LDMC, the only trait that did not converge, is also the trait for which we have the least available data. We were unable to obtain trait values for nearly one third of the species included in our study. Most of these species rarely accounted for more than 5% of the relative cover in a given year and are unlikely to be significant drivers of the abundance-dependent CWMs we calculated. However, the omission of *Helianthus pauciflorus* and *Solidago altissima* has an unknown but potentially significant impact on the community-weighted mean LDMC. Both of these species had relatively high abundances and were unequal contributors to the communities of Sequence I and II. Therefore we recommend caution when interpreting the persistent divergence observed in this trait. That said, if LDMC is indeed persistently divergent it could have important functional implications, as LDMC may be a better predictor of aboveground net primary productivity than SLA (Smart et al., 2017). The second caveat is that our trait values are derived from species-level mean trait values and therefore reflect differences in species composition between the sequences, not intraspecific variation. Ideally, both interspecific and intraspecific trait variation should be accounted for when considering trait responses to environmental conditions (Albert et al, 2011; Siefert et al., 2015; Fajardo and Siefert, 2018). While we were unable to directly measure our trait values to account for intraspecific variation, our results are likely still robust. Interspecific variation accounts for approximately 75% of the total trait variation within communities (Siefert et al., 2015) and tends to account for a higher proportion of total trait variation when species abundance is considered (Kichenen et al., 2013). Furthermore, intraspecific variation is less important along climatic gradients than edaphic gradients (Siefert et al., 2014) and less important for leaf-level traits than whole-plant traits (Siefert et al., 2015). Additionally, we expect that intraspecific variation should be making the CWMs of the two sequences more similar over time if the species in these communities respond in the same direction to environmental conditions, since the plants in each community accumulate more years of shared climate conditions with each passing year. Therefore we are confident that the above-ground function of these two communities is indeed converging, and suspect that the trait values should be even more similar than we observed. While we were unable to include belowground traits in our analyses, and belowground traits such as specific root length and root architecture can impact ecosystem functions including nutrient cycling and soil formation (Bardgett et al., 2014), the similar ratio of perennial to annual grasses between the sequences suggests that any belowground functional differences should be relatively minor.

Taking the above points into consideration, we may still draw several noteworthy conclusions. When considering the effects of plant traits on ecosystem function, it is important to consider shifts across a suite of traits, rather than examining single traits (Kichenen et al., 2013). We found that in both sequences, deterministic processes drive similar trait responses over time that leads to a similar suite of trait values in the two communities. Leaf N and SLA decrease in the first few years before stabilizing, while LDMC and C:N ratio increase before stabilizing, as the weedy species that initially colonize both sequences are replaced by higher proportions of intentionally-sown species (**Fig. 1**). While the identity of species in the two communities remains divergent, the suite of functional traits exhibited by each community becomes more similar over the decadal timescale of our study, a pattern consistent with the increasing dominance of late-successional species and the convergence of grass to forb ratios. This result is similar to the findings of Fukami at al. (2005) that functional group composition converges due to deterministic processes despite stochastic processes leading to divergent species compositions, and provides further evidence that community assembly is guided by different processes at different scales of organization. In light of the diverse experiments demonstrating that stochastic processes lead to divergent restoration trajectories (Fukami et al., 2005; Matthews and Spyreas, 2010; Stuble et al., 2017) and evidence that climate change and invasive species will continue to influence restoration success (Funk et al., 2008; Suding et al., 2008), our results lend further support to the idea that restoration goals should focus on functional groups and ecosystem functions rather than target species compositions (Palmer et al., 1997; Suding, 2011). Assembly history has a lower impact at higher levels of organization such as community biomass and net ecosystem exchange (Kardol et al., 2013), so community function may be a much more realistic restoration goal than species composition, one that is robust to even extreme climate events such as the drought in the establishment year of Sequence II.

Conclusions

The impact of stochastic processes such as drought is strongest in the first few years after the event. These impacts may drive persistent differences in species identity but appear to be less important in driving community function. Our results suggest that deterministic processes (i.e., shared seed mixes, restoration procedures, long-term climate patterns, and the local species pool) can override even extreme climate events to lead to convergence in the function of restored communities. This finding should give restoration practitioners confidence that when their focus is community function, the long-term outcomes of their efforts can be robust to year-to-year variation in precipitation.

Chapter 2: References

Ackerly, D. D. and Cornwell, W. K. (2007). A trait-based approach to community assembly: partitioning of species trait values into within- and among-community components. *Ecology Letters* 10, 135–145.

Albert, C.H., Grassein, F., Schurr, F.M., Vieilledent, G., and Violle, C. (2011). When and how should intraspecific variability be considered in trait-based plant ecology? *Perspectives in Plant Ecology, Evolution, and Systematics*, 13: 217–225. doi:10.1016/j.ppees.2011.04.003

Atkin O.K., Bloomfield, K.J., Reich, P.B., Tjoelker, M.G., and Asner, G.P., et al. (2015). Global variability in leaf respiration among plant functional types in relation to climate and leaf traits. *New Phytologist* DOI: 10.1111/nph.13253

Bakker, J.D., Wilson, S.D., Christian, J.M., Li, X., Ambrose, L.G. and Waddington, J. (2003). Contingency of grassland restoration on year, site, and competition from introduced grasses. *Ecological Applications*, 13: 137-153. doi:10.1890/1051-0761(2003)013[0137:COGROY]2.0.CO;2

Bardgett, R.D., Mommer, L., and De Vries, F.T. (2014). Going underground: root traits as drivers of ecosystem processes. *Trends in Ecology and Evolution*, 29(12): 692-699, https://doi.org/10.1016/j.tree.2014.10.006

Chase, J.M. (2003). Community assembly: when should history matter? *Oecologia* 136(4): 489-498. DOI: 10.1007/s00442-003-1311-7.

Chase, J.M. (2010). Stochastic community assembly causes higher biodiversity in more productive environments. *Science* 328(5984): 1388-1391 DOI: 10.1126/science.1187820

Ciccarelli D. (2015). Mediterranean coastal dune vegetation: Are disturbance and stress the key selective forces that drive the psammophilous succession? *Estuarine, Coastal and Shelf Science* 165(5):247–253 doi: 10.1016/j.ecss.2015.05.023

Cornelissen, J. H. C. (1996). An experimental comparison of leaf decomposition rates in a wide range of temperate plant species and types. *Journal of Ecology* 84:573-582.

Craine, J. M., Lee, W.G., Bond, W.J., Williams, R.J., and Johnson, L.C. (2005). Environmental constraints on a global relationship among leaf and root traits of grasses. *Ecology* 86:12-19.

Craine, J. M., Elmore, A.J., Aidar, M.P.M., Bustamante, M., Dawson, T.E., et al. (2009). Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. *New Phytologist* 183:980-992.

Craine, J.M., Nippert, J.B., Towne, E.G., Tucker, S., Kembel, S.W., Skibbe, A, and McLauchlan, K.K. (2011). Functional consequences of climate-change induced plant species loss in a tallgrass prairie. *Oecologia* 165: 1109-1117

Craine, J.M., Towne, E.G., Ocheltree, T.W., and Nippert, J.B. (2012). Community traitscape of foliar nitrogen isotopes reveals N availability patterns in a tallgrass prairie. *Plant Soil* 356: 395-403

De Deyn, G. B., Cornelissen, J. H. and Bardgett, R. D. (2008). Plant functional traits and soil carbon sequestration in contrasting biomes. *Ecology Letters* 11: 516 – 531.

Díaz, S., Hodgson, J.G., Thompson, K., Cabido, M., Cornelissen, J.H.C., et al. (2004). The plant traits that drive ecosystems: Evidence from three continents. *Journal of Vegetation Science* 15:295-304.

Díaz, S., Settele, J., Brondízio, E., et al. (2019). Pervasive human-driven decline of life on Earth points to the need for transformative change. *Science* 366. doi: 10.1126/science.aax3100

Drenovsky, R.E., Grewell, B.J., D'Antonio, C.M., Funk, J.L., James, J.J., Molinari, N., Parker, I.M., and Richards, C.L. (2012). A functional trait perspective on plant invasion. *Annals of Botany*, 110(1): 141-153 https://doi.org/10.1093/aob/mcs100

Fajardo, A. and Siefert, A. (2018). Intraspecific trait variation and the leaf economics spectrum across resource gradients and levels of organization. *Ecology*, 99: 1024-1030. doi:10.1002/ecy.2194

Fukami, T., Martijn Bezemer, T., Mortimer, S.R. and van der Putten, W.H. (2005). Species divergence and trait convergence in experimental plant community assembly. *Ecology Letters*, 8: 1283-1290. doi:10.1111/j.1461-0248.2005.00829.x

Fukami, T., Dickie, I.A., Paula Wilkie, J., Paulus, B.C., Park, D., Roberts, A., Buchanan, P.K. and Allen, R.B. (2010). Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. *Ecology Letters*, 13: 675-684. doi:10.1111/j.1461-0248.2010.01465.x

Funk, J.L., Cleland, E.E., Suding, K.N., and Zavaleta, E.S. (2008). Restoration through reassembly: plant traits and invasion resistance. *Trends in Ecology and Evolution*, 23(12): 695 - 703. doi:10.1016/j.tree.2008.07.013

Funk, J.L., Larson, J.E., Ames, G.M., Butterfield, B.J., Cavender-Bares, J., Firn, J., Laughlin, D.C., Sutton-Grier, A.E., Williams, L. and Wright, J. (2017). Revisiting the Holy Grail: using plant functional traits to understand ecological processes. *Biological Reviews*, 92: 1156-1173. doi:10.1111/brv.12275

Garnier, E., Navas, M.-L., and Grigulis, K. (2016). Chapter 4: Gradients, response traits, and ecological strategies. pp. 64 - 93 in *Plant Functional Ecology*,. Oxford University Press, Oxford, UK.

Grigulis, K., Lavorel, S., Krainer, U., Legay, N., Baxendale, C., Dumont, M., Kastl, E., Arnoldi, C., Bardgett, R. D., Poly, F., Pommier, T., Schloter, M., Tappeiner, U., Bahn, M. and Clement, J.-C. (2013). Relative contributions of plant traits and soil microbial properties to mountain grassland ecosystem services. *Journal of Ecology* 101: 47–57.

Heil, G. W. 2004. Chapter 11. Modeling of plant community assembly in relation to deterministic and stochastic processes. pp. 230–244 in Temperton, V., Hobbs, R., Nuttle, T. and Halle, S., eds. *Assembly rules and restoration ecology: bridging the gap between theory and practice.* Island Press, Washington, D.C., USA.

Kardol, P., Souza, L. and Classen, A.T. (2013). Resource availability mediates the importance of priority effects in plant community assembly and ecosystem function. *Oikos*, 122: 84-94. doi:10.1111/j.1600-0706.2012.20546.x

Kattge, J., W. Knorr, T. Raddatz, and C. Wirth. (2009). Quantifying photosynthetic capacity and its relationship to leaf nitrogen content for global-scale terrestrial biosphere models. *Global Change Biology* 15:976-991.

Kattge, J., Bönisch, G., Díaz, S., Lavorel, S., Prentice, I. C., Leadley, P., Tautenhahn, S., Werner, G., et al. (2020). TRY plant trait database - enhanced coverage and open access. *Global Change Biology*, 26(1), 119-188. doi:10.1111/gcb.14904.

Kerkhoff, A. J., Fagan, W.F., Elser, J.J., and Enquist, B.J. (2006). Phylogenetic and growth form variation in the scaling of nitrogen and phosphorus in the seed plants. *American Naturalist* 168:E103-E122.

Kichenin, E., Wardle, D.A., Peltzer, D.A., Morse, C.W. and Freschet, G.T. (2013). Contrasting effects of plant inter‐ and intraspecific variation on community‐level trait measures along an environmental gradient. *Functional Ecology*, 27: 1254-1261. doi:10.1111/1365-2435.12116

Kleyer, M., Bekker, R.M., Knevel, I.C., Bakker, J.P., Thompson, K., et al., (2008). The LEDA Traitbase: a database of life-history traits of the Northwest European flora. *Journal of Ecology,* 96:1266-1274.

La Pierre, K.J. and Smith, M.D. (2015) Functional trait expression of grassland species shift with short- and long-term nutrient additions. *Plant Ecology* 216: 307 doi:10.1007/s11258-014-0438-4

Laughlin, D. C., Leppert, J.J., Moore, M.M., and Sieg, C.H. (2010). A multi-trait test of the leaf-height-seed plant strategy scheme with 133 species from a pine forest flora. *Functional Ecology,* 24:493-501.

Lavorel, S. and Grigulis, K. (2012). How fundamental plant functional trait relationships scale-up to trade-offs and synergies in ecosystem services. *Journal of Ecology,* 100, 128–140.

Lhotsky, B., Csecserits, A., Kovács, B., and Botta-Dukát, Z. (2016). New plant trait records of the Hungarian flora, *Acta Botanica Hungarica*, 58(3-4), 397-400, https://doi.org/10.1556/abot.58.2016.3-4.8

Li, Y. and Shipley, B. (2018). Community divergence and convergence along experimental gradients of stress and disturbance. *Ecology*, 99: 775-781. doi:10.1002/ecy.2162

Loranger, J., Meyer, S. T., Shipley, B., Kattge, J., Loranger, H., Roscher, C. and Weisser, W. W. (2012). Predicting invertebrate herbivory from plant traits: evidence from 51 grassland species in experimental monocultures. *Ecology* 93, 2674 – 2682.

Maccherini S., Bacaro G., and Marignani, M. (2018). Beneficial effects of restoration practices can be thwarted by climate extremes. *Science of The Total Environment,* 626:851–859, https://doi.org/10.1016/j.scitotenv.2018.01.117

Magnago, L.F.S., Edwards, D.P., Edwards, F.A., Magrach, A., Martins, S.V. and Laurance, W.F. (2014). Functional attributes change but functional richness is unchanged after fragmentation of Brazilian Atlantic forests. *Journal of Ecology*, 102: 475-485. doi:10.1111/1365-2745.12206

Maire V, Wright, I.J., Prentice, I.C., Batjes, N.H., Bhaskar, R., et al. (2015) Data from: Global effects of soil and climate on leaf photosynthetic traits and rates. *Dryad Digital Repository*. http://dx.doi.org/10.5061/dryad.j42m7

Manning, G. C., and Baer, S. G.(2018). Interannual variability in climate effects on community assembly and ecosystem functioning in restored prairie. *Ecosphere* 9(6):e02327. 10.1002/ecs2.2327

Matthews, J.W. and Spyreas, G. (2010). Convergence and divergence in plant community trajectories as a framework for monitoring wetland restoration progress. *Journal of Applied Ecology,* 47: 1128-1136. doi:10.1111/j.1365-2664.2010.01862.x

Miller, J.E.D., Ives, A.R., Harrison, S.P., and Damschen, E.I. (2018). Early- and late-flowering guilds respond differently to landscape spatial structure. *Journal of Ecology*, 106:1033–1045. https://doi.org/10.1111/1365-2745.12849

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos. P., Stevens, M.H.H., Szoecs, E., and Wagner, H., (2019). vegan: community ecology package. R package version 2.5-6. https://CRAN.R-project.org/package=vegan

Palmer, M. A., Ambrose, R.F., and Poff, N.L. (1997). Ecological theory and community restoration ecology. *Restoration Ecology,* 5:291–300, https://doi.org/10.1046/j.1526-100X.1997.00543.x

Polley, H.W., Isbell, F.I. and Wilsey, B.J. (2013). Plant functional traits improve diversity-based predictions of temporal stability of grassland productivity. *Oikos*, 122: 1275-1282. doi:10.1111/j.1600-0706.2013.00338.x

Prentice, I.C., Meng, T., Wang, H., Harrison, S.P., Ni, J., and Wang, G., (2011). Evidence for a universal scaling relationship of leaf CO2 drawdown along a moisture gradient. New *Phytologist* 190: 169Ð180

Shipley, B. (1995). Structured Interspecific Determinants of Specific Leaf-Area in 34 Species of Herbaceous Angiosperms. *Functional Ecology,* 9:312-319.

Shipley, B. and Lechowicz, M.J. (2000). The functional co-ordination of leaf morphology, nitrogen concentration, and gas exchange in 40 wetland species. *Ecoscience,* 7:183-194.

Shipley, B. (2002). Trade-offs between net assimilation rate and specific leaf area in determining relative growth rate: relationship with daily irradiance. *Functional Ecology,* (16) 682-689

Shipley, B. and Vu, T.T. (2002). Dry matter content as a measure of dry matter concentration in plants and their parts. *New Phytologist,* 153:359-364.

Siefert, A., Fridley, J.D., and Ritchie, M.E. (2014). Community Functional Responses to Soil and Climate at Multiple Spatial Scales: When Does Intraspecific Variation Matter? *PLoS ONE,* 9(10): e111189. https://doi.org/10.1371/journal.pone.0111189

Siefert, A., Violle, C., Chalmandrier, L., Albert, C.H., Taudiere, A., et al. (2015). A global meta-analysis of the relative extent of intraspecific trait variation in plant communities. *Ecology Letters,* 18: 1406-1419. doi:10.1111/ele.12508

Smart, S.M., Glanville, H.C., Blanes, M.d.C., Mercado, L.M., Emmett, B.A., et al. (2017). Leaf dry matter content is better at predicting above-ground net primary production than specific leaf area. *Functional Ecolology*, 31: 1336-1344. doi:10.1111/1365-2435.12832

Smith, N. G. and Dukes, J. S. (2017). LCE: leaf carbon exchange data set for tropical, temperate, and boreal species of North and Central America. *Ecology*, 98: 2978. doi:10.1002/ecy.1992

Steffen, W., Sanderson, A., Tyson, P.D. et al. (eds) (2004). *Global Change and the Earth System: A Planet Under Pressure*. Springer-Verlag, Berlin.

Stuble, K.L., Fick, S.E. and Young, T.P. (2017). Every restoration is unique: testing year effects and site effects as drivers of initial restoration trajectories. *Journal of Applied Ecology*, 54: 1051-1057. doi:10.1111/1365-2664.12861

Suding, K.N., Gross, K.L., and Houseman, G.R. (2004). Alternative states and positive feedbacks in restoration ecology. *Trends in Ecology and Evolution* 19(1): 46 - 53. doi:10.1016/j.tree.2003.10.005

Suding, K.N., Lavorel, S., Chapin, F.S., III, Cornelissen, J.H.C., Díaz, S., Garnier, E., Goldberg, D., Hooper, D.U., Jackson, S.T. and Navas, M.-L. (2008). Scaling environmental change through the community-level: a trait-based response-and-effect framework for plants. *Global Change Biology*, 14: 1125-1140. doi:10.1111/j.1365-2486.2008.01557.x

Suding, K.N. (2011). Toward an era of restoration in ecology: successes, failures, and opportunities ahead. *Annual Review of Ecology, Evolution, and Systematics,* 42(1): 465-487, https://doi.org/10.1146/annurev-ecolsys-102710-145115

Tabachnick, B.G., and Fidell, L.S. (2013). Profile analysis: the multivariate approach to repeated measures. pp 314 - 376 in *Using Multivariate Statistics*, 6th edition, Pearson, Boston.

Tribouillois, H., Fort, F., Cruz, P., Charles, R., Flores, O., Garnier, E., et al. (2015). A Functional Characterisation of a Wide Range of Cover Crop Species: Growth and Nitrogen Acquisition Rates, Leaf Traits and Ecological Strategies. *PLoS ONE,* 10(3): e0122156. doi:10.1371/journal.pone.0122156

Trowbridge, W. B. (2007). The role of stochasticity and priority effects in floodplain restoration. *Ecological Applications*, 17:1312–1324.

Tucker, S.S., Craine, J.M., and Nippert, J.B. (2011). Physiological drought tolerance and the structuring of tallgrass assemblages. *Ecosphere,* 2(4): 48, doi:10.1890/ES11-00023.1

Van Buskirk, J. and Smith, D.C. (1991). Density‐Dependent Population Regulation in a Salamander. *Ecology*, 72: 1747-1756. doi:10.2307/1940973

van der Plas, F. and Olff, H. (2014). Mesoherbivores affect grasshopper communities in a megaherbivore-dominated South African savannah. *Oecologia,* 175: 639. doi:10.1007/s00442-014-2920-z

Vergutz, L., Manzoni, S., Porporato, A., Novais, R.F., and Jackson, R.B. (2012). A Global Database of Carbon and Nutrient Concentrations of Green and Senesced Leaves. Data set. Oak Ridge National Laboratory Distributed Active Archive Center, Oak Ridge, Tennessee, U.S.A. http://dx.doi.org/10.3334/ORNLDAAC/1106

Vile, D. (2005). Significations fonctionnelle et ecologique des traits des especes vegetales: exemple dans une succession post-cultural mediterraneenne et generalisations, Ph.D. Thesis.

Villeger, S., Mason, N. W. H. and Mouillot, D. (2008). New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology* 89, 2290–2301.

Wang, H., Harrison, S.P., Prentice, I.C., Yang, Y., Bai, F., Furstenau Togashi, H., Wang, M., Zhou, S., and Ni, J. (2017). The China Plant Trait Database. *PANGAEA*, https://doi.org/10.1594/PANGAEA.871819

White, M. A., Thornton, P.E., Running, S.W., and Nemani, R.R. (2000). Parameterization and sensitivity analysis of the BIOME-BGC terrestrial ecosystem model: Net primary production controls. *Earth Interactions* 4:1-85.

Wright, I. J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., et al., (2004). The worldwide leaf economics spectrum. *Nature,* 428:821-827.

Wright, J.P., Sutton-Grier, A. (2012). Does the leaf economic spectrum hold within local species pools across varying environmental conditions? *Functional Ecology,* 26: 1390-1398 doi: 10.1111/1365-2435.12001

Chapter 1: Tables and Figures

Table 1. Results from SIMPER analysis addressing species-specific contributions to the overall dissimilarity in species composition between uninvaded (Zero) and highly invaded (High) plots. The species contributing 50% of the total dissimilarity in the species composition between Zero and High invasion plots are shown. No other pairs of cover categories differed significantly in their species composition. "Frequency" gives the proportion of plots in each cover category in which the species was present; "% Contribution" gives the percent of the dissimilarity between Zero and High invasion plots accounted for by the frequency of each species; "Cumulative Dissimilarity" gives the percent of dissimilarity between Zero and High invasion plots accounted for by each species and all species listed above. Note that species that are abundant at all invasion levels (such as *Ambrosia psilostachya*) are not major contributors to dissimilarity.

Species (Functional Group)	**Frequency (Zero Invasion)**	**Frequency (High Invasion)**	**Percent Contribution**	**Cumulative Dissimilarity**
Dalea purpurea (legume)	0.93	0.13	4.87	4.87
Psoralidium tenuiflorum (legume)	1.00	0.38	3.64	8.51
Dichanthelium oligosanthes (C_3 grass)	0.57	0.13	3.34	11.84
Unknown Forb (forb)	0.43	0.75	3.20	15.04
Liatris squarrosa (forb)	0.93	0.50	3.17	18.21
Chamaecrista fasciculata (legume)	0.21	0.63	3.11	21.31
Stenaria nigricans (forb)	0.71	0.50	3.04	24.35
Unknown Grass	0.36	0.63	3.03	27.38
Erigeron strigosus (forb)	0.86	0.50	3.02	30.40
Croton monanthogynus (forb)	0.43	0.50	2.90	33.30
Tragia betonicifolia (forb)	0.43	0.50	2.88	36.18
Oenothera serrulata (forb)	0.43	0.38	2.78	38.97
Solidago rigida (forb)	0.36	0.38	2.77	41.73
Desmanthus illinoensis (legume)	0.64	0.63	2.71	44.44
Sorghastrum nutans (C_4 grass)	0.93	0.63	2.57	47.01
Amphiachyris dracunculoides (forb)	0.00	0.38	2.51	49.52
Solidago nemoralis (forb)	0.93	0.63	2.46	51.98

Figure 1. Increasing cover of the invasive *Bothriochloa ischaemum* reduces the abundance of the native *Schizachyrium scoparium*. The relationship is stronger when all plots are included (blue line; blue + red points), but there is still a linear decrease in *S. scoparium* abundance when the analysis is restricted to only include plots where the invasive *B. ischaemum* is present (red line; red points only), indicating an increase in impact severity as invader abundance increases.

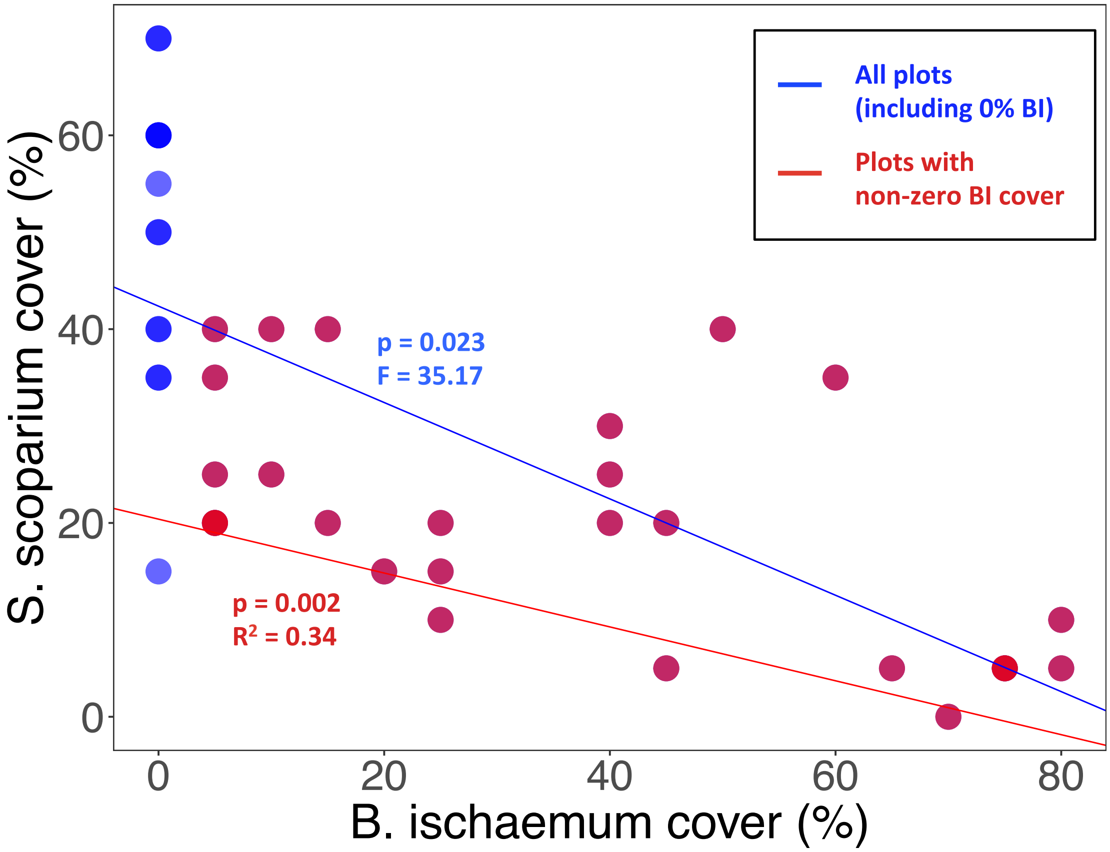


Figure 2. Principal coordinate ordination for visualizing functional trait composition of the native grass, *Schizachyrium scoparium*, across increasing foliar cover classes of the invasive grass *Bothriochloa ischaemum*: Zero, Low (1–15% cover), Medium (16–49% cover), and High (50% or higher cover). The traits represented are height, individual foliar cover, specific leaf area (SLA), and leaf dry matter content (LDMC). PERMANOVA and SIMPER analysis indicate the trait composition of plots in the High invasive cover class is significantly different from the trait composition of plots in the Zero and Low invasive cover classes. Points represent the centroids of each invasion category on the PCO axes and error bars represent the standard error.

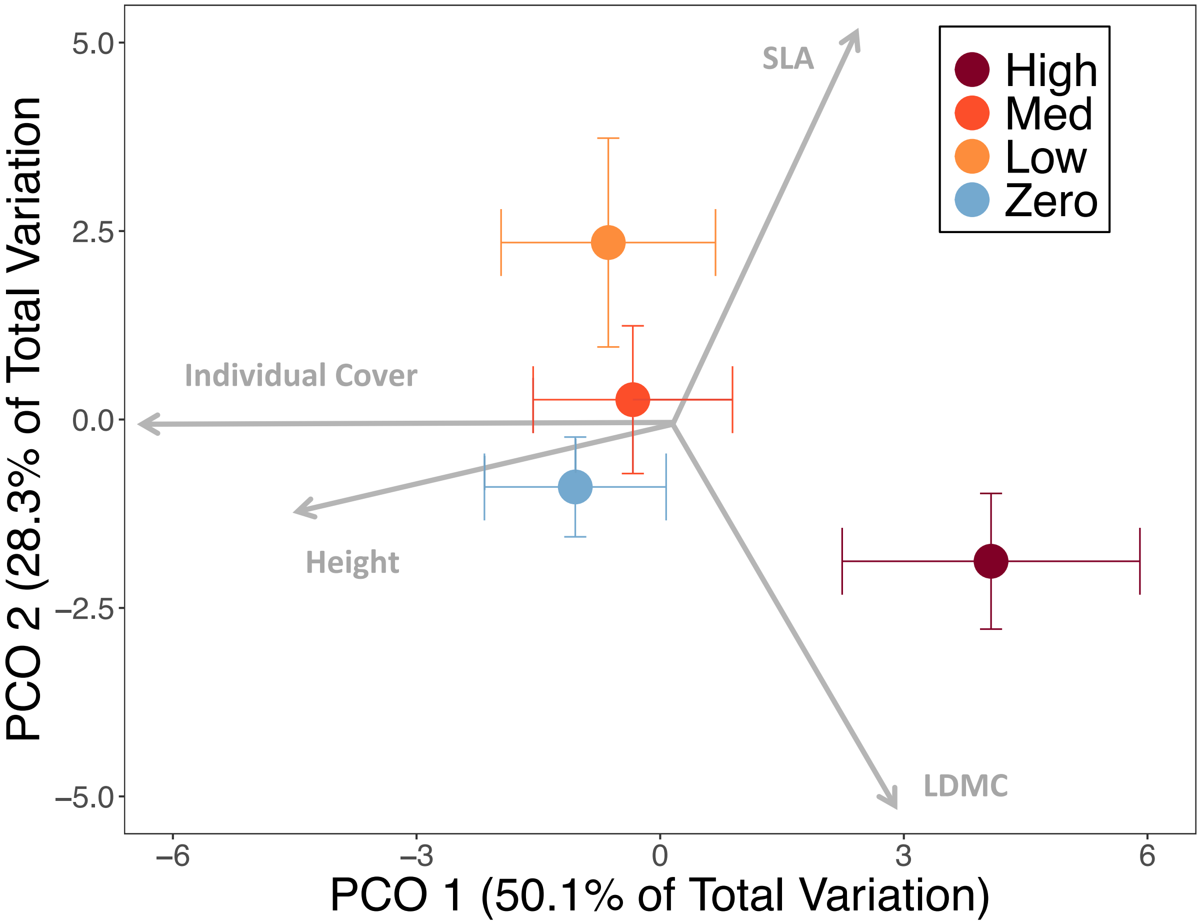


Figure 3. Principal coordinate ordination for visualizing species composition (presence/absence) of the native plant community across increasing foliar cover classes of the invasive grass *Bothriochloa ischaemum*: Zero, Low (1–15% cover), Medium (16–49% cover), and High (50% or higher cover). PERMANOVA and SIMPER analysis indicates the species composition of plots in the High invasion class is significantly different from the species composition of plots in the Zero invasion class. Points represent the centroids of each invasion category on the PCO axes and error bars represent the standard error. Vectors show species with a correlation of r > 0.7 to the axes: Amphiachyris dracunculoides, *Prunus angustifolia*, *Psoralidium tenuiflorum*, *Solidago sp.*, *Verbena halei*, an unidentified grass, and an unidentified forb. Note that the axes explain only 31.8% of the variation in species composition, but the SIMPER analysis accounted for 100% of the variation.

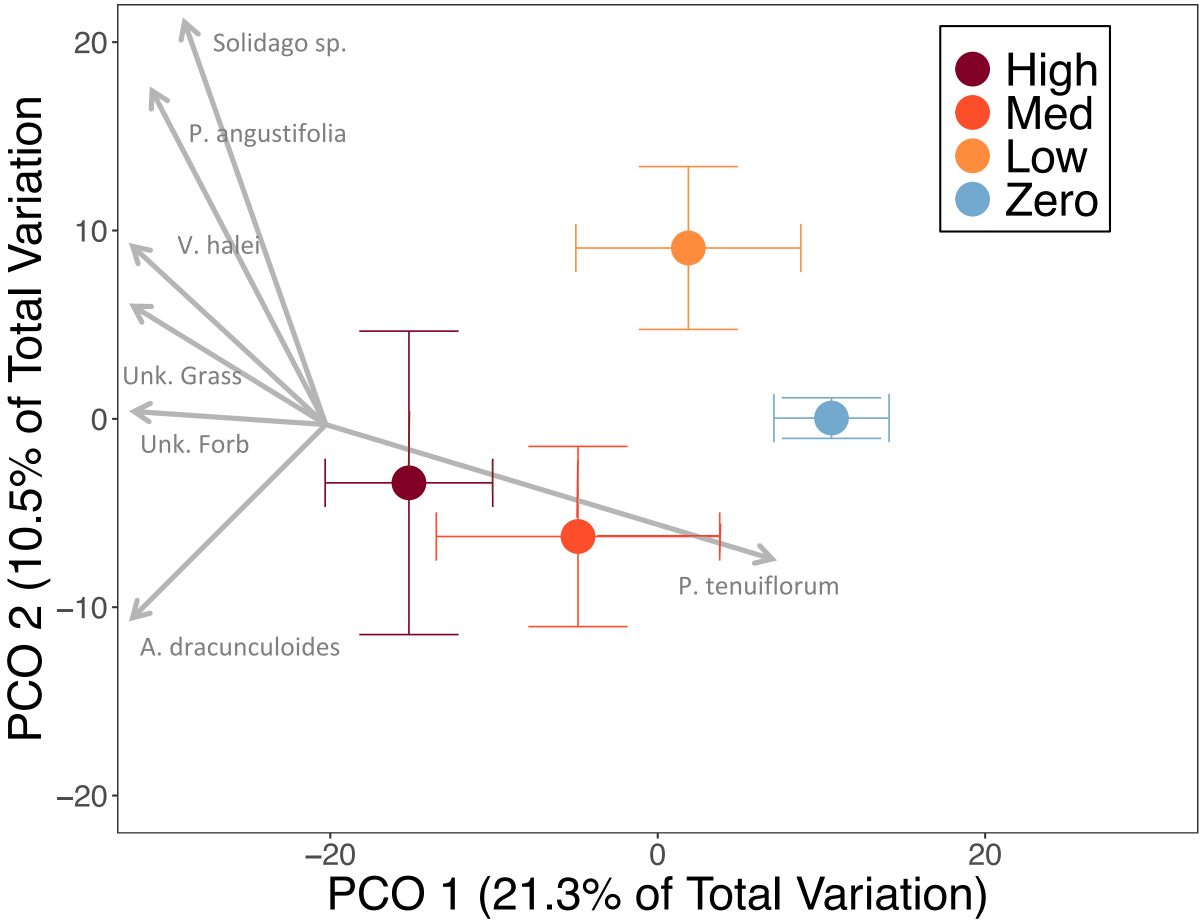


Figure 4. Increasing cover of the invasive grass *Bothriochloa ischaemum* reduces the total foliar cover of the community (blue) as well as foliar cover of subdominant groups (C_3 grasses, forbs, woody plants, and legumes; red). This indicates that while the invader replaces native C_4 grasses at a 1:1 ratio it has a negative effect on the overall community compared to native C_4 grasses at the same level of abundance. Total foliar cover can exceed 100% because the foliage of the different functional groups overlaps.

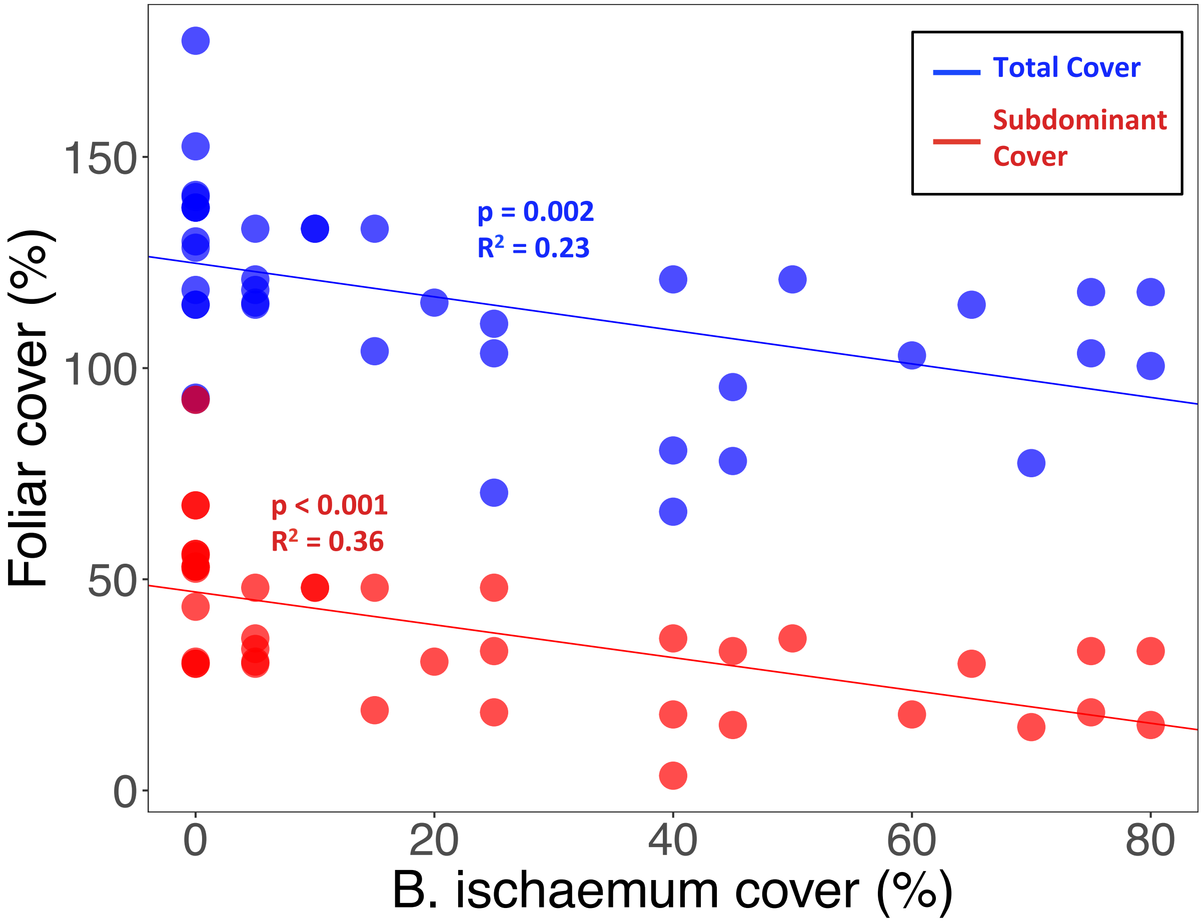
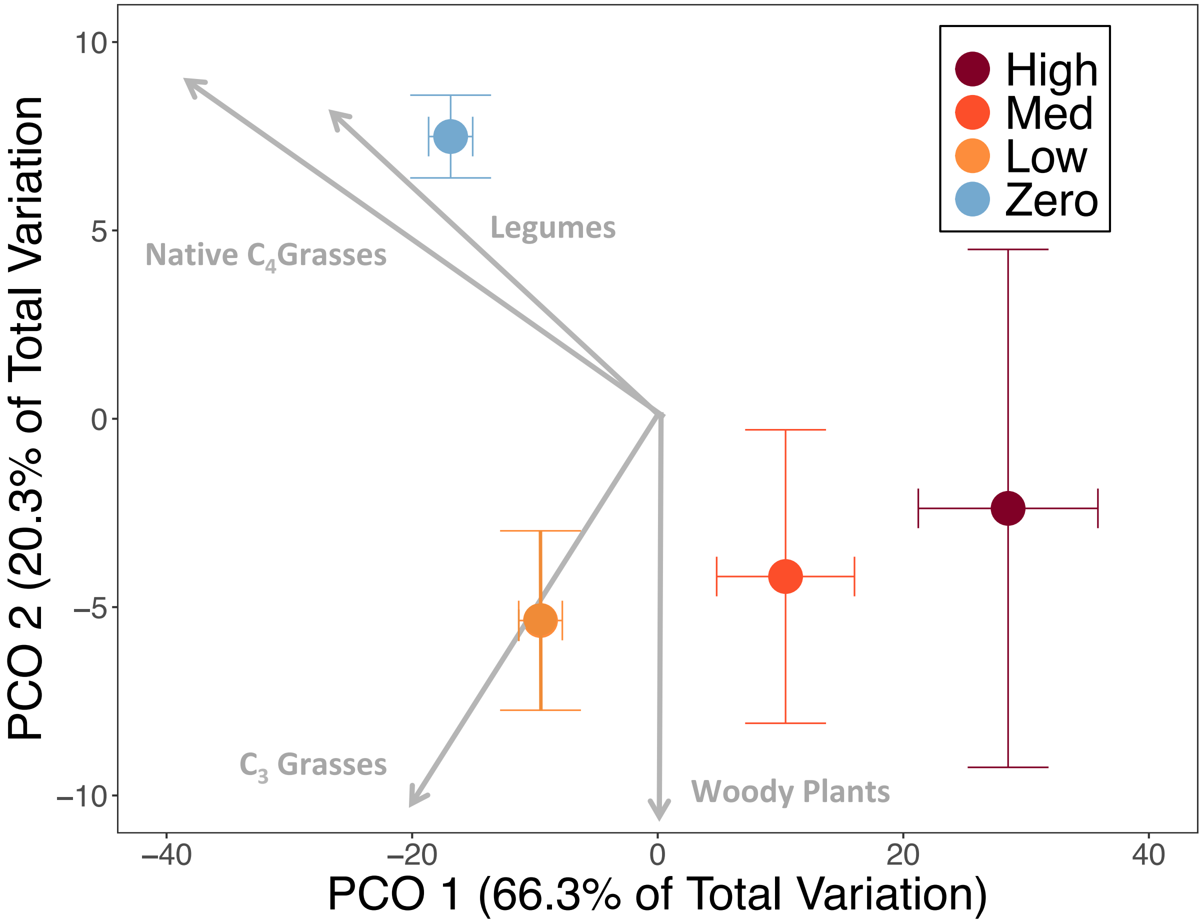


Figure 5. Principal coordinate ordination for visualizing plant functional group composition of the native plant community across increasing foliar cover classes of the invasive grass *Bothriochloa ischaemum*: Zero, Low (1–15% cover), Medium (16–49% cover), and High (50% or higher cover). PERMANOVA and SIMPER analysis indicates all pairs of invasive cover categories were significantly different from one another except for plots in the Medium and High invasion classes. Points represent the centroids of each invasion category on the PCO axes and error bars represent the standard error. Vectors show functional groups with a correlation of r > 0.5 to the axes.



Chapter 2: Tables and Figures

Figure 1. The ratio of volunteer to sown species cover declined over time as the weedy species that initially dominated each restoration sequence were replaced by the intentionally-sown late-successional species as the sequences aged. The ratio of volunteer to sown species cover did not differ between the two sequences (p = 0.303). We performed a natural log transformation on the ratios to better illustrate this pattern. The initial years of both sequences were heavily dominated by volunteer species that naturally colonized the plots from the seed bank or surrounding area. After two to three years the sown species reached approximately equal abundance, and thereafter they dominated both sequences.

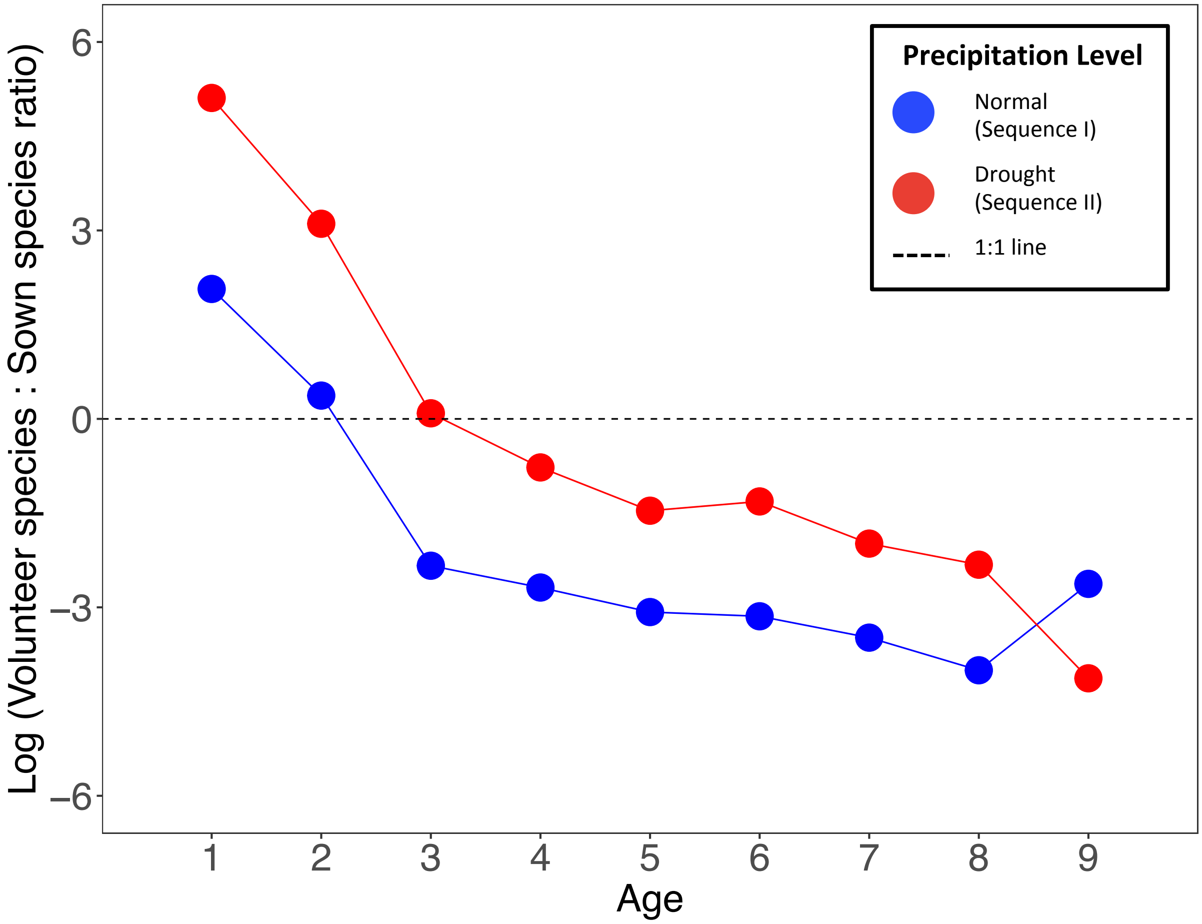


Figure 2. Although both Sequence I (normal precipitation) and Sequence II (drought) were initially dominated by fast-growing, weedy species such as *Erigeron canadensis* (**A**), intentionally-sown, late-successional grasses accounted for a greater proportion of relative cover in Sequence I (**B**). This initial difference may explain why Sequence I was dominated by grasses in most years, while Sequence II was dominated by forbs (although it was close to a 1:1 ratio in most years).

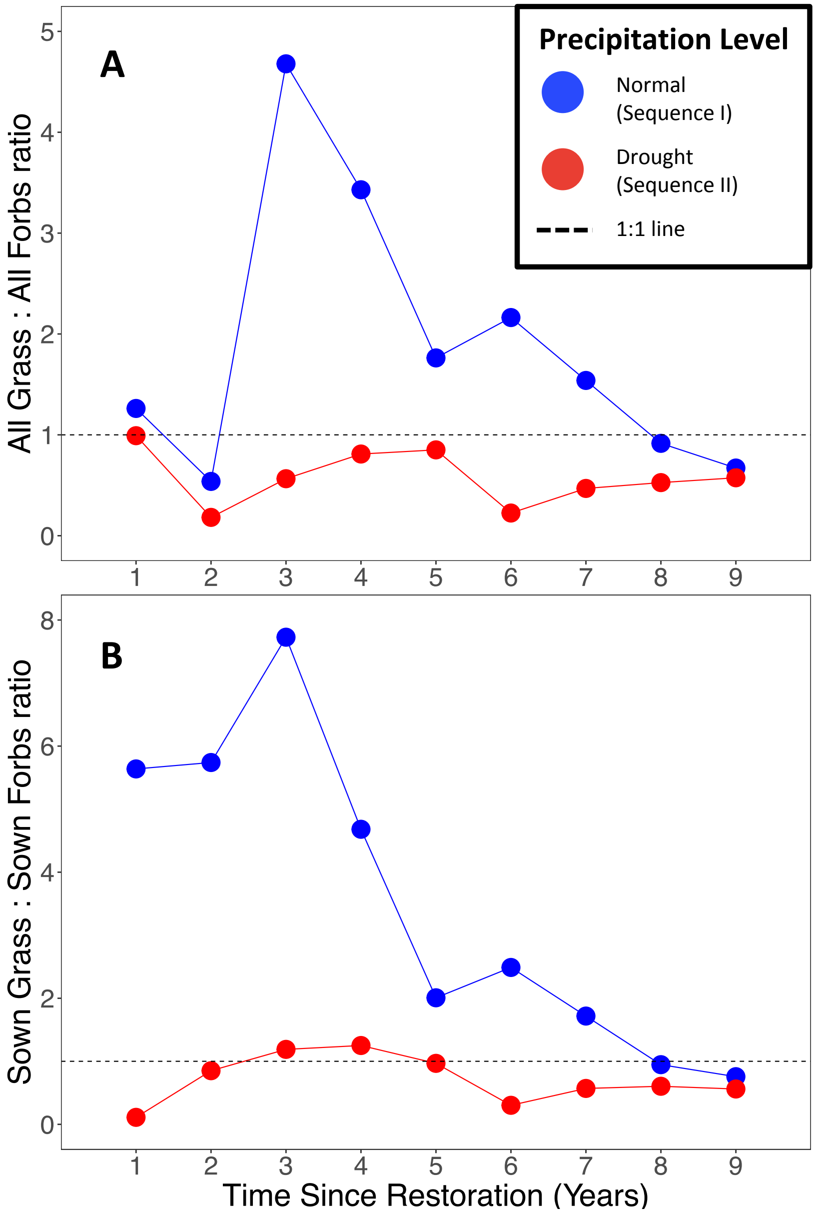
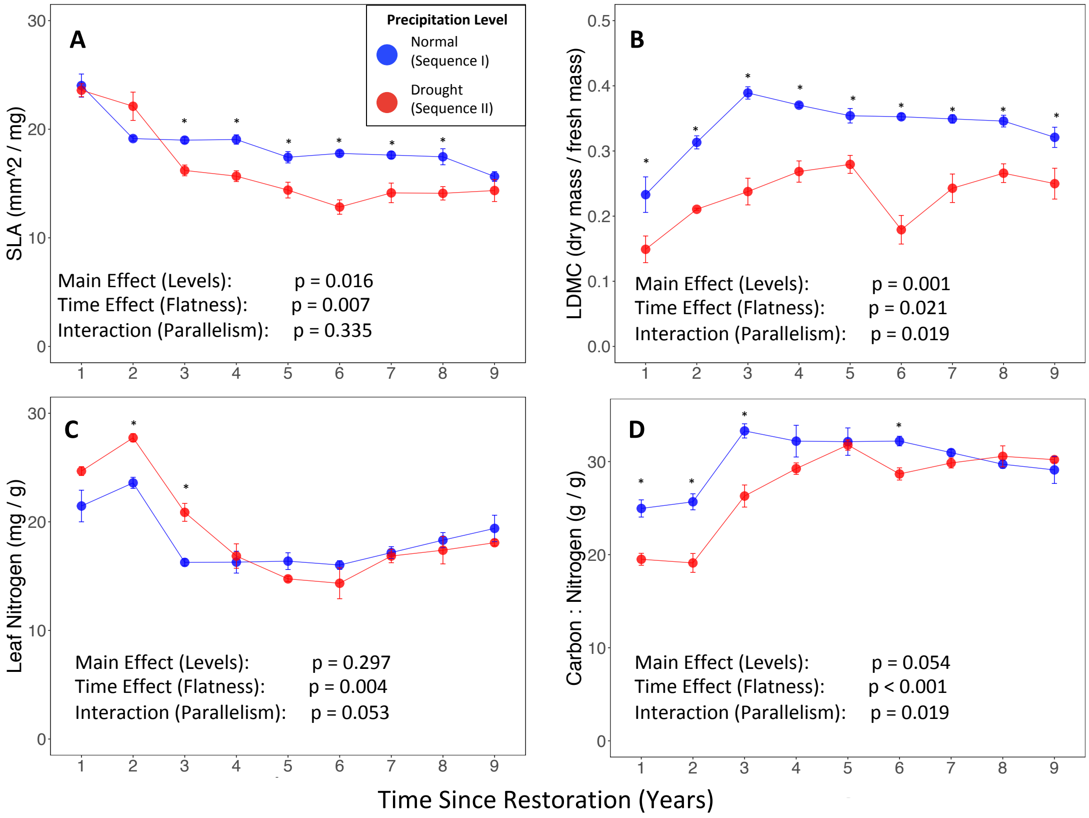


Figure 3. Profile analyses revealed at least one significant effect on each of the four functional traits calculated for the two restored communities. There was an effect of establishment year precipitation on (**A**) specific leaf area (SLA; p = 0.016, F = 10.98) and (**B**) leaf dry matter content (LDMC; p = 0.001, F = 32.38). Sequence I, restored during a year with normal precipitation, had significantly higher SLA and LDMC than Sequence II, restored during a drought. There was an effect of time on all four traits: SLA, LDMC, (**C**) leaf nitrogen content, and (**D**) leaf carbon : nitrogen ratio (C:N ratio). There was also an interaction of time and establishment year precipitation on LDMC and C:N ratio, with both traits changing more gradually in Sequence II. There is evidence for functional convergence in SLA, leaf N, and C:N ratio, but LDMC values are persistently divergent between the restoration sequences. An * marks individual years where trait values differ, and error bars equal the standard error.



Chapter 1: Supplementary Materials

Table S1. Parameters from all analyses run for this project. All linear models were run with the percent foliar cover of the invasive grass *Bothriochloa ischaemum* as the independent variable and within-species (population level) or across-species (community-level) metrics as responses. The four *B. ischaemum* cover categories (Zero, Low (1–15% cover), Medium (16–49% cover), and High (50% or higher cover)) were used for the PERMANOVAs and PERMDISPs. "SS" = *Schizachyrium scoparium*; "BI" = *Bothriochloa ischaemum*; "SLA" = specific leaf area; "LDMC" = leaf dry matter content.

Linear Fixed Effects Models	p	R-squared	Intercept	Slope	F
SS cover ~ non-zero BI cover	0.002	0.340	20.393	-0.278	12.370
mean SS height	0.031	0.120	46.867	-0.057	5.028
mean SS SLA	0.411	0.018	170.118	-0.202	0.690
SS height variance	0.986	<0.001	47.019	0.005	<0.001
SS ind. cover variance	0.151	0.055	0.299	0.006	2.150
SS SLA variance	0.713	0.004	2749.533	-20.014	0.137
SS LDMC variance	0.294	0.030	0.005	< -0.001	1.132
total C4 grass median cover	0.904	0.004	77.828	-0.009	0.015
native C4 grass median cover	<0.001	0.841	77.828	-1.009	200.700
legume median cover	<0.001	0.318	24.300	-0.309	17.730
legume median cover ~ non-zero BI cover	0.209	0.065	10.865	-0.064	1.668
total foliar cover	0.002	0.225	124.822	-0.397	11.040
subdominant foliar cover	<0.001	0.360	46.994	-0.389	21.380

Linear Mixed Effects Models	p	Intercept	Slope	F
SS cover	0.023	41.597	-0.474	35.170
mean SS individual cover	0.139	2.015	-0.006	3.276
mean SS LDMC	0.536	0.429	<0.001	0.435
species richness	<0.001	18.818	-0.098	13.255
C3 grass median cover	0.039	4.936	-0.079	5.256
herbaceous forb median cover	0.466	14.966	-0.029	0.753
woody plant median cover	0.062	6.668	-0.088	3.692
bare ground	0.713	10.268	-0.028	0.311
litter	0.355	21.078	0.096	1.205

PERMANOVA	p	DF - BI	DF - resids.	pseudo-F
spp. composition	0.013	3	36	1.600
functional group composition	<0.001	3	36	12.806
SS trait composition	0.031	3	109	2.137

PERMDISP	p	DF - BI	DF - resids.	F
species composition	0.006	3	36	6.539
functional group composition	0.001	3	36	15.019
SS trait composition	0.951	3	109	0.149

Figure S1. Map of plot locations at sub-site 1 at the Kessler Atmospheric and Ecological Field Station. Plots are arranged in short transects (10 – 15m) of four to five plots. Each plot is 2m x 2m. Twenty-five plots are located at sub-site 1. Plot color indicates invasion level: blue = Zero invasion, yellow = Low (1 – 15%) invasion, orange = Medium (16 – 49%) invasion, and red = High (50% and over) invasion. This sub-site is centered at N34.9816 W-97.5323.

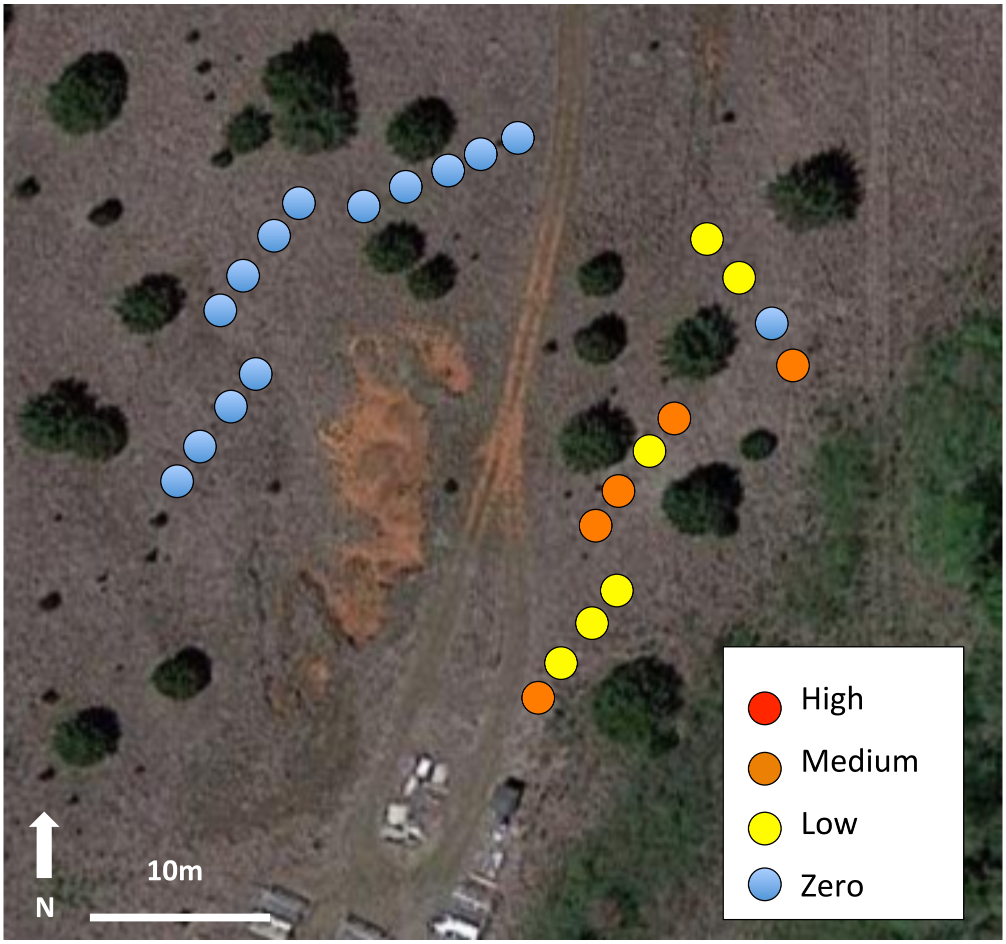


Figure S2. Map of plot locations at sub-site 2 at the Kessler Atmospheric and Ecological Field Station. Plots are arranged in short transects (10 – 25m) of seven to eight plots. Each plot is 2m x 2m. Fifteen plots are located at sub-site 21. Plot color indicates invasion level: blue = Zero invasion, yellow = Low (1 – 15%) invasion, orange = Medium (16 – 49%) invasion, and red = High (50% and over) invasion. This sub-site is centered at N34.9755 W-97.5222.

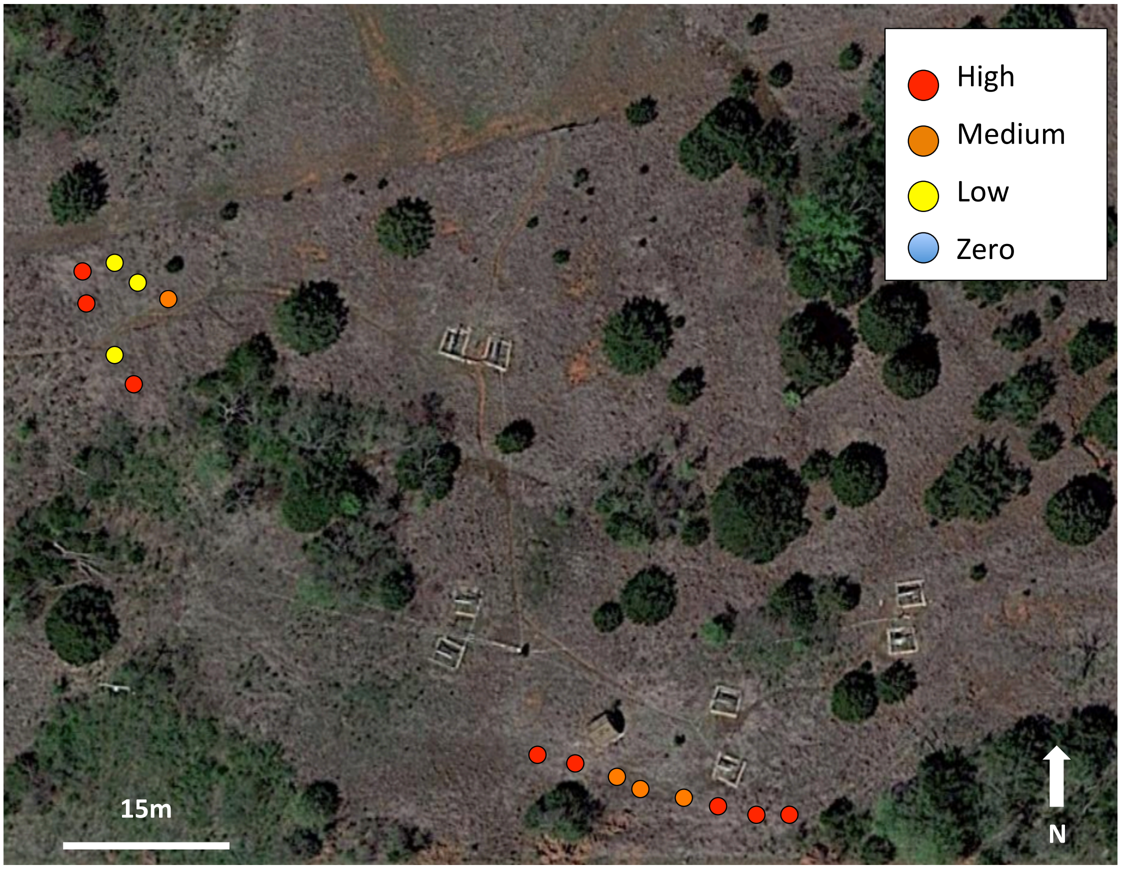


Figure S3. Species richness declines as foliar cover of the invasive grass *Bothriochloa ischaemum* increases (p = 0. 036, R2 = 0.11). Plots with Zero or Low *B. ischaemum* cover had an average of 16.43 ± 1.87 and 18.33 ± 4.69 species, respectively. Plots with Medium or High *B. ischaemum* cover had an average of 14.67 ± 2.92 and 14.25 ± 5.15 species.

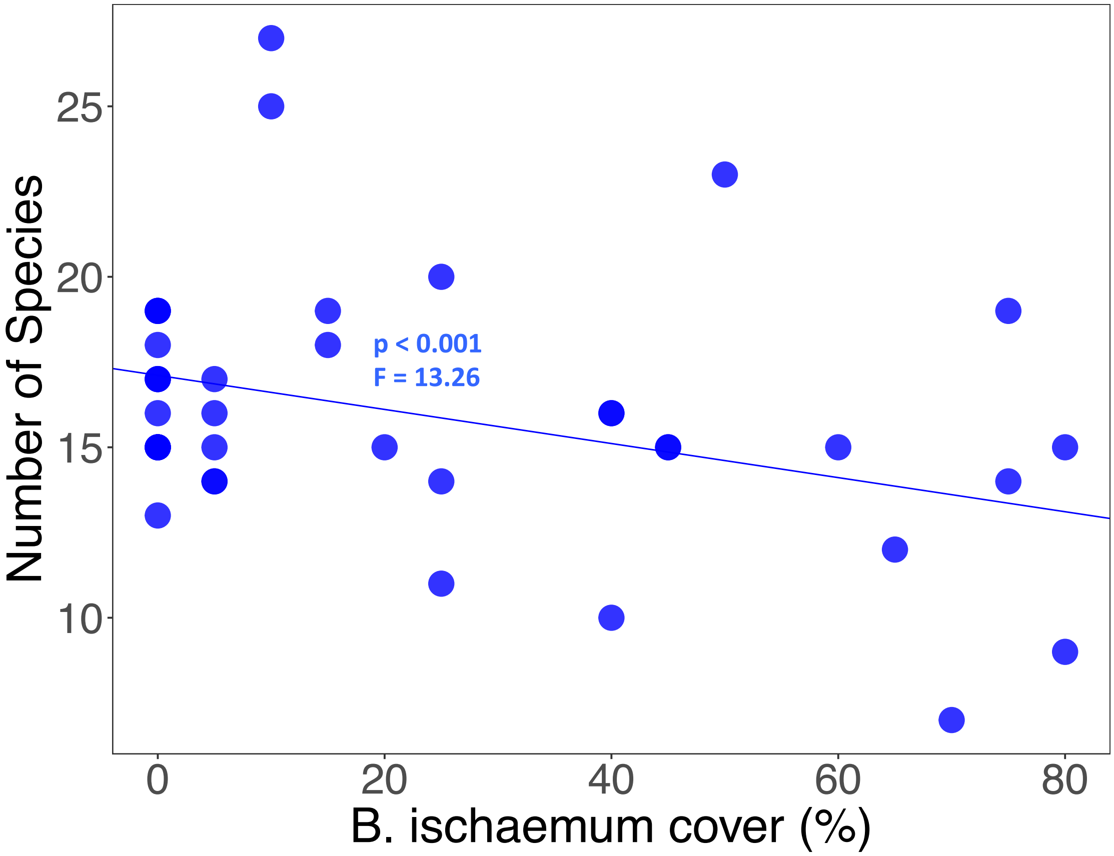
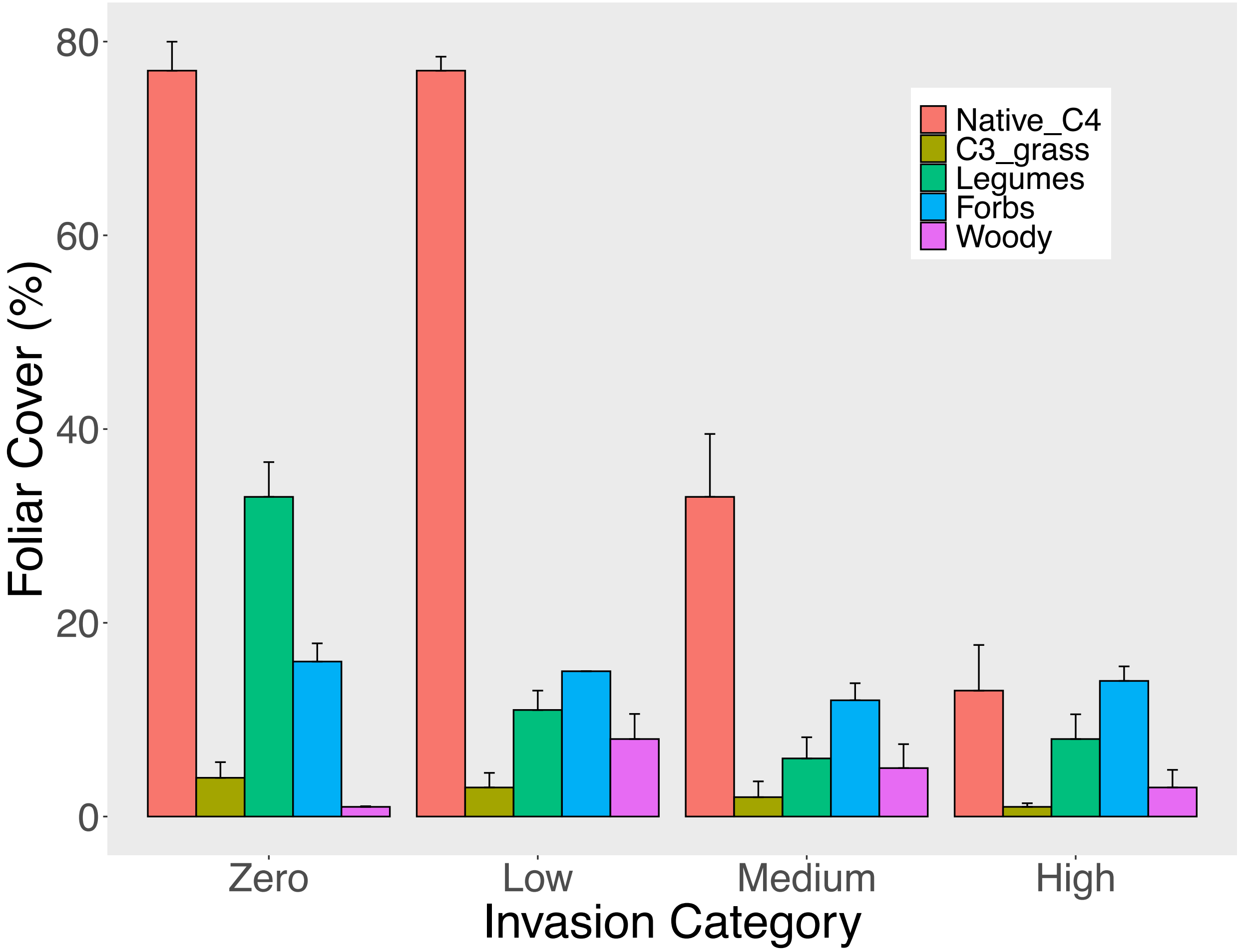


Figure S4. Functional group cover varied with cover category of the invasive grass *Bothriochloa ischaemum*. The cover of herbaceous forbs and woody plants was unaffected by invasion. Native C_3 and C_4 grass cover declined linearly as invasion increased, while the decline in legume cover was driven by the difference between invaded and uninvaded plots. A one-way ANOVA showed there was a difference in legume cover based on *B. ischaemum* invasion category, and a Tukey HSD test indicated that plots with Zero invasion differed from all invasion levels (all p-values < 0.001). Error bars give standard error.



Chapter 2: Supplementary Materials

Table S1. We analyzed data from the 33 most abundant species in our restored communities. We downloaded data from four functional traits from the TRY plant trait database. The traits we analyzed are leaf nitrogen content (leaf N, in mg / g), leaf dry matter content (LDMC, dry mass / fresh mass), leaf carbon : nitrogen ratio (C:N ratio, in g / g) and specific leaf area (SLA, in mm2 / mg). Trait values are the average value from the entries for each species downloaded from the TRY database. The 33 species can be divided into four groups: sown forbs (SF), volunteer forbs (VF), sown grasses (SG), and volunteer grasses (VG). Origin indicates if the species is native (N) or introduced (I).

Species	Origin	Group	leaf N	LDMC	C:N ratio	SLA
Baptisia australis	N	SF	48.160	0.184	13.500	13.988
Desmanthus illinoensis	N	SF	30.260	0.481	14.466	14.419
Helianthus pauciflorus	N	SF	11.690	NA	33.962	6.944
Lespedeza capitata	N	SF	23.435	0.361	24.621	20.884
Liatris punctata	N	SF	15.680	0.301	27.819	5.390
Silphium integrifolium	N	SF	18.080	0.268	20.197	9.970
Solidago rigida	N	SF	12.010	0.258	33.761	7.622
Abutilon theophrasti	I	VF	45.455	NA	8.214	30.847
Ambrosia artemisiifolia	N	VF	26.860	0.249	12.335	16.869
Amaranthus tuberculatus	N	VF	16.320	NA	24.699	16.260
Cirsium altissimum	N	VF	11.530	NA	36.470	10.868
Dalea multiflora	N	VF	18.270	NA	24.304	7.257
Erigeron canadensis	N	VF	28.620	0.246	17.460	20.077
Euphorbia maculata	N	VF	18.830	NA	23.651	13.228
Galium sp.	N	VF	24.374	0.143	27.452	34.508
Helianthus annuus	N	VF	35.550	0.122	13.739	11.550
Lepidium sp.	N	VF	34.248	0.171	12.557	23.279
Mollugo verticillata	N	VF	25.736	NA	13.997	20.408
Solidago altissima	N	VF	NA	NA	21.417	16.856
Solidago canadensis	N	VF	18.143	0.294	25.370	17.026
Solanum rostratum	N	VF	41.540	NA	10.571	12.574
Andropogon gerardii	N	SG	13.311	0.369	34.692	23.866
Bouteloua curtipendula	N	SG	16.170	0.504	46.717	15.783
Elymus canadensis	N	SG	27.446	0.378	16.930	21.632
Panicum virgatum	N	SG	17.601	0.479	24.304	20.845
Schizachyrium scoparium	N	SG	12.681	0.388	43.356	18.318
Sorghastrum nutans	N	SG	13.471	0.385	35.384	18.088
Bromus japonicus	I	VG	NA	0.289	NA	10.879
Digitaria sanguinalis	I	VG	21.963	0.172	33.091	37.165
Eragrostis sp.	N	VG	17.383	0.364	25.430	23.932
Hordeum pusillum	N	VG	26.180	NA	15.968	21.544
Setaria pumila	I	VG	25.090	0.238	16.209	23.739
Setaria sp.	I	VG	22.922	0.255	18.363	27.013

Table S2. We calculated the community-weighted means (CWMs) of four plant functional traits across the first nine years of each restoration sequence. We calculated the CWM of each trait for all four plots within each sequence and used these values for the profile analyses. Here we show the average of the four CWMs for each sequence for each year, which are shown in **Fig. 2**. The CWMs of Leaf N, LDMC, and SLA were calculated from the "Complete" trait set while the CWM of the C:N Ratio was calculated from the "Refined" trait set.

Sequence	Age	Leaf N	LDMC	C:N Ratio	SLA
1	1	21.459	0.233	24.963	24.022
1	2	23.590	0.313	25.679	19.141
1	3	16.266	0.389	33.295	18.996
1	4	16.290	0.370	32.194	19.055
1	5	16.382	0.354	32.142	17.420
1	6	16.024	0.353	32.203	17.777
1	7	17.169	0.349	30.958	17.620
1	8	18.315	0.346	29.714	17.464
1	9	19.396	0.321	29.105	15.647
2	1	24.674	0.149	19.503	23.592
2	2	27.738	0.211	19.112	22.116
2	3	20.873	0.238	26.298	16.209
2	4	16.853	0.268	29.240	15.679
2	5	14.749	0.279	31.782	14.396
2	6	14.343	0.179	28.670	12.836
2	7	16.852	0.243	29.851	14.141
2	8	17.389	0.266	30.564	14.100
2	9	18.079	0.250	30.205	14.357

Table S3. Results from the profile analyses performed on each of the four functional traits. The first panel shows results from analyses of the long-term patterns (trait values from every other year across the first nine years). The second panel shows the post-hoc analyses of fine-scale patterns (trait values from every year over the first seven years).

Profile Analyses: every other year for the first nine years of each sequence

Main Effects (Levels)	**p**	**F**	**df (groups)**	**df (residuals)**
SLA	0.016	10.98	1	6
LDMC	0.001	32.38	1	6
Leaf N	0.297	1.30	1	6
C:N	0.054	5.74	1	6

Interaction (Parallelism)	**p**	**Approx. F**	**Wilks' Lambda**	**df (numerator)**	**df (denominator)**
SLA	0.335	1.76	0.299	4	3
LDMC	0.019	18.05	0.04	4	3
Leaf N	0.053	8.76	0.079	4	3
C:N	0.019	18.26	0.039	4	3

Time Effect (Flatness)	**p**	**F**	**df1**	**df2**
SLA	0.007	38.25	4	3
LDMC	0.021	17.27	4	3
Leaf N	0.004	55.5	4	3
C:N	<0.001	394.19	4	3

Profile Analyses: every year for the first seven years of each sequence

Main Effects (Levels)	**p**	**F**	**df (groups)**	**df (residuals)**
SLA	0.009	14.51	1	6
LDMC	<0.001	71.83	1	6
Leaf N	0.109	3.54	1	6
C:N	0.007	16.18	1	6

Interaction (Parallelism)	**p**	**Approx. F**	**Wilks' Lambda**	**df (numerator)**	**df (denominator)**
SLA	0.11	48.05	0.003	6	1
LDMC	0.355	4.26	0.038	6	1
Leaf N	0.027	827.56	<0.001	6	1
C:N	0.009	6611.30	<0.001	6	1

Time Effect (Flatness)	**p**	**F**	**df1**	**df2**
SLA	0.157	23.45	1	6
LDMC	0.174	19.00	1	6
Leaf N	0.005	20711.33	1	6
C:N	0.002	146199.40	1	6

Figure S1. There were no discernable patterns between annual precipitation (bars, left) or growing season precipitation (bars, right) and the CWMs of the four functional traits we calculated (lines).