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# Mechanisms altering exotic-native proportions in plant communities, and impacts on biodiversity and ecosystems

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**Mechanisms altering exotic-native proportions in plant communities, and impacts on  
biodiversity and ecosystems**

by

**Leanne Michelle Martin**

A dissertation submitted to the graduate faculty  
in partial fulfillment of the requirements for the degree of  
**DOCTOR OF PHILOSOPHY**

Major: Ecology and Evolutionary Biology

Program of Study Committee:  
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**ABSTRACT**

Understanding the causes and consequences of variation in species composition and diversity across space and time are basic questions in ecology that have strong implications for conservation and restoration. However, exotic-dominated ecosystems, in which species with potentially novel interactions occur, are altering the context in which we ask these questions. The work presented here tested for the first time whether 1) experimental community assembly history manipulations of the seeds of native species could alter species composition, diversity, and ecosystem measures in grasslands dominated by exotic species, and 2) whether exotic-dominated grasslands differ from native-dominated grasslands for multiple aspects of diversity and ecosystems across a latitudinal gradient and over a growing season. Differences in community assembly history resulted in either a more diverse, native state when native seeds were added in spring and without priority effects, or a low-diversity, exotic-dominated state when seeds were added in summer or with priority effects. The exotic-dominated state persisted for eight years, even with an experimental seed addition of native species, and the differences resulted in altered productivity and fire temperatures. Across the latitudinal gradient, exotic-dominated grasslands had lower levels of species diversity, but had higher levels of tissue N concentrations. Productivity in exotic grasslands was higher in the south but it was lower in the north, where biomass peaked earlier in the growing season than in native grasslands. Exotic-dominated grasslands were more strongly dominated by C<sub>4</sub> species in the south and C<sub>3</sub> species in the north, with a strong shift at 34-36 degrees, implying that an important measure of functional diversity was altered. Experimental results from a common garden experiment indicated that the difference in functional diversity was at least partially caused by species themselves. Pollinator communities did not differ between exotic and native grasslands, implying that other factors may

be important in structuring pollinator communities. Overall, results from this work imply that novel ecosystems should be considered when testing ecological theory in the field and when considering impacts of species on ecosystems. Managing grasslands and restorations for high levels of species diversity will require understanding the impacts of multiple exotic species.

## CHAPTER 1: GENERAL INTRODUCTION

High levels of variation in species composition and biodiversity exist across space and time on Earth. Some of the most basic questions in community and ecosystem ecology, therefore, try to address 1) the causes of variation in species composition and biodiversity across space and time and 2) the ecosystem consequences of this variation (e.g., Whittaker 1960, Anderson et al. 2011). A deeper understanding of these basic questions could have important implications for how we conserve and restore the variation of biota on Earth, and how those activities will affect species diversity, the functional diversity that is related to ecosystem functioning, and ecosystem services, or “the benefits people receive from ecosystems” (Millennium Ecosystem Assessment 2005). For example, mounting evidence suggests that higher amounts of biodiversity can provide higher levels of ecosystem services and functioning over longer time frames and across many places (e.g., Naeem et al. 1994, Isbell et al. 2011). Declines in biodiversity worldwide, therefore, generate a need to understand what generates and maintains biodiversity, its patterns of variation, and its role in sustaining ecosystem functioning and services (Naeem et al. 1994, Symstad et al. 2003, Gamfeldt et al. 2008). Ecologists, however, now face the important task of addressing these important questions in ecosystems that are highly altered by anthropogenic activities. Specifically, little work has been conducted on how both biodiversity and variation in species composition arise in the now common condition of exotic-dominated ecosystems, in which multiple exotic species with novel ecological interactions occur. Consequently, we have little knowledge of how novel ecosystems in general impact ecosystem functioning and services. Therefore, we need to address the most basic questions in community and ecosystem ecology in the context of novel ecosystems.

Biodiversity is composed of multiple spatial components, including alpha, beta, and gamma diversity. All of these components contribute to global biodiversity, and understanding how they are generated at high levels may help conserve current and restore lost species diversity. Alpha, or local, diversity is important because it is the scale at which species interactions of plants and many resulting ecosystem functions occur (Martin et al. 2005, Wilsey 2010). Mechanisms generating beta diversity, defined as turnover or variation in species composition across space, have been of theoretical and practical interest to ecologists for half a century (Whittaker 1960, Gering 2003, Martin et al. 2005, Wilsey et al. 2005, Legendre et al. 2009, Chase 2010, Anderson et al. 2011). Theoretically, beta diversity allows us to link alpha (local) and regional (gamma) diversity (Wilsey 2010). High levels of beta diversity can also reduce rates of extinction in fragmented landscapes to levels below those predicted by island biogeography theory, and conservation biologists increasingly recognize that conserving beta diversity across many sites may sometimes preserve more species than will conserving one large site (Gering 2003, Wilsey et al. 2005). However, despite its importance to ecosystems, we lack an understanding of the relative importance of alternative mechanisms generating beta diversity, as well as the generation of community composition and diversity of the local communities (alpha diversity) therein.

Several different processes can generate beta diversity, but the relative importance of these processes remains poorly understood (Chase 2003). Abiotic conditions, dispersal limitation, and differences in community assembly history are all mechanisms by which beta diversity can be generated. First, species could sort deterministically according to differences in abiotic environments, such as soil type or amount of precipitation (Whittaker 1960). Second, dispersal limitation could generate beta diversity because all species would not be found



everywhere if dispersal were limited. Thus, differences in community composition across space could emerge (Mouquet and Loreau 2003). Finally, the theory of community assembly history predicts that the order and timing of arrival of species after a disturbance can impact species diversity and community composition, leading to high levels of beta diversity, or alternate states (Drake 1991, Chase 2003). Specifically, the timing of arrival of species (e.g., season of arrival), priority effects, and the identity of an early-arriving species could all impact species composition and diversity of developing communities. Priority effects alter species composition when an early-arriving species grows and out-competes later arriving species (Alford and Wilbur 1985). Differences in identities of an early-arriving species could generate high levels of beta diversity if a species from a particular functional group limits recruitment and establishment of other species from the same functional group (Diamond 1975; Fox 1987; Gotelli & McCabe 2002; Fargione et al. 2003). Thus, assembly history differences could theoretically lead high levels of beta diversity and alternate states (Chase 2003). Understanding how all of these factors impact both alpha and beta diversity could greatly improve our ability to restore lost levels of biodiversity. However, the relative importance of these three factors on species composition and diversity is poorly understood. In particular, the interacting effects of timing of species arrival, identity of early-arriving species, and priority effects during community assembly history remain unexplored.

The context within which important basic ecological questions are being asked, such as whether community assembly history can generate alternate states that increase beta diversity, is rapidly changing. Climate change, habitat fragmentation, and anthropogenic alteration of ecosystems are forcing ecologists to ask how our understanding of ecological patterns and processes may be altered by these global changes. One important consequence of human

alteration of ecosystems is that exotic-dominated plant communities consisting of recently assembled non-native species (here defined as species that are not native to North America), with potentially novel ecological interactions, now exist on nearly one-third of Earth's terrestrial surface (Hobbs et al. 2006; Kulmatiski 2006; Ellis 2011; Wilsey et al. 2011). In degraded environments with high exotic species propagule pressure, however, we have little understanding of how community assembly of native seeds might take place during the restoration process.

In addition to having a poor understanding of how species assemble in exotic-dominated landscapes, we also lack information about the community and ecosystem impacts of existing exotic-dominated, novel ecosystems on landscapes over broad spatial scales and over time. We have a large body of evidence from studies looking at single or a few species suggesting that many of these species can become dominant and subsequently generate ecosystem impacts in their non-native ranges (e.g. Vitousek 1990; Levine et al. 2003; Vilà et al. 2011; Pyšek et al. 2012). For example, Vilà et al. (2011) conducted a meta-analysis and reported that single exotic species invasions result in decreased plant diversity, but that ecosystem process rates can decrease, increase, or remain unchanged on average. The differences can depend on the traits of the exotic species (Liao et al. 2008, van Kleunen et al. 2010). This suggests that single species invasions can have inconsistent effects. However, exotic species do not exist in their non-native ranges alone (Kuebbing et al. 2013). Rather, they have novel interactions with multiple other exotic and native species (Hobbs et al. 2006). Studying only one exotic species at a time will not answer questions about broader effects on communities and ecosystems (Wilsey 2005). Thus, when considering broad spatial scales, multiple exotic species could have greater collective impacts than would be supposed from studying individual species.

The effects of multiple, interacting exotic species on biodiversity and ecosystem functioning have only recently been addressed (Wilsey et al. 2009, 2011, Isbell and Wilsey 2011a, Mascaro et al. 2012). Recent experimental work revealed that communities composed of all exotic species caused rapid diversity declines compared to paired communities composed of all native species in a common garden environment (Wilsey et al. 2009, 2011, Isbell and Wilsey 2011a). Furthermore, these changes did not consistently lead to reduced biomass production (Wilsey et al. 2011, Isbell and Wilsey 2011b). Multiple species invasions in Hawaii, however, have resulted in more diverse exotic-dominated sites with increased productivity compared to native-dominated sites (Mascaro et al. 2012). Thus, over broad spatial scales, where identities and abundances of multiple exotics vary across landscapes, the effects of many exotic species could accumulate to have community and ecosystem effects that are important to biodiversity conservation, ecosystem functioning, and the management of ecosystem services. For example, functional diversity, such as  $C_3:C_4$  proportions, can affect ecosystem functioning. Specifically, plant species with the  $C_4$  mode of photosynthesis have higher temperature growth optima, water and nitrogen use efficiency, and carbon uptake than  $C_3$  species. These differences can impact patterns of seasonal growth that may affect higher trophic levels, element cycling, and decomposition rates (e.g. Teeri and Stowe 1976; Tieszen et al. 1997; Sage and Monson 1999). Although we know that the proportion of  $C_3$  biomass increases linearly with latitude in the northern hemisphere for native species (e.g. Epstein et al. 1997), we do not know how this pattern may have changed with the increase in exotic-dominated systems. Additionally, most exotic species were introduced by humans for specific purposes such as forage production and quality, and thus they may provide higher levels of desirable ecosystem services (Mack and Lonsdale 2001). If exotic-dominated ecosystems contain lower species diversity, which would

be consistent with experimental results (Wilsey et al. 2009, 2011, Isbell and Wilsey 2011a) but higher levels of other ecosystem services such as forage quality, then this could lead to management tradeoffs (Tallis et al. 2008).

Experimental evidence suggesting that exotic-dominated systems may exhibit altered phenology relative to native-dominated systems is also accumulating. This is important because differences in species' phenologies, or timing of biological events such as growth, reproduction, and senescence, are important for regulating ecosystem processes and functioning. For example, phenology of organisms can regulate timing of peak primary production, flowering events important for pollinators, and elemental cycling (e.g., Morissette et al. 2009, Peñuelas et al. 2009). Wilsey et al. (2011) found that experimental grassland plots planted with exotic species mixtures greened up two weeks earlier compared to plots planted with native species. Surprisingly, the earlier green up effects of exotics were consistent across different climate change scenario treatments (i.e., altered levels of irrigation), suggesting that effects of exotics on changing phenology may be as or more important than climate change effects (Wilsey et al. 2011). Fridley (2012) also found altered phenology between exotic and native species in deciduous forests, where exotic species exhibited extended leaf phenologies compared to native species.

Effects of climate change on phenology have been a primary avenue for research because warming temperatures are linked to earlier phenology, including green-up and flowering dates, of plant species (Cleland et al. 2007). In turn, research has emerged attempting to determine whether effects of earlier phenology of plants impacts higher trophic levels, including pollinators (Hegland et al. 2009, Bartomeus et al. 2011, Rafferty and Ives 2011, Kudo and Ida In Press). Understanding plant-pollinator dynamics is of increasing concern given that pollinators are reportedly in decline and provide essential ecosystem services (Losey and Vaughan 2006, Klein

et al. 2007, Isaacs et al. 2009, Burkle et al. 2013). Theoretically, ecological mismatches between plants and their pollinators should emerge if earlier phenology due to climate warming impacts plants, but not pollinators (Hegland et al. 2009). Recent evidence suggesting that exotics also exhibit earlier phenology implies that plants and pollinators could also exhibit temporal differences between exotic versus native systems. However, timing of ecosystem and community dynamics of multiple trophic levels in exotic- versus native-dominated systems are currently poorly understood (Wolkovich and Cleland 2011).

### **Dissertation Organization**

This dissertation addresses questions related to 1) community assembly processes and ecosystem impacts occurring within grasslands with high exotic species propagule pressure, and 2) community and ecosystem consequences of the replacement of native- by exotic-dominated grasslands. In Chapters 2 and 3, I present results from a community assembly history experiment that asks how restored prairie plant communities assemble in landscapes with high exotic species propagule pressure. In Chapters 4 and 5, I ask whether exotic-dominated grasslands differ from native-dominated grasslands over a latitudinal gradient and within a growing season for multiple measures related to diversity, ecosystem functioning, and ecosystem services.

In Chapter 2, I present results from a study in which we manipulated community assembly history of native prairie species in two separate locations that differed in productivity. Both of the sites were located in landscapes with high propagule pressure of exotic species. We were thus able to ask how the history of community assembly impacts beta diversity, as well as differences in alpha species diversity, native prairie species abundance, and proportion of exotic

species in restored prairie plant communities. We found that communities developed into either low-diversity, exotic-dominated communities or more diverse, native communities based on differences in community assembly history. In Chapter 3, I ask whether the exotic-dominated states that developed during the community assembly history experiment were persistent in the face of native species seed pressure. More specifically, I ask whether a native species seed addition, added six to seven years after the communities developed, was able to shift the exotic-dominated communities to a more native-dominated state.

In Chapters 4 and 5, I investigate the implications of exotic- versus native-dominated states on species and functional diversity and ecosystem services in existing landscapes. Thus, in these chapters, I compare alpha-level diversity at the site scale rather than the scale of an experimental plot. In Chapter 4, I present results comparing multiple measures related to diversity, functional diversity, and ecosystem services between exotic- and native-dominated grasslands across a latitudinal gradient and in an ongoing companion experiment in the tallgrass prairie region, USA. Specifically, I compared multiple measures that are important in grasslands and rangelands, including plant species richness and diversity,  $C_3/C_4$  proportions, aboveground net primary productivity, multiple aspects of forage quality to herbivores including tissue N concentrations, and potential pollinator (bee) abundances, richness and diversity. This is one of the first studies attempting to compare multiple measures of diversity and ecosystem services between entire grasslands dominated by either exotic or native species over a broad spatial extent. I also ask whether differences across the gradient in an important aspect of functional diversity,  $C_3/C_4$  proportions, could be at least partially caused by species themselves, by utilizing an ongoing experiment comparing exotic versus native communities in a common garden environment. In Chapter 5, I ask whether systems dominated by exotic versus native species

exhibit altered ecosystem and community dynamics of plant and bee pollinator communities over a growing season. Thus, unlike the latitudinal study, where I compared differences at peak biomass, in this chapter I sampled multiple times over a growing season in grasslands in central Iowa, USA.

Finally, in Chapter 6, I summarize results from all chapters and offer directions for future research and concluding remarks.

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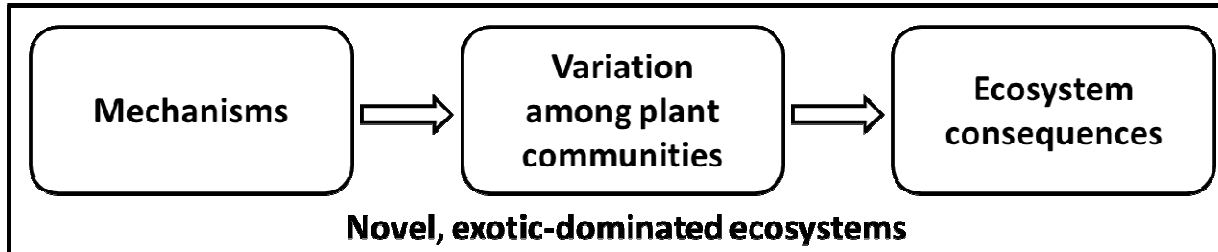


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**Figure 1.** Dissertation research framework. This dissertation addresses the mechanisms generating variation among plant communities, and the ecosystem consequences of variation. The research framework is unique because it addresses these questions in the context of novel ecosystems.



**CHAPTER 2. ASSEMBLY HISTORY ALTERS ALPHA AND BETA DIVERSITY,  
EXOTIC-NATIVE PROPORTIONS AND FUNCTIONING OF RESTORED PRAIRIE  
PLANT COMMUNITIES**

A paper published in *Journal of Applied Ecology*

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**Summary**

1. Restorations provide a test of community assembly history theory, and practitioners require information on how assembly history might help to restore diverse native species communities. Variation in community assembly history (historical order of species arrival) is hypothesized to generate beta diversity by producing alternate states, but restorations are hindered because there have been few tests using long-term field experiments.
2. We experimentally altered assembly history of native species into formerly exotic-dominated grassland sites while simultaneously removing dispersal limitation to test whether alternate states or a single equilibrium would develop, and whether alternate states would generate varying ecosystem-level effects. Assembly history was altered by varying the identity of early-emerging species, timing of seed additions after disturbance (early-emerging species added in spring or summer), and priority effects (common 30-species seed mixture added either at the same time or after an early-emerging species canopy developed). The experiment was conducted at two sites that differed in productivity.
3. Altering timing and priority effects during assembly history had large effects on species composition and diversity. On average, diversity was highest and the proportion of exotic species was lowest in plots seeded in spring and without priority effects. Identity of early-emerging species did not significantly affect community structure.

4. Differences in species composition affected fuel mass, fire temperatures, and peak above-ground primary productivity, key ecosystem processes in tallgrass prairie.
5. *Synthesis and applications.* Our results indicate that grassland communities can reach alternate exotic- or native-dominated states in uniform environments when perennial exotic species are present. These states were strongly affected by timing of native species arrival and priority effects. Thus, assembly history is a key process that can give rise to beta diversity, and our results suggest that native species should be established early in the restoration process before exotics become fully established.

### **Introduction**

Beta diversity, or turnover in species composition across space, is an important component of diversity (Wilsey *et al.* 2005), yet the relative importance of processes generating turnover are poorly understood. Without a better understanding of these processes, our ability to restore beta diversity is hindered. Variation in abiotic conditions, dispersal limitation, and community assembly history are main hypotheses for how communities differentiate (Belyea & Lancaster 1999; Chase 2003). First, species may sort deterministically according to variation in abiotic conditions (e.g. similar soil types or climate), consistent with an abiotic niche model (Whittaker 1960). This hypothesis implies that species composition should reach a single stable equilibrium within a uniform environment but exhibit high beta diversity among environments (Chase 2003). Observational studies suggest, however, that much variation in species composition is left unexplained after relating it to abiotic conditions (e.g. Condit *et al.* 2002; Legendre *et al.* 2009). Second, differences in dispersal among species, where some species fail to reach all suitable locations, may generate beta diversity. With unlimited dispersal and similar

abiotic conditions, all species would be found everywhere and beta diversity would approach zero (Mouquet & Loreau 2003). Finally, assembly history theory predicts that if the historical order of species arrival differs, then multiple stable equilibria can arise within uniform environments, thus generating high beta diversity (Drake 1991; Chase 2003). Although abiotic filters, dispersal processes, and species arrival order are often combined into the general term, “community assembly,” here we use “community assembly history” to refer solely to the historical order of species arrival. Seasonal differences in abiotic conditions during establishment, identity of earlier arriving species, and variations in arrival order could lead to variation in species composition within an area. Chase (2003) hypothesized that a single stable equilibrium is more likely to arise when the regional species pool is small, dispersal and disturbance rates are high, and local productivity is low. Alternatively, multiple stable equilibria are hypothesized to develop within a uniform environment when the regional species pool is large, dispersal and disturbance rates are low, and local productivity is high (Chase 2003).

Community assembly history is poorly understood because long-term field experiments incorporating many species are few in number (Anderson *et al.* 2011), and this is particularly true for plant communities (but see e.g. Fargione, Brown & Tilman 2003; Fukami *et al.* 2005, Collinge and Ray 2009, Myers and Harms 2011). Here we test whether the identity of early emerging species, the timing of seed addition, and priority effects alter the outcomes of tallgrass prairie restorations in uniform environments with high exotic species propagule pressure. Differences in the identity of early-emerging species could increase beta diversity if species permit establishment from other functional groups more readily than their own during the assembly history process (Diamond 1975; Fox 1987; Gotelli & McCabe 2002; Fargione, Brown & Tilman 2003). The seasonal timing of disturbances by ungulates or by digging by fossorial

mammals could enhance beta diversity if species exploit disturbances at different times of the year according to temporal niche requirements (Questad & Foster 2008). Bazzaz (1996) hypothesized that early species may alter local abiotic conditions and affect subsequent community composition. For example, spring disturbance could favour C<sub>4</sub> plant species because soil temperatures are increasing, but summer disturbance could favour C<sub>3</sub> species because temperatures are decreasing (Sage & Monson 1999). Priority effects occur when an early-establishing species is able to attain large size before its competitors arrive, which enables it to out-compete later-arriving species (Alford & Wilbur 1985). Priority effects could therefore alter eventual species composition and ecosystem processes. Alternatively, competition–colonization theory predicts that a species with the highest competitive ability will eventually out-compete those with a better colonizing ability, despite differences in priority effects or timing of disturbance (Tilman 1994). If competition–colonization processes dominate, then it would not matter when propagules are added to the system during a restoration. However, if the assembly history processes described above are more important, then the order of introduction is important.

Evidence is emerging that priority effects differ between native and exotic species, and this could be important in human-altered systems where novel, exotic-dominated systems can exist as alternate states to native systems (Christian and Wilson 1999; Hobbs *et al.* 2006; Wilsey, Daneshgar & Polley 2011). Exotic species have been shown to green-up several weeks before comparable native species and to have stronger priority effects than natives (Marushia, Cadotte & Holt 2010; Wilsey, Daneshgar & Polley 2011; Wainwright, Wolkovich & Cleland 2012; Dickson *et al.* 2012). Meta-analyses showed that above-ground growth rates are higher among exotic than native species (Leishman *et al.* 2007; van Kleunen, Weber & Fischer 2010). These



results imply that fundamentally different communities, or alternate states, may develop if exotics establish before natives, and this is important in restorations if the goal is to maximize native species diversity (Martin, Moloney & Wilsey 2005).

We used a long-term field experiment to test the importance of assembly history in generating beta diversity. We varied assembly history of native prairie species into systems with high exotic propagule pressure by altering identity of early-emerging species, timing of seed additions after disturbance (spring or summer), and priority effects (diverse prairie mix added with early emerging species or the following spring) in more than one environment. These factors have not been varied simultaneously before. Manipulations took place at an intermediate scale between small-scale disturbances (e.g. gopher mounds, ant hills) and field-size restoration in grasslands. We measured species composition and diversity to assess community responses to treatments. We also assessed fire temperature, fuel mass, and above-ground net primary productivity to determine if community changes had ecosystem-level ramifications that are important in grasslands. We predicted that if aspects of community assembly history were important, generation of multiple states would arise by altering 1) identity of early-emerging species, which would allow members of other functional groups into the plots more readily than their own, 2) timing of disturbance and seed additions, where spring timing would favour  $C_4$  establishment and summer timing would favour  $C_3$  establishment, and 3) priority effects, where adding a diverse native prairie mixture either with early-emerging species or after they established would alter species composition. Alternatively, if abiotic site conditions, dispersal limitation and competition–colonization mechanisms were most important, then the historical order of species arrival would not affect communities. We found that timing and priority effects

can generate alternate states of native- and exotic-dominated communities, and that these states have ecosystem-level consequences.

## Materials and Methods

### *Study Sites*

The experiment was conducted on two separate Iowa State University-owned field sites that differed in abiotic conditions and above-ground net primary productivity in Iowa, USA. Conducting the experiment in two sites allowed us to test whether assembly history processes were stronger in the more productive site as predicted by Chase (2003), although this is an unreplicated test. Previous measurements verified that the central site (Horticulture Research Station, hereafter HRS) is approximately two times more productive than the western site (Western Research Farm, hereafter WRF) in a separate study at the same sites (Blong 2007). The WRF is located in the Iowa Loess Hills region (lat. 42°03'N, long. 95°49'W) and is more xeric relative to HRS, which is located in the Des Moines Lobe (lat. 42°6'N, long. 93°35'W). The 30-year annual precipitation average prior to the study was  $777 \pm 201$  mm for WRF and  $855 \pm 208$  mm for HRS. Both sites were within normal ranges during 2005 and 2006, the establishment years of the experiment. WRF contains Ida silt loam, well-drained calcareous loess, with 14–20% slopes and 2.5% organic matter, while HRS contains Storden loam soils with 9–14% slopes and 2.5–3.5% organic matter. Plots at both sites were located on abandoned pasture dominated by the perennial exotic species *Bromus inermis* Leysser (nomenclature follows Eilers & Roosa 1994) and were established by disking the area to bare ground twice in early spring 2005 prior to adding treatments. Plots had no *B. inermis* present at the beginning of the study, but it reestablished from seed in the second year. A previous study at the same two

sites found that the seed bank was predominately exotic in origin (Blong 2007), thus allowing a test of hypotheses under high exotic propagule pressure.

### *Experimental Design*

We used a split-plot experiment to test the influence of three community assembly history factors. Factors included variation in the identity of early-emerging species, timing of seed addition following disturbance, and priority effects. Six treatments were randomly assigned to 5 x 5 m main plots with five replicates at each site to alter the identities of early-emerging tallgrass prairie species (Fig. 1). Species were selected because they establish early compared to other members of their functional groups. Main plot (“identity”) treatments included the C<sub>3</sub> annual *Chamaecrista fasciculata* (Michx.) Greene, C<sub>3</sub> perennial *Elymus canadensis* L., C<sub>3</sub> biennial *Rudbeckia hirta* L., C<sub>4</sub> perennial *Bouteloua curtipendula* (Michx.) Torrey, a mixture of all four species, and a control (no early-emerging species). Main plot identity treatments were seeded at a rate of 11.5 kg ha<sup>-1</sup>. We randomly assigned subplot treatments of seed timing and priority effects in a 2 x 2 factorial design to four, 2 x 2 m subplots (with 1-m wide alleyways) within each main plot (Fig. 1). The timing treatment simulated differences in species propagule arrival to bare ground following disturbance. Timing was altered by adding seeds either in April 2005 (spring timing) after initial disking of the entire area, or August 2005 (summer timing) after removing negligible amounts of biomass that established to ground level using a gas-powered, hand-held string trimmer. Plants that were trimmed did not regrow, and thus seeds were added to bare ground in both spring and summer. Priority effects were altered by either adding a common 30-species prairie seed mixture (hereafter “prairie mix”) simultaneously with early-emerging species in 2005 (no priority effect) or a year later in the following spring (April) 2006

(with priority effect). Thus, plots with priority effects allowed time for early-emerging species (or volunteers in control plots) to establish before the prairie mix was added. The 30-species prairie mix, which included 32 seeds per species of tallgrass prairie forbs and grasses totalling 960 seeds per subplot, was broadcast by hand to all subplots (see Table S1 in Supporting Information). There were 6 main plot treatments with 5 replicates across 2 sites giving 60 plots in total, and 4 subplots in each of the 60 main plots for 240 subplots in total. However, one main plot at each site was removed from all analyses due to accidental mowing or incorrect seeding, which reduced main plots to  $n = 58$  and subplots to  $n = 232$ .

Priority effect treatments are confounded with time to varying degrees in all priority effect studies. This is because when seeds are added before or after a canopy of other species develops, they are also added at two different time periods, which could vary in granivore activity, rainfall or temperature. However, we hypothesize that this led to minimal confounding in the current study for three reasons. First, there was very little recruitment from the prairie mix during the time period in question (i.e. recruitment occurred in 2006 and possibly afterwards). Second, there was little difference in temperature and precipitation between 2005 and 2006 in this study. Finally, similarity in results at both sites (as we found) would suggest that any confounding factor, such as granivore activity or rainfall, would also need to be similar at both sites. We suggest that this is unlikely because sites are located 185 km apart in different geologic regions. Thus, our treatments should be interpreted as primarily a priority effect, but other potentially important factors might have also differed between the time periods when seedlings established.

Plots were not weeded in order to allow communities to realistically assemble in a restoration context. Plots were immediately surrounded by fields dominated by exotic pasture

grasses in a larger agricultural matrix (corn and soybean fields), and were separated from native, unplowed remnant grasslands by 5 and 13 km at HRS and WRF, respectively. Consequently, we assumed that prairie species establishment was solely from the seeded prairie mix.

### *Sampling Design*

Species diversity, proportion of exotic species, and establishment from the prairie mix were estimated in each subplot using abundance data from a non-destructive point intercept sampling technique, which is highly correlated with biomass (Jonasson 1988; Wilsey, Daneshgar & Polley 2011). At the end of 2005, spring timing plots were dominated by annual species that were not present in the prairie mix (HRS 82% and WRF 89% annual species, based on cover estimates) and that did not persist in appreciable quantities. Establishment of species from the prairie mix did not begin until the 2006 growing season in all plots. Therefore, sampling was conducted in July 2006–2008 and 2010, the second to fourth and sixth years of the experiment. Species abundances were estimated by dropping 1-m long point intercept pins 24 times in a systematic fashion within a 50 x 100 cm point intercept frame placed in the centre of each subplot and counting the number of times pins hit each species, allowing multiple hits per species. Points were raised to contact plants greater than 1 m tall in a few cases. Plant species present in the frame that were not hit were given a value of 0.5 hits (Wilsey, Daneshgar & Polley 2011). Species diversity was calculated using Simpson's diversity ( $1/\sum p_i^2$ ). Species were designated as either native or exotic (Eilers & Roosa 1994) and the proportion of exotic species was estimated by dividing the number of exotic species hits by total number of hits. Establishment of species from the prairie mix was estimated by summing total number of hits

from the mix. Early-emerging species were omitted from response variables because we were interested only in responses of developing communities.

Species establishing early may alter abiotic conditions for future species, providing a mechanism to explain long-term community structure (Bazzaz 1996). We measured litter mass and soil water at both sites and soil temperature at HRS in subplots in June 2006 to test this hypothesis when plant seedling establishment was highest. Litter was collected in a 20 x 50 cm quadrat in one random location in each subplot, dried, and weighed. Soil water was estimated gravimetrically ((wet mass - dry mass)/dry mass) by taking one, 2.5-cm diameter soil core to 10 cm depth from each subplot and drying cores at 105° C. Soil temperature was estimated using a Hobo® data logger (Onset Computer Corporation, Bourne, MA) and thermocouples.

#### *Ecosystem-level Effects*

Fuel mass, fire temperatures, and peak above-ground primary productivity were estimated in each subplot to determine if assembly history altered ecosystem-level effects among communities. Fuel mass was estimated by clipping biomass in a 20 x 50 cm quadrat prior to burning all plots in spring, 2008. Fire temperatures were estimated by placing ceramic tiles painted with Omegalaq® Liquid Temperature Lacquers (Omega Engineering Inc., Stamford, Connecticut) on the ground in each subplot prior to burning and comparing developed colours to a standard. Fire temperature results were qualitatively similar to results from thermocouples (Dylan Schwilk unpublished data). Peak above-ground biomass was estimated by clipping biomass in a 20 x 50 cm quadrat in late August or early September 2010, the sixth year of establishment. Green biomass was dried at 65° C for 48 hours to constant mass and weighed.

### *Statistical Analyses*

Response variables measured during 2006–2008 and 2010 were analysed using split-plot repeated measures ANOVA. All plots at HRS were burned and the original seed mixture added to half the main plots in spring, 2010 for an additional study not described here, but these treated plots did not differ from untreated plots in 2010 (seeded versus non-seeded contrast: diversity, abundance of prairie mix, and proportion of exotics all  $F_{1,17} < 1.00$ ,  $P > 0.30$ ), so seeded and unseeded plots were combined for analyses. Site and early-emerging species identity were tested with the main plot error term, and timing and priority effect treatments and interactions were tested using the subplot error term using type III sums of squares in Proc Mixed in SAS (Littell *et al.* 2002). *A priori* contrasts were conducted on timing (spring vs. summer) and priority effects (with vs. without) and timing x priority interactions. Split-plot ANOVA was also used to analyse litter, soil water, soil temperature, fuel mass, fire temperature, and ANPP. Proportion of exotics was logit transformed ( $\log(p+\square/1-p+\square)$ , where  $p$  is proportion of exotics and  $\square$  is the lowest non-zero proportion, to improve normality prior to analysis (Warton & Hui 2011). Abundance from the mix contained many zeros in 2006 because establishment was low in some treatments; therefore it was analysed without the 2006 data and remaining data were  $(ln+1)$ -transformed prior to analyses to reduce heteroscedasticity and to improve normality. Data from 2006 are presented visually. Litter, fuel mass, and ANPP were  $ln$ -transformed prior to analyses to reduce heteroscedasticity and to improve normality.

We used multivariate tests to assess overall variation in community composition (i.e. beta diversity) among treatments (Anderson *et al.* 2011). We omitted early-emerging species and used Bray-Curtis dissimilarity, which excludes joint absences, on species relative abundances for all multivariate analyses. We used non-parametric permutation-based MANOVA

(perMANOVA) to test for overall community composition differences among main and subplot treatments using the *adonis* function in package *vegan* version 2.0-1 in R, which uses type I sums of squares (Anderson 2001), using data averaged across years to test for differences. Main plot treatment differences also used data averaged across subplots within each main plot. We included site and early-emerging species treatments with all interactions in the subplot model, and site and interactions with early-emerging species in the main plot model so that multivariate and univariate tests would use the same error d.f. Data were permuted 999 times within each main plot for subplot tests and within each site for main plot tests. Finally, if perMANOVA results were significant, we conducted NMDS analysis using the *metaMDS* function in *vegan* version 2.0-1 in R to visualize variation in species composition among treatments at each site for each year of sampling (Anderson *et al.* 2011).

## Results

HRS had higher diversity and proportion of natives than WRF (diversity 3.2 at HRS, 2.5 at WRF, SE = 0.09; exotics 0.60 at HRS, 0.69 at WRF, SE = 0.01) (Table 1). Identity of early-emerging species did not significantly affect any of the community response variables (Table 1).

Diversity, relative abundance from the prairie mix, and proportion of exotics were all significantly affected by timing and priority effects. Interactions between these two effects produced differences that were stronger than between sites or identity treatments. Removing dispersal limitation did not lead to convergent species compositions, and communities diverged similarly among assembly history treatments at both sites. Abundance from the prairie mix was four times higher and proportion of exotics was 20% lower in spring than summer timing treatments. All variables were strongly affected by priority effects, with diversity and abundance



from the prairie mix being 16% and 68% lower, respectively, and proportion of exotics being 20% higher when the prairie mix was added later than early-emerging species (i.e. with priority effects) (Table 1, Fig. 2). Timing and priority effects interacted, with diversity being 37% higher and relative abundance of species from the prairie mix being six times higher in the spring timing–no priority effect treatment compared to all other treatments by the end of year six (Table 1, Fig. 2). The spring timing–no priority effect treatment was 47% native by year six, whereas other treatments were dominated by exotics (82–86% exotic) (Table 1, Fig. 2). All treatments were dominated by perennials (>94%), and the proportion of C<sub>3</sub> biomass was 35% and 26% lower in spring timing-no priority effect treatments compared to other treatments at WRF and HRS, respectively, by the end of year six (see Table S2).

Species composition varied among timing and priority effect treatments (perMANOVA  $F_{3,138} = 22.2$ ,  $P < 0.01$ ), but early-emerging species did not result in changes to species composition ( $F_{5,46} = 0.88$ ,  $P = 0.58$ ). Ordinations revealed that the spring timing-no priority effect treatment diverged sharply from other treatments (Fig. 3).

#### *Abiotic Conditions*

HRS had higher litter and soil moisture than WRF at the beginning of 2006 (litter  $F_{1,46} = 76.96$ ,  $P < 0.01$ ; soil water  $F_{1,46} = 84.86$ ,  $P < 0.01$ ), but identity treatments had no significant effect on abiotic variables (litter  $F_{5,46} = 0.69$ ,  $P = 0.63$ ; soil water  $F_{5,46} = 1.17$ ,  $P = 0.34$ ; soil temperature  $F_{5,23} = 0.79$ ,  $P = 0.57$ ). Timing treatments altered all abiotic variables at the beginning of 2006 (Fig. 4). Litter was much higher in spring than summer timing treatments at HRS and WRF (timing contrast  $F_{1,138} = 309.8$ ,  $P < 0.01$ , site x timing and priority interaction  $F_{1,138} = 3.42$ ,  $P = 0.02$ ). Litter was not significantly affected by priority effects ( $F_{1,138} = 0.24$ ,  $P$

= 0.62) on average, but litter was increased slightly by priority effects in spring timing treatments (timing x priority interaction  $F = 6.7$ ,  $P = 0.01$ ). Soil water content was 29% higher in spring than summer timing treatments overall, (timing contrast  $F_{1,138} = 94.64$ ,  $P < 0.01$ ) and this was largely driven by HRS, which had 49% higher soil moisture in spring than summer (site x timing and priority interaction  $F_{1,138} = 16.31$ ,  $P < 0.01$ ). Soil moisture was not affected by priority effects or interactions (priority  $F_{1,138} = 1.49$ ,  $P = 0.22$ , timing x priority  $F_{1,138} = 0.05$ ,  $P = 0.83$ ). Average soil temperature at HRS was 11% lower in spring than summer timing treatments ( $F_{1,69} = 112.91$ ,  $P < 0.01$ ) and was not affected by other treatments (priority  $F_{1,69} = 0.40$ ,  $P = 0.53$ , timing x priority  $F_{1,69} = 1.40$ ,  $P = 0.24$ ).

#### *Ecosystem-level Effects*

Timing and priority effects significantly altered ecosystem variables consistently at both sites (all site x timing and priority interactions  $F < 1.9$ , all  $P \geq 0.15$ ). None of these were affected by identity treatments (fuel mass  $F_{5,46} = 0.68$ ,  $P = 0.64$ , fire temperature  $F_{5,45} = 1.28$ ,  $P = 0.29$ , peak ANPP  $F_{5,46} = 0.59$ ,  $P = 0.71$ ). Fuel mass and fire temperatures were 34% and 19% higher, respectively, in spring timing treatments (fuel mass  $F_{1,134} = 47.8$ ,  $P < 0.01$ ; temperature  $F_{1,134} = 50.4$ ,  $P < 0.01$ , Fig. 4). The spring timing–no priority effect treatments had 51% higher peak ANPP than other treatments (timing x priority  $F_{1,138} = 12.35$ ,  $P < 0.01$ , Fig. 4). Peak ANPP was 47% lower at WRF than HRS ( $F_{1,46} = 18.65$ ,  $P < 0.01$ ).

## **Discussion**

Community assembly history in our long term field experiment generated alternate states characterized by considerable differences in diversity and native-exotic proportions, and

consequently in ecosystem processes. Long-lived perennial species dominated all communities, suggesting that a future disturbance or death of long-lived individuals would be required to reinitiate the assembly history process. We found higher biodiversity overall at the more productive site, similar to Chase (2010). However, in contrast to predictions by Chase (2003), we found that alternate states were just as likely to develop in the site with low productivity. Our sites with different productivity levels were not replicated, so we were unable to determine whether the interesting trend we found with alternate states and productivity was general. Therefore, we suggest that further tests of this hypothesis should be conducted across replicated sites. Wilsey (2010) reported in a forum article on diversity partitioning that beta diversity was higher among assembly history treatments within sites than between the two sites in this experiment. We found here that the high beta diversity developed early, and it was caused by interacting timing and priority effects, with the spring timing–no priority effect treatment having vastly lower proportions of exotic species and greater species diversity on average. Although priority effects can be confounded with year in priority effect studies, these effects were minimal in the current study because virtually no recruitment from the seed mix was found until the second year and there were similarities in precipitation and temperature between years. After communities diverged, they remained significantly different through five growing seasons, and these differences affected fuel mass, fire temperatures, and ANPP. Altering identity of early emerging species had no effect on any of the community or ecosystem variables. Our results suggest that identity of the early invader during community assembly history may not be as important as timing of species arrival and priority effects in altering species composition.

We predicted that timing of disturbance in subplots (creating bare ground in spring or summer) would differentially affect  $C_3$ – $C_4$  proportions, but our results suggest that exotic

invasions complicated matters when exotic propagule pressure was high as it is in our system. Specifically, summer disturbance and concurrent seeding favoured C<sub>3</sub> species as predicted. Disturbance and seeding in spring favoured C<sub>4</sub> species, but only when they established before C<sub>3</sub> species (which were mostly exotic). When C<sub>4</sub> species did not establish first, C<sub>3</sub> species eventually dominated communities. Overall, the percentage of C<sub>4</sub> species was much higher in spring timing plots when the prairie mix was added simultaneously compared to all other treatments. Our results are consistent with Dickson *et al.* (2010), who found that an exotic legume was able to out-compete native perennial C<sub>4</sub> prairie grasses because it established earlier. If exotic species green-up earlier, have stronger priority effects, and have higher above-ground growth rates than natives (Leishman *et al.* 2007; Wilsey, Daneshgar & Polley 2011; Dickson *et al.* 2012), then exotics may have large and underappreciated consequences for predicting community assembly history outcomes (Wolkovich & Cleland 2011).

Variation in abiotic conditions between spring and summer timing treatments suggests that early invaders can modify their environments and contribute to community divergence. Localized species–environment feedbacks were more important than site conditions in generating community structure. Polley *et al.* (2006) found that early-season annuals regulated perennial plant composition and ecosystem functioning in grasslands. In our study, spring disturbance and seeding favoured early invasion of annual volunteer species. These annuals produced higher litter mass which generated cool, moist soil conditions favourable for prairie species to grow early in the second year of the study, when most establishment from prairie species seeded without a priority effect occurred. Conversely, summer seeding led to fewer annuals, less litter mass and warmer, drier soil conditions. These conditions, along with priority

effect treatments, favoured early establishment and resulting dominance of exotic perennial C<sub>3</sub> species and the suppression of native prairie species.

Our results provide stronger support for assembly history than for competition–colonization mechanisms in structuring restored tallgrass prairie communities in areas of high exotic propagule pressure (Dickson *et al.* 2010). Competition–colonization theory predicts that a single equilibrium will develop as long as propagules of a competitive species are available. Native perennial C<sub>4</sub> grass species have some of the highest competitive abilities (Wedin & Tilman 1993), yet these species established in only one assembly history treatment despite being seeded in all plots. Pacala & Rees (1998) suggest as an alternative model to competition–colonization tradeoffs that niche differences permit early-colonizing species to establish and maintain dominance even when the better competitor is present. Miles and Knops (2009) observed that exotic C<sub>3</sub> perennials persisted over very long time periods in the presence of competitive native species. In our study, perennial exotic species established early in all treatments, and they continued to dominate plots when they established fully before natives.

The community differences that we found resulted in important long-term effects on ecosystem variables. Greater fuel mass and higher fire temperatures were found in spring timing treatments, and these variables are important to functioning (e.g. Stronach & McNaughton 1989). Above-ground productivity was higher in the more diverse plots containing a greater proportion of native species that resulted from the assembly history treatments. Our results support the few existing studies (Foster & Dickson 2004; Körner *et al.* 2008; Fukami *et al.* 2010; Dickson *et al.* 2010) that found that community assembly history has ecosystem-level ramifications.

Community assembly history theory can link a range of questions in community, invasion, and restoration ecology (Suding, Gross & Houseman 2004; Temperton *et al.* 2004),

and it is an underappreciated mechanism contributing to beta diversity. We found that alternate states of perennials can occur by altering assembly history. These states persisted into the sixth year, and only longer-term monitoring will determine whether they are transient or stable (Fukami & Nakajima 2011). For example, Collinge & Ray (2009) found that strong priority effects of vernal pool communities diminished after four to five years and suggested that assembly may be transient. Furthermore, our results imply that generation of beta diversity in restorations may be undesirable if it results in exotic dominance in some areas. We suggest that establishing native species before exotics is crucial for restoring diverse native prairie communities in situations where *perennial* exotics are present, and that using cover crops (early-emerging species in our study) may not be as beneficial as expected (Padilla & Pugnaire 2006) in less stressful environments.

### *Conclusions*

Our results indicate that variation in species composition among communities that appears random and idiosyncratic in field conditions may in fact be the predictable result of historical assembly processes (Drake 1991). Beta diversity arose when disturbance and priority effects were altered during assembly history in the field, and communities of perennial exotic versus native species established deterministically, not stochastically. Further work on how timing of disturbance and priority effects alter native–exotic proportions, especially in the face of global climate change and other human impacts, will help us develop an understanding of when communities will assemble into diverse native communities and when they will collapse into low diversity communities of exotics. Our management recommendation is for restoration projects

to establish native species as early as possible when in situations with high exotic species abundance.

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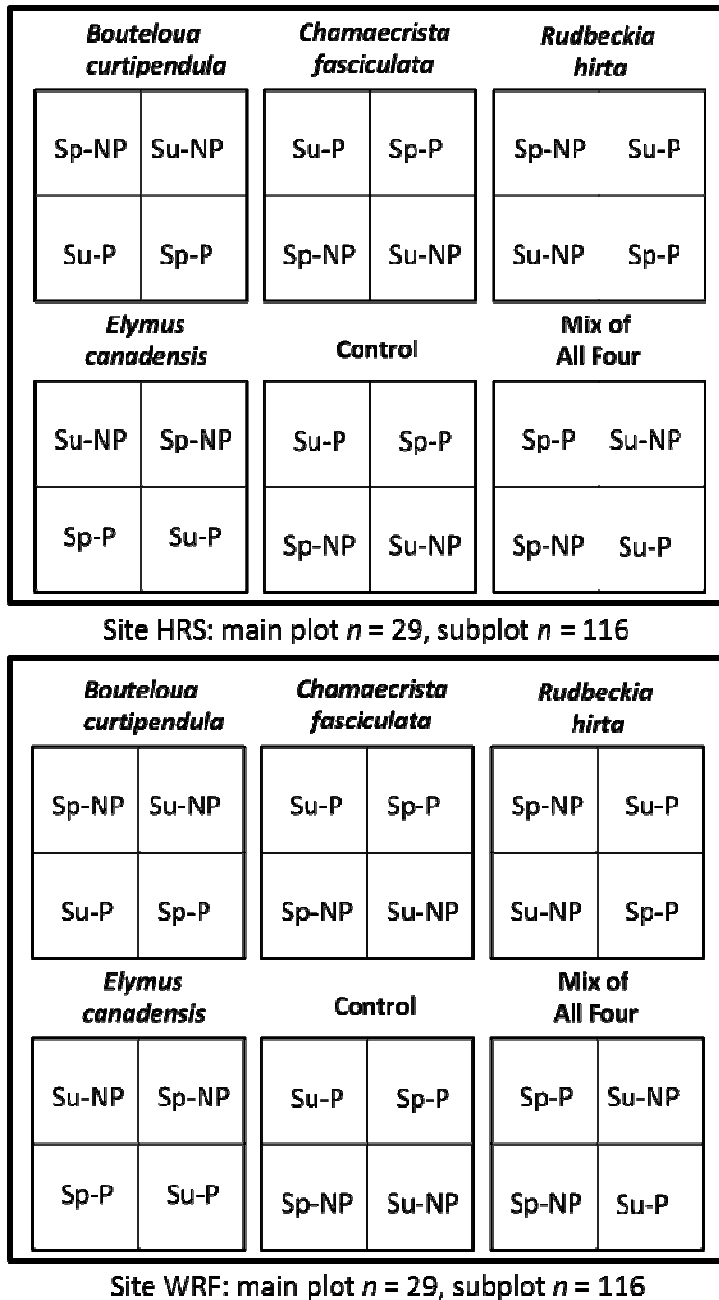
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**Table 1.** ANOVA results ( $F$ ,  $P$ ) for community assembly history experiment effects on diversity (Simpson's 1/D), proportion of exotic species abundance, and abundance from the 30-species prairie mixture. Values significant at the  $\alpha \leq 0.05$  level are in bold.

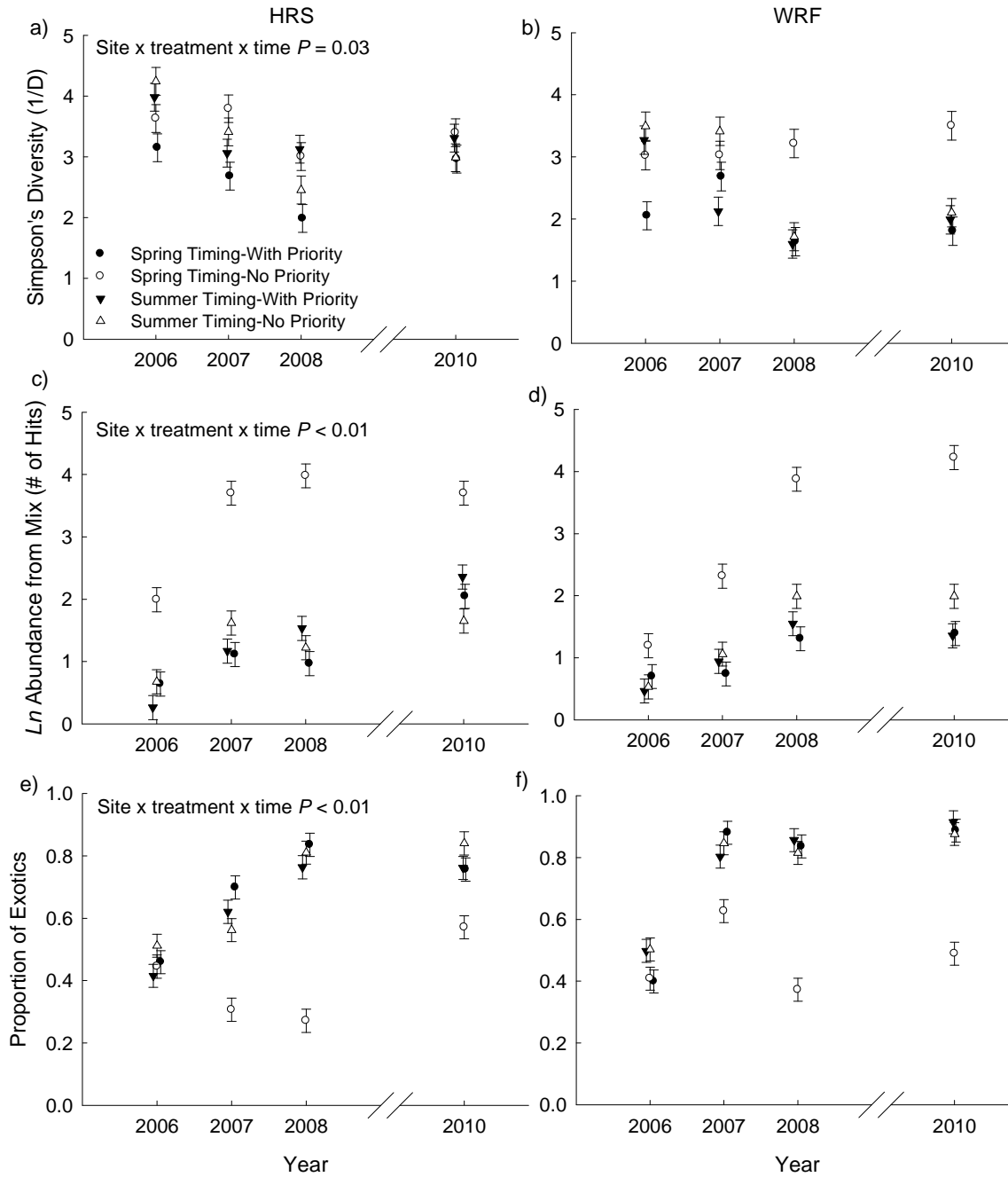
Source	d.f.*	Diversity	Proportion Exotic	Prairie Mix
Site (S)	1	<b>30.66(&lt;0.01)</b>	<b>19.12(&lt;0.01)</b>	2.41(0.13)
History (Identity) (HI)	5	1.64(0.17)	2.23(0.07)	1.63(0.17)
S x HI	5	2.35(0.06)	2.01(0.10)	1.21(0.32)
Error (Plot x S x HI)	46			
History (Timing and Priority) (HTP)	3	<b>14.33(&lt;0.01)</b>	<b>81.40(&lt;0.01)</b>	<b>108.69(&lt;0.01)</b>
S x HTP	3	<b>3.30(0.02)</b>	1.14(0.34)	1.51(0.21)
HI x HTP	15	0.53(0.92)	1.02(0.44)	1.39(0.16)
S x HI x HTP	15	0.47(0.95)	1.09(0.37)	1.62(0.08)
Error (Plot x S x HI x HTP)	138			
Time	3(2)	<b>44.56(&lt;0.01)</b>	<b>123.69(&lt;0.01)</b>	<b>40.64(&lt;0.01)</b>
S x Time	3(2)	1.02(0.39)	<b>17.30(&lt;0.01)</b>	<b>13.82(&lt;0.01)</b>
HI x Time	15(10)	1.63(0.06)	<b>2.54(&lt;0.01)</b>	1.74(0.07)
S x HI x Time	15(10)	1.00(0.46)	1.09(0.36)	0.76(0.67)
HTP x Time	9(6)	<b>5.22(&lt;0.01)</b>	<b>17.11(&lt;0.01)</b>	<b>2.26(0.04)</b>
S x HTP x Time	9(6)	<b>2.13(0.03)</b>	<b>3.60(&lt;0.01)</b>	<b>6.70(&lt;0.01)</b>
HI x HTP x Time	45(30)	0.83(0.77)	0.86(0.73)	1.02(0.43)
S x HI x HTP x Time	45(30)	0.87(0.72)	0.67(0.95)	0.79(0.78)
Error (Plot x S x HI x HTP x Time)	552(368)			
<i>Contrasts</i>				
Priority	1	<b>23.00(&lt;0.01)</b>	<b>82.57(&lt;0.01)</b>	<b>137.07(&lt;0.01)</b>
Timing	1	0.11(0.74)	<b>64.59(&lt;0.01)</b>	<b>74.11(&lt;0.01)</b>
Timing x Priority	1	<b>19.88(&lt;0.01)</b>	<b>97.04(&lt;0.01)</b>	<b>114.90(&lt;0.01)</b>
Error (Plot x S x HI x HTP)	138			

\*Different d.f. for Prairie Mix are reported in parentheses due to removing the first year (2006) of data because of the large number of zero values.

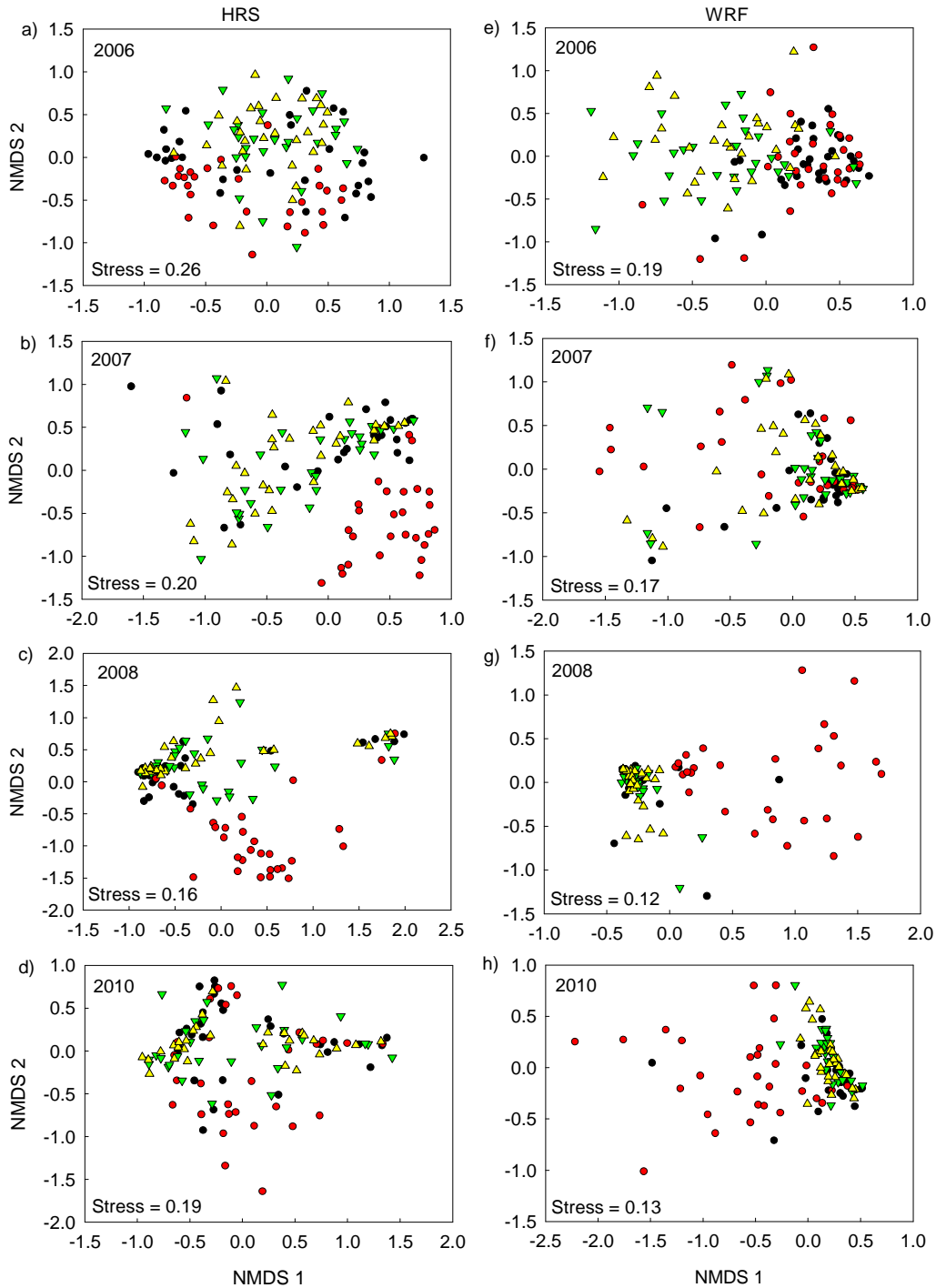


**Figure 1.** Schematic of assembly history treatments at a mesic site, HRS, and a dry site, WRF. Main plots (5 x 5 m) with six early-emerging species identity treatments (labelled above main plots), and subplot treatments (2 x 2 m with 1-m alleys) with altered timing and priority effects: spring timing–no priority (Sp-NP), early-emerging species added in spring with the 30-species prairie mix; spring timing–with priority (Sp-P), early-emerging species added in spring with the 30-species prairie mix added the following year; summer timing–no priority (Su-NP), early-emerging species added in summer with the 30-species prairie mix; summer timing–with priority (Su-P), early-emerging species added in summer with the 30-species prairie mix added the following year.

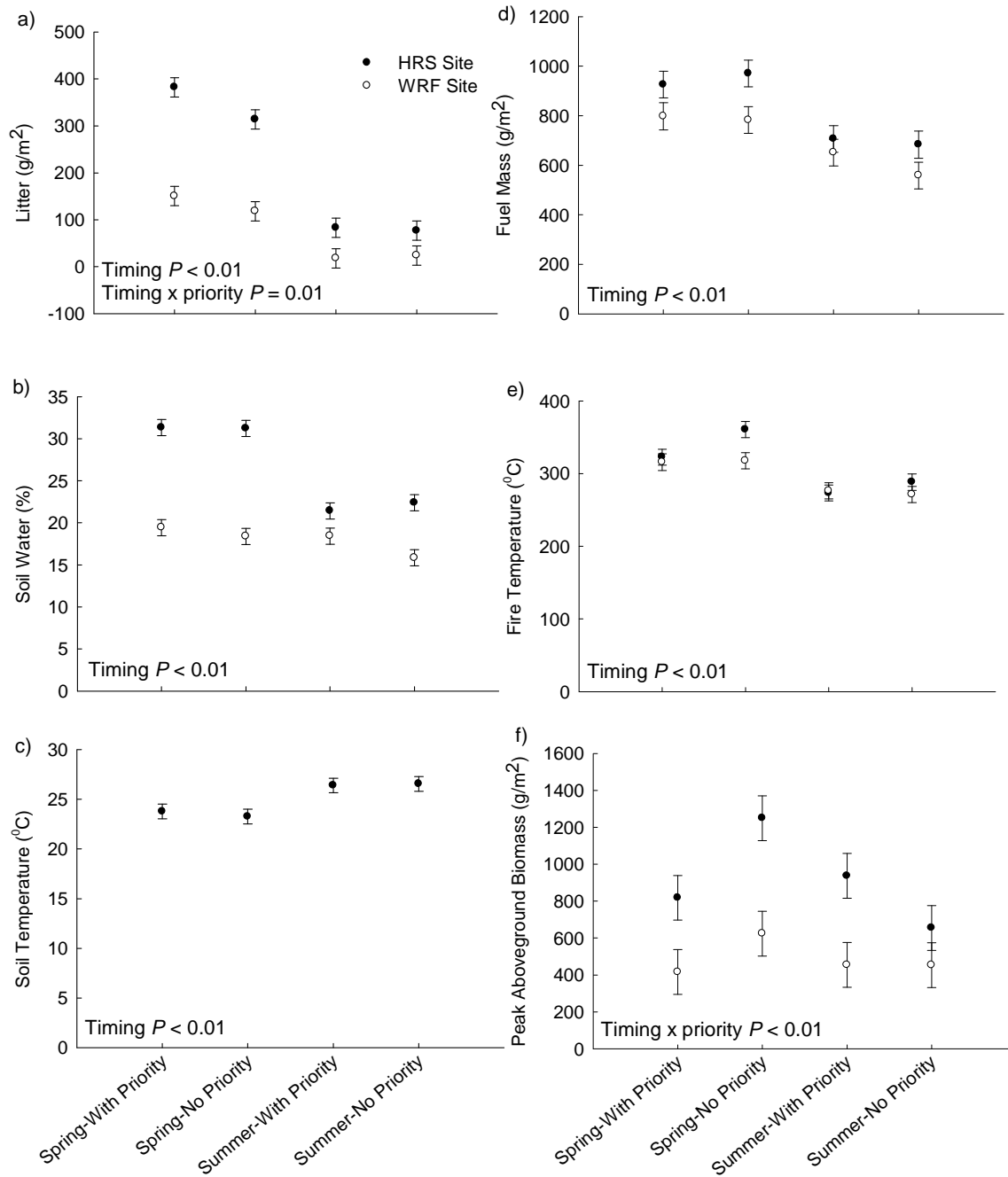
**Figure 2.** Simpson's diversity at a mesic site, HRS (a), and a dry site, WRF (b), abundance from the 30-species prairie mixture at HRS (c) and WRF (d), and proportion of exotic abundance at HRS (e) and WRF (f) in community assembly history treatments (LS means  $\pm$  SE).



**Figure 3.** Non-metric multidimensional scaling ordinations using Bray-Curtis dissimilarity on species relative abundances from the prairie mix and invaders, showing the four assembly history treatments at HRS and WRF in 2006 (a, e), 2007 (b, f), 2008 (c, g), and 2010 (d, h). Points represent spring timing–priority (black circles), spring timing–no priority (red circles), summer timing–priority (inverted green triangles), and summer timing–no priority (yellow triangles) plots.



**Figure 4.** Litter (a) and soil water content at HRS and WRF (b) and soil temperature at HRS in the second year (c), and ecosystem processes at both sites including fuel mass (d) and fire temperatures in the fourth year (e) and peak above-ground biomass in the sixth year (f) of the community assembly history experiment (LS Means  $\pm$  SE). “Spring” and “Summer” refer to the timing treatments.





## Supporting Information

**Table S1.** Seed mix of native prairie species. All species are perennial except *Oenothera biennis* (biennial). Nomenclature follows Eilers and Roosa (1994).

Species	Family
Warm season (C <sub>4</sub> ) grasses	
1. Little bluestem, <i>Schizachyrium scoparium</i>	Poaceae
2. Big bluestem, <i>Andropogon gerardii</i>	Poaceae
3. Indian grass, <i>Sorghastrum nutans</i>	Poaceae
4. Switch grass, <i>Panicum virgatum</i>	Poaceae
5. Tall dropseed, <i>Sporobolus asper</i>	Poaceae
Cool season (C <sub>3</sub> ) grasses	
6. June grass, <i>Koeleria macrantha</i>	Poaceae
7. Porcupine grass, <i>Stipa spartea</i>	Poaceae
Forbs	
8. Wild bergamot, <i>Monarda fistulosa</i>	Lamiaceae
9. Bottle gentian, <i>Gentiana andrewsii</i>	Gentianaceae
10. Butterfly milkweed, <i>Asclepias tuberosa</i>	Asclepiadaceae
11. Dotted <sup>1</sup> or Rough <sup>2</sup> blazing star, <i>Liatris punctata and aspera</i>	Asteraceae
12. Ground plum, <i>Astragalus crassicaarpus</i>	Fabaceae
13. Hoary vervain, <i>Verbena stricta</i>	Verbenaceae
14. Lead plant, <i>Amorpha canescens</i>	Fabaceae
15. Pale purple <sup>1</sup> or Narrow leaved <sup>2</sup> Coneflower, <i>Echinacea pallida</i> and <i>angustifolia</i>	Asteraceae
16. New Jersey tea, <i>Ceanothus americanus</i>	Rhamnaceae
17. Ox-eye, <i>Heliopsis helianthoides</i>	Asteraceae
18. Prairie phlox, <i>Phlox pilosa</i>	Polemoniaceae
19. Prairie larkspur, <i>Delphinium virescens</i>	Ranunculaceae
20. Prairie rose, <i>Rosa arkansana</i>	Rosaceae
21. Purple prairie clover, <i>Dalea purpurea</i>	Fabaceae
22. Red root, <i>Ceanothus herbaceus</i>	Rhamnaceae
23. Round-headed bush clover, <i>Lespedeza capitata</i>	Fabaceae
24. Smooth aster, <i>Aster laevis</i>	Asteraceae
25. Stiff goldenrod, <i>Solidago rigida</i>	Asteraceae
26. White prairie clover, <i>Dalea candidum</i>	Fabaceae
27. Yellow coneflower, <i>Ratibida pinnata</i>	Asteraceae
28. Primrose, <i>Oenothera biennis</i>	Onagraceae
29. Compass plant, <i>Silphium laciniatum</i>	Asteraceae
30. Pasque flower, <i>Anemone patens</i>	Ranunculaceae

<sup>1</sup> dry site (WRF)

<sup>2</sup> mesic site (HRS)

**Table S2.** Abundances (number of hits using point-intercept method) and standard errors (in parentheses) of species from the prairie mix in subplot treatments in the community assembly experiment comprising 90% of the total number of hits, averaged over main plots and over four years of sampling (2006, 2007, 2008, 2010). Exotic species are in bold, species from the mix are denoted with an asterisk (\*), and C<sub>3</sub> or C<sub>4</sub> status is denoted with the subscripts 3 or 4, respectively. All species are perennial except *C. canadensis* (annual), *A. artemisiifolia* (annual), and *O. biennis* (biennial). All treatments were dominated by perennials (94% or higher biomass at both sites) by the end of year six. Nomenclature follows Eilers and Roosa (1994).

Scientific Name	Spring-Priority	Spring-No Priority	Summer-Priority	Summer-No Priority
Site HRS				
<b><i>Bromus inermis</i></b> <sub>3</sub>	31.86 (2.97)	26.04 (3.04)	30.48 (3.49)	31.78 (3.02)
<b><i>Coronilla varia</i></b> <sub>3</sub>	17.35 (4.12)	12.57 (3.40)	14.97 (3.86)	20.18 (4.63)
<b><i>Poa pratensis</i></b> <sub>3</sub>	7.35 (2.13)	7.07 (2.18)	19.13 (3.52)	17.11 (2.81)
<i>Heliopsis helianthoides</i> * <sub>3</sub>	2.59 (0.65)	20.41 (2.09)	2.31 (0.48)	0.68 (0.31)
<i>Sorghastrum nutans</i> * <sub>4</sub>	2.80 (1.80)	14.17 (2.65)	0.90 (0.46)	0.02 (0.02)
<i>Conyza canadensis</i> <sub>3</sub>	2.87 (0.90)	1.06 (0.30)	5.72 (1.01)	5.92 (1.03)
<i>Andropogon gerardii</i> * <sub>4</sub>	0.76 (0.38)	6.59 (1.47)	2.51 (1.23)	0.77 (0.69)
<i>Panicum virgatum</i> * <sub>4</sub>	0.07 (0.07)	9.22 (1.92)	0.05 (0.04)	0.00 (0.00)
<b><i>Pastinaca sativa</i></b> <sub>3</sub>	1.90 (0.43)	1.26 (0.28)	3.19 (0.58)	2.95 (0.55)
<i>Oenothera biennis</i> * <sub>3</sub>	1.28 (0.51)	1.30 (0.42)	1.13 (0.35)	2.54 (0.58)
<i>Oxalis stricta</i> <sub>3</sub>	1.75 (0.49)	1.54 (0.48)	1.54 (0.41)	1.07 (0.25)
<i>Physalis heterophylla</i> <sub>3</sub>	1.41 (0.28)	0.86 (0.16)	1.98 (0.40)	1.48 (0.29)
<i>Solidago canadensis</i> <sub>3</sub>	0.94 (0.39)	1.81 (0.84)	1.84 (1.27)	0.84 (0.38)
<i>Physalis virginiana</i> <sub>3</sub>	1.41 (0.34)	0.81 (0.17)	0.84 (0.15)	2.03 (0.38)
<i>Aster pilosus</i> <sub>3</sub>	1.30 (0.68)	0.30 (0.13)	1.23 (0.31)	1.38 (0.48)
<b><i>Phalaris arundinacea</i></b> <sub>3</sub>	1.13 (0.68)	0.00 (0.00)	1.40 (0.85)	1.13 (0.63)
<i>Lactuca sp.</i> <sub>3</sub>	0.47 (0.11)	0.36 (0.16)	0.94 (0.20)	1.06 (0.31)
Average Total Number of Hits	87.01	116.16	99.81	101.19
Site WRF				
<b><i>Bromis inermis</i></b> <sub>3</sub>	44.17 (4.01)	28.29 (2.50)	46.78 (4.06)	47.00 (3.94)
<b><i>Poa pratensis</i></b> <sub>3</sub>	4.49 (1.13)	3.94 (0.92)	9.48 (1.95)	7.75 (1.59)
<i>Ambrosia artemisiifolia</i> <sub>3</sub>	6.10 (1.06)	5.07 (0.96)	3.78 (0.81)	2.91 (0.54)
<i>Heliopsis helianthoides</i> * <sub>3</sub>	0.96 (0.43)	8.70 (1.33)	0.73 (0.24)	1.21 (0.34)
<i>Schizachyrium scoparium</i> * <sub>4</sub>	0.65 (0.40)	10.06 (3.14)	0.00 (0.00)	0.00 (0.00)
<i>Andropogon gerardii</i> * <sub>4</sub>	0.12 (0.07)	9.38 (1.73)	0.14 (0.07)	0.00 (0.00)
<b><i>Trifolium pratense</i></b> <sub>3</sub>	2.19 (0.43)	2.01 (0.41)	2.54 (0.55)	2.41 (0.48)
<b><i>Medicago lupulina</i></b> <sub>3</sub>	1.52 (0.22)	1.31 (0.25)	3.29 (0.54)	2.82 (0.46)
<i>Conyza canadensis</i> <sub>3</sub>	0.47 (0.11)	0.80 (0.23)	2.93 (0.64)	3.16 (0.80)
<i>Verbena stricta</i> <sub>3</sub>	1.49 (0.21)	1.05 (0.14)	1.89 (0.26)	1.47 (0.20)
<i>Sorghastrum nutans</i> * <sub>4</sub>	0.62 (0.36)	4.70 (0.93)	0.02 (0.01)	0.10 (0.10)
Average Total Number of Hits	66.28	84.77	77.70	77.62

### **CHAPTER 3: NATIVE SPECIES SEED ADDITIONS DO NOT CAUSE RESTORED PRAIRIE PLANT COMMUNITIES TO SHIFT FROM EXOTIC TO NATIVE STATES**

A paper submitted to *Ecological Applications*

Leanne M. Martin and Brian J. Wilsey

#### **Abstract**

Exotic- and native- dominated ecosystems can exist as alternate states on landscapes, but whether exotic-dominated states are persistent in the face of native species seed pressure is not well known. Here, we asked whether low diversity, exotic-dominated states could shift to a more diverse, native community state by adding native seeds. We used an existing long-term community assembly history experiment in which alternate exotic and native states of perennial species had previously developed to test this hypothesis. Plots originally seeded in spring and without priority effects developed into more diverse, native communities, but plots seeded in summer or with priority effects developed into lower-diversity, exotic-dominated communities. We added native seeds to plots in the spring, which was the optimal time for native establishment in previous years, and we added seeds to plots after removing aboveground biomass with fire. We found that an experimental seed addition did not cause a shift from exotic states to native states. Plots seeded eight years earlier in spring and without a priority effect continued to have the highest native species abundance and diversity and lowest proportion of exotics. Our results suggest that exotic-dominated states can persist in the face of native species seed pressure. Community assembly history can play a strong role in generating and maintaining alternate states over long time frames that are relevant to restoration. New restoration projects in exotic-dominated landscapes should maximize effort towards establishing high levels of native species diversity and low proportions of exotics during initial stages of restoration.

## Introduction

The persistence of alternate ecosystem states (also referred to as alternative attractors), in which two or more ecosystem states exist in the same environmental conditions on landscapes, is a topic of increasing interest to ecologists (Chase 2003, Scheffer and Carpenter 2003, Suding et al. 2004). For example, novel, exotic-dominated ecosystems are common on landscapes across the globe, but ecosystems dominated by native species also occur within the same geologic region and under similar precipitation and temperature regimes (Hobbs et al. 2006, Kulmatiski 2006, Ellis 2011, Wilsey et al. 2011). Thus, exotic- and native-dominated systems can exist as alternate states on landscapes. Although exotic and native states can occur simultaneously, we currently have little understanding regarding the long-term persistence of these states in the face of native species seed pressure (but see Seabloom 2010). Specifically, it is unclear whether increased native seed pressure might shift exotic states to native states, yet this is an important question for restoration. If exotic systems are not persistent over the long-term, they should allow native seeds to colonize. However, if exotic states are persistent over the long-term, they should resist colonization by native seeds.

Community assembly history is increasingly recognized as a way in which alternate states can develop under similar abiotic conditions (e.g., Drake 1991, Fukami et al. 2005, Ejrnæs et al. 2006, Chase 2007, Körner et al. 2008, Chase 2010, Fukami et al. 2010, Martin and Wilsey et al. 2012). Specifically, differences in timing and arrival order of species can influence communities that develop (Körner et al. 2008, Martin and Wilsey et al. 2012, Kardol et al. 2013). Community assembly processes have recently been found to generate alternate states of perennial exotic versus native species in the same abiotic conditions (Martin and Wilsey 2012). However, it is poorly understood whether alternate states generated by differences in community

assembly history are persistent in the face of seed pressure. Theory predicts that if assembly history mechanisms are strong, then seeds should not be able to establish in alternate states due to colonization resistance (Law 1999, Fukami and Nakajima 2011). Thus, alternate community states that develop during community assembly should remain divergent. However, if assembly history mechanisms are weak and are not persistent, then seeds could establish and cause convergence of the alternate states (Law 1999, Chase 2003, Fukami and Nakajima 2011).

Understanding whether seed additions to exotic-dominated states can cause shifts to native states is important to restoration. For example, restoring ecosystems requires purposefully attempting to shift a degraded ecosystem state, such as a low diversity, exotic-dominated system, to a diverse state dominated by native species (Suding et al. 2004, Scheffer and Carpenter 2003, Martin et al. 2005, Foster et al. 2007). Repeated additions of seeds are predicted to increase establishment success of added species compared to single additions (e.g., Von Holle and Simberloff 2005, but see Collinge and Ray 2009). However, more information is necessary to address whether adding native seeds can easily shift exotic-dominated states to native states in a restoration context (e.g., Reinhardt Adams and Galatowitsch 2008, Seabloom 2010).

We tested whether seed additions can cause a low-diversity, exotic state to shift to a higher diversity, native state with an ongoing community assembly history experiment (Martin and Wilsey 2012). Relatively diverse native states established when a 30-species prairie seed mix was seeded to bare ground in spring without priority effects (Martin and Wilsey 2012). In contrast, lower diversity, exotic-dominated states arose when the same 30-species seed mix was planted on bare ground in late summer or after an early-arriving species was seeded first (Martin and Wilsey 2012). Thus, all plots received the 30-species prairie mix, but they developed into more native or exotic states based on differences in timing of seeding (spring or summer) and

priority effects (prairie mix added to plots that had an early-arriving species seeded first or not). The states also differed in ecosystem measures, including ANPP, litter production, and fire temperatures (Martin and Wilsey 2012). We hypothesize that if assembly history mechanisms are weak and exotic states are not persistent, then a second seed addition will shift the exotic states to a more native state (e.g., Funk et al. 2008). Alternatively, if the community assembly history mechanisms are strong and exotic states are persistent, then the native seed additions will not shift the exotic states to a more native state. We tested these alternate hypotheses and found strong support for the latter hypothesis.

## Methods

### *Study Sites*

The experiment was conducted in the Northern Plains tallgrass prairie region, Iowa, USA, on two separate sites owned by Iowa State University. The Horticulture Research Station (HRS) is located in the Des Moines Lobe geologic region, and is a highly productive site. The Western Research Farm (WRF) is located in the Loess Hills geologic region, where soils consist predominantly of loess. The WRF is consequently much more xeric and less productive relative to HRS. Experiments at both sites were conducted in abandoned pasture formerly dominated by the exotic forage grass *Bromus inermis* Leysser, and the sites are surrounded by an agricultural corn and soybean matrix. Exotic seed pressure is therefore relatively high at both sites (Blong 2007). See Martin and Wilsey (2012) for full site descriptions.

### *Experimental Design*

The experiment was initiated in 2005 at both sites using a split-plot design (Martin and Wilsey 2012). Main plots were 5 x 5 m and consisted of randomly assigning six treatments with altered early-emerging species identities. Main plot treatments included the C<sub>3</sub> perennial grass *Elymus canadensis* L., the C<sub>3</sub> biennial forb *Rudbeckia hirta* L., the C<sub>3</sub> annual legume *Chamaecrista fasciculata* (Michx.) Greene, the C<sub>4</sub> perennial grass *Bouteloua curtipendula* (Michx.) Torrey, a mixture of all four species, and a control with no early-emerging species. Main plot treatments were seeded at a rate of 11.5 kg/ha. Four subplot treatments within each main plot were randomly assigned to 2 x 2 m subplots with 1-m wide alleyways, and consisted of altering the timing of seed arrival and priority effects in a 2x2 factorial design. A common 30-species native prairie seed mix (32 seeds per species for 960 seeds total per subplot; called “mix” to differentiate from the early-emerging species “mixture” in main plots) was added to all subplots (Supplemental Table 1). Timing was altered by adding seeds to plots in either early spring or late summer to bare ground (Martin and Wilsey 2012). Priority effects were altered by either adding the 30-species prairie mix simultaneously with the early-emerging species (without priority effects), or the following year in spring (with priority effects). The priority effect treatment therefore allowed early-emerging species to establish before adding the prairie seed mix. The six main plot treatments were replicated five times each within each site (6 treatments x 5 replicates x 2 sites = 60 main plots total), and there were four sub-plot treatments within each main plot (60 main plots x 4 subplots = 240 subplots total) (see Martin and Wilsey 2012 for details and experimental design figure).

### *Seed Addition*

To test whether a native seed addition could shift the low-diversity, exotic states to a higher diversity, native state, we randomly selected three of the five replicates from each of the six early-emerging species main plot treatments at each site. We subsequently added the same 30-species prairie seed mix at the same rate as in the initial experiment (32 seeds per species, less *Anemone patens* L. and *Ceanothus americanus* L. due to lack of seed availability) to the subset of main plots in 2010 at HRS, and in 2011 at WRF. Seeds were added directly to the subset of main plots immediately after burning all main plots at each site. Thus, seed additions took place in communities that were established for six (HRS) and seven (WRF) years. One *R. hirta* plot at WRF and one *E. canadensis* plot at HRS were removed from all analyses due to accidental mowing and incorrect seeding, so only two main plots each were randomly selected for seed addition treatments at the respective sites. Thus, the splitting of original main plot identity treatments into seed addition versus no seed addition resulted in a factorial arrangement of 12 treatments (6 identity treatments x 2 seed addition treatments).

### *Sampling*

We used a point intercept sampling technique to sample species composition in all plots at both sites. This sampling technique is highly correlated with biomass (Wilsey et al. 2011). This method consisted of dropping 1 m-long pins 48 times in a systematic manner in the center of a 50 x 100 cm point-intercept frame in each subplot, and counting the number of times pins hit each plant species in each subplot. Multiple hits were allowed per species, and pins were raised above one meter to count hits on tall species in a few instances. Species found in the quadrat but not hit by pins were assigned a value of 0.5 hits (Wilsey et al. 2011). Final abundances were



estimated by summing the number of hits per species in each plot. Plots were sampled in late July in 2012, two (WRF) or three (HRS) years after seed additions. We measured abundance from the mix as the total number of hits from species in the 30-species prairie mix, Simpson's Diversity as  $1/D$ , and proportion of exotics as the number of exotic species hits/total number of hits using abundance data from each plot. We used Simpson's  $1/D$  to assess diversity of communities because this allowed us to test more definitively whether species from the mix actually established successfully. Species richness, in contrast, is more heavily weighted towards rare species, including individuals that are more likely to go locally extinct (Wilsey et al. 2005).

### *Statistical Analysis*

Species diversity, abundance from the mix, and proportion exotic were analyzed using a split-plot ANOVA. Assembly history outcomes did not differ between sites in the initial experiment (see Martin and Wilsey 2012 for analyses of differences between sites), so for this analysis we used site as a blocking term to account for variation at the two sites. Early-emerging species and the seed addition treatments were tested with the main plot error term (Plot[Site x Cover x Seed Addition]), and timing and priority effects and their interactions were tested with the subplot error term (Plot[Site x Trt x Cover x Seed Addition]). *A priori* contrasts were also conducted on timing (spring vs. summer) and priority effects (with vs. without) and their interactions. Proportion exotic was logit-transformed ( $\log(p+\square/1-p+\square)$ ), where  $p$  is proportion of exotics, and  $\square$  is the lowest nonzero proportion, and recruitment from the mix was  $\ln+1$  transformed to improve normality and reduce heteroscedasticity. Untransformed least-squared means are presented in figures.

All analyses were conducted using Proc Mixed in SAS 9.3 using Type III SS (Littell et al. 2002). We also tested whether the final species composition was altered by the seed addition with perMANOVA using Bray-Curtis dissimilarity on species relative abundances. This analysis was conducted using the *adonis* function, which uses Type I SS, from the *vegan* package in R, version 2.13.2 (Anderson et al. 2011). Main plots (early emerging species identities, seed additions, and their interactions) were tested by averaging over subplots, and subplots were tested using the same error d.f. (138) that was used in the univariate analyses. Data were permuted 999 times within sites for main plot tests and within main plots for subplot tests.

## Results

Plots with the seed addition did not significantly differ from non-seeded plots for abundance from the mix, diversity, or proportion of exotics (Table 1, Figure 1). Adding native species seeds also did not shift the original exotic and native alternate community states (Table 1, Figure 1). The timing and priority effect treatments continued to have the strongest effects on native species abundance, species diversity, and proportion of exotics (Table 1, Figure 1). Plots seeded eight years earlier in spring and without priority effects (i.e., timing x priority effect interactions) continued to be the most diverse with the highest abundance of species from the mix and the lowest proportion of exotics compared to plots seeded in summer or with priority effects, and this trend continued after the seed addition (a priori contrasts, Table 1, Figure 1) (see also Martin and Wilsey 2012).

A seed addition did not alter species composition compared to no seed addition (perMANOVA seed addition  $F_{1,45} = 0.8$ ,  $P = 0.60$ ), and species composition did not significantly differ among early-emerging species treatments (perMANOVA  $F_{5,45} = 1.2$ ,  $P = 0.22$ ). However,

species composition continued to significantly differ between timing and priority effect treatments (perMANOVA  $F_{3,138} = 9.1$ ,  $P < 0.001$ ) (Supplementary Table 2).

## Discussion

Experimentally adding seeds to established stands did not cause a shift from a low diversity, exotic-dominated community state to a more diverse, native-dominated state. This occurred despite adding seeds to plots in spring, which was the optimal time for native establishment in previous years (Martin and Wilsey 2012), and adding seeds to burned plots with an open canopy. Seeds had two or three years to establish, which should be an adequate amount of time based on our initial treatments (Martin and Wilsey 2012) and other seed addition studies (e.g., Fargione et al. 2003). Thus, our results imply that exotic-dominated communities were persistent in the face of native species seed pressure.

After eight years, community assembly history treatments continued to have the strongest effects on native species abundance, species diversity, proportion of exotics, and species composition in restored grassland plant communities. Plots seeded eight years earlier in spring and without a priority effect continued to have the highest native species abundance and diversity and lowest proportion of exotics even with an experimental seed addition. Other community assembly history experiments have showed that priority effects can have strong and long-term effects on community composition even when natural seed dispersal is allowed between experimental communities (Chase 2007). Here, we experimentally removed seed limitation in addition to allowing natural seed dispersal. Thus, our results imply that community assembly history effects may generate positive feedbacks that can be very strong in the face of seed

pressure (Suding et al. 2004). Further research should be conducted to determine the mechanisms underlying persistence of alternate states in the face of seed pressure.

Seed limitation, microsite limitation, or a combination of these factors can limit establishment of plant species in a community (e.g., Turnbull et al. 2000, Foster et al. 2004, Martin and Wilsey 2006, Myers and Harms 2011). Although seeds have been found to limit species richness in many systems, including in grasslands (Foster et al. 2007, Myers and Harms 2009), our results suggest that seed limitation by itself was not the only factor limiting higher levels of species diversity and native abundance in exotic-dominated communities. Native seeds established relatively easily in spring at both sites in the initial assembly history experiment, implying this should have been a satisfactory time and an adequate rate of seed supply for species to establish. A more likely explanation, therefore, is that high levels of competition from perennial species that established as a result of initial timing and priority effects limited native seed establishment (Martin and Wilsey 2012, Kardol et al. 2013). Disturbances, such as digging or grazing by animals, can increase seed establishment beyond seed additions alone by providing microsites for establishment (Martin and Wilsey 2006, Myers and Harms 2009), although that was not tested in our study. Reducing the competitive effects of established species on seeds might therefore be necessary to shift exotic-dominated states to more native-dominated states (Petraitis and Latham 1999). This might be particularly relevant in perennial grasslands, where species quickly re-emerge from below-ground meristems in post-burn environments (Benson and Hartnett 2006). Established species might be quickly utilizing resource pulses needed for seeds to establish (Collins 1987, Benson and Hartnett 2006).

Our results are highly relevant to restoration projects. Our plots were managed in a manner typical of tallgrass prairie restoration (i.e., fire every two – three years in spring) (Howe

1994) and our experiment was conducted in a typical restoration context for the tallgrass prairie (i.e., on bare ground, with high exotic species seed pressure, and in an agricultural matrix). The results of our long-term experiment suggest that new restoration projects should allocate maximum effort to establishing high native species diversity and low proportions of exotics during initial stages of the restoration process when in exotic-dominated landscapes. If exotic-dominated states are persistent with native seed pressure, then shifting exotic-dominated states to native states could require more intensive management techniques than simply adding native seeds to assembled communities (but see Foster et al. 2007 for less productive environments). Likewise, disturbance alone is unlikely to increase native recruitment if native seeds are not available for recruitment in restorations (Martin and Wilsey 2006).

In conclusion, our results indicate that exotic states can be persistent in the face of native species seed pressure. Species composition and diversity in restored grassland communities strongly depend on priority and timing effects early in establishment. Thus, community assembly history can play a strong role in generating and maintaining alternate states over long time frames that are relevant to restoration (Temperton et al. 2004). We recommend that restoration projects should pay careful attention to the earliest phases of community assembly history and establishment in order to establish the most diverse, native communities in the long-term. More fully understanding the persistence of novel, exotic-dominated communities in the context of community assembly history should allow us to more confidently restore diverse, native communities in the future.

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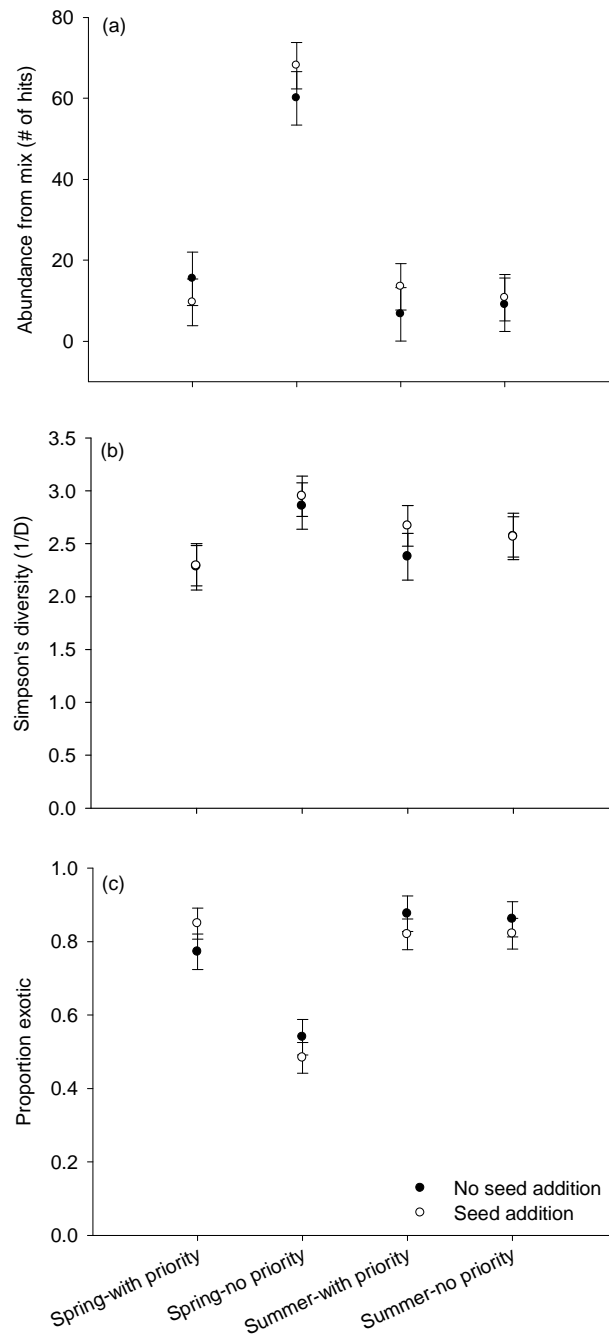


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**Table 1.** ANOVA results [F (P)] for tests of effects of assembly history and a seed addition (Seed) on species diversity (Simpson's 1/D), proportion of exotics, and recruitment from a native prairie mix. Values significant the  $\alpha \leq 0.05$  are in bold.

Source	Diversity	Proportion Exotic	Prairie Mix
History (Identity) (HI)	F <sub>5,45</sub> =0.69 (0.63)	F <sub>5, 45</sub> =1.15 (0.35)	F <sub>5,45</sub> =0.97 (0.45)
Seed (S)	F <sub>1,45</sub> =0.27 (0.61)	F <sub>1, 45</sub> =0.32 (0.57)	F <sub>1,45</sub> =0.83 (0.37)
HI x S	F <sub>5,45</sub> =1.47 (0.22)	F <sub>5,45</sub> =1.02 (0.42)	F <sub>5,45</sub> =2.10 (0.08)
History (Timing and Priority) (HTP)	F <sub>3,138</sub> =3.86 ( <b>0.01</b> )	F <sub>3,138</sub> =21.8 ( <b>&lt;0.01</b> )	F <sub>3,138</sub> =26.3 ( <b>&lt;0.01</b> )
HI x HTP	F <sub>15,138</sub> =1.09 (0.37)	F <sub>15,138</sub> =0.61 (0.86)	F <sub>15,138</sub> =0.44 (0.97)
S x HTP	F <sub>3,138</sub> =0.28 (0.84)	F <sub>3,138</sub> =0.66 (0.58)	F <sub>3,138</sub> =0.39 (0.76)
HI x S x HTP	F <sub>15,138</sub> =0.98 (0.48)	F <sub>15,138</sub> =0.68 (0.80)	F <sub>15,138</sub> =0.73 (0.75)
<i>Contrasts</i>			
Priority	F <sub>1,138</sub> =6.53 ( <b>0.01</b> )	F <sub>1,138</sub> =22.2 ( <b>&lt;0.01</b> )	F <sub>1,138</sub> =28.4 ( <b>&lt;0.01</b> )
Timing	F <sub>1,138</sub> =0.15 (0.70)	F <sub>1,138</sub> =24.6 ( <b>&lt;0.01</b> )	F <sub>1,138</sub> =22.7 ( <b>&lt;0.01</b> )
Timing x Priority	F <sub>1,138</sub> =4.91 ( <b>0.03</b> )	F <sub>1,138</sub> =18.4 ( <b>&lt;0.01</b> )	F <sub>1,138</sub> =27.8 ( <b>&lt;0.01</b> )

**Figure 1.** Abundance from the 30-species prairie mix (a), Simpson's diversity (b), and proportion of exotics (c) in a long-term community assembly history experiment when plots were seeded (open circles) or not (closed circles) six to seven years after treatments were initiated. Initial treatments consisted of adding a seed mix in spring or summer, and either with an early-emerging species (no priority) or the following year after an early-emerging species was seeded (with priority). Figure depicts least squared means  $\pm$  1 SE.



**Supplementary Table 1.** Seed mix of native prairie species. All species are perennial except *Oenothera biennis* (biennial). Nomenclature follows Eilers and Roosa (1994).

Species	Family
Warm season (C <sub>4</sub> ) grasses	
1. Little bluestem, <i>Schizachyrium scoparium</i>	Poaceae
2. Big bluestem, <i>Andropogon gerardii</i>	Poaceae
3. Indian grass, <i>Sorghastrum nutans</i>	Poaceae
4. Switch grass, <i>Panicum virgatum</i>	Poaceae
5. Tall dropseed, <i>Sporobolus asper</i>	Poaceae
Cool season (C <sub>3</sub> ) grasses	
6. June grass, <i>Koeleria macrantha</i>	Poaceae
7. Porcupine grass, <i>Stipa spartea</i>	Poaceae
Forbs	
8. Wild bergamot, <i>Monarda fistulosa</i>	Lamiaceae
9. Bottle gentian, <i>Gentiana andrewsii</i>	Gentianaceae
10. Butterfly milkweed, <i>Asclepias tuberosa</i>	Asclepiadaceae
11. Dotted <sup>1</sup> or Rough <sup>2</sup> blazing star, <i>Liatris punctata and aspera</i>	Asteraceae
12. Ground plum, <i>Astragalus crassicaarpus</i>	Fabaceae
13. Hoary vervain, <i>Verbena stricta</i>	Verbenaceae
14. Lead plant, <i>Amorpha canescens</i>	Fabaceae
15. Pale purple <sup>1</sup> or Narrow leaved <sup>2</sup> Coneflower, <i>Echinacea pallida and angustifolia</i>	Asteraceae
16. New Jersey tea, <i>Ceanothus americanus</i>	Rhamnaceae
17. Ox-eye, <i>Heliopsis helianthoides</i>	Asteraceae
18. Prairie phlox, <i>Phlox pilosa</i>	Polemoniaceae
19. Prairie larkspur, <i>Delphinium virescens</i>	Ranunculaceae
20. Prairie rose, <i>Rosa arkansana</i>	Rosaceae
21. Purple prairie clover, <i>Dalea purpurea</i>	Fabaceae
22. Red root, <i>Ceanothus herbaceus</i>	Rhamnaceae
23. Round-headed bush clover, <i>Lespedeza capitata</i>	Fabaceae
24. Smooth aster, <i>Aster laevis</i>	Asteraceae
25. Stiff goldenrod, <i>Solidago rigida</i>	Asteraceae
26. White prairie clover, <i>Dalea candidum</i>	Fabaceae
27. Yellow coneflower, <i>Ratibida pinnata</i>	Asteraceae
28. Primrose, <i>Oenothera biennis</i>	Onagraceae
29. Compass plant, <i>Silphium laciniatum</i>	Asteraceae
30. Pasque flower, <i>Anemone patens</i>	Ranunculaceae

<sup>1</sup> dry site (WRF)

<sup>2</sup> mesic site (HRS)

**Supplementary Table 2.** Average ( $\pm 1$  Standard Error) number of hits for each species in timing (spring or summer) and priority effect (priority or no priority) treatments in 2012. Species represent the top 95% of the total number of hits when averaged across all four treatments and are listed in order of most abundant to least abundant. Species in bold are exotic, and asterisks denote species from the prairie seed mix. Nomenclature follows Eilers and Roosa (1994).

Scientific Name	Spring- Priority	Spring- No Priority	Summer- Priority	Summer- No Priority
<b><i>Bromus inermis</i></b>	54.07 (4.28)	45.47 (4.56)	55.16 (4.45)	58.53 (4.04)
<b><i>Poa pratensis</i></b>	13.53 (2.08)	9.43 (1.56)	25.69 (3.31)	21.34 (2.51)
<b><i>Coronilla varia</i></b>	5.17 (2.02)	9.50 (3.53)	11.17 (4.49)	7.16 (2.83)
<i>Schizachyrium scoparium*</i>	3.57 (2.04)	18.98 (4.57)	0.59 (0.40)	0.21 (0.13)
<i>Andropogon gerardii*</i>	1.98 (1.17)	16.52 (4.26)	2.13 (1.44)	0.02 (0.01)
<i>Sorghastrum nutans*</i>	3.26 (1.10)	9.73 (2.05)	2.25 (0.85)	1.14 (0.63)
<i>Solidago canadensis</i>	2.33 (0.82)	4.63 (2.09)	2.35 (1.39)	2.66 (1.12)
<i>Panicum virgatum*</i>	0.01 (0.01)	10.26 (3.45)	0.01 (0.01)	0.05 (0.05)
<i>Heliopsis helianthoides*</i>	1.99 (0.79)	2.49 (0.71)	2.02 (0.61)	1.88 (0.50)
<b><i>Medicago lupulina</i></b>	1.82 (0.54)	0.66 (0.20)	2.41 (0.72)	2.88 (0.73)
<b><i>Phalaris arundinacea</i></b>	2.05 (1.74)	0.31 (0.31)	3.72 (2.75)	1.12 (0.82)
<i>Silphium laciniatum*</i>	0.16 (0.12)	1.98 (0.68)	1.17 (0.62)	2.09 (1.14)
<b><i>Trifolium pratense</i></b>	1.06 (0.52)	0.47 (0.24)	1.73 (0.88)	1.28 (0.56)
<b><i>Pastinaca sativa</i></b>	0.76 (0.34)	0.54 (0.22)	0.93 (0.32)	1.59 (0.55)
<b><i>Melilotus spp.</i></b>	1.86 (1.53)	0.62 (0.32)	0.45 (0.21)	0.76 (0.49)
Total	97.20	137.28	116.30	110.09

**CHAPTER 4: BIODIVERSITY, PHOTOSYNTHETIC MODE, AND ECOSYSTEM SERVICES DIFFER BETWEEN NATIVE AND NOVEL ECOSYSTEMS**

A paper submitted to *Oecologia*

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**Abstract**

Human activities have caused non-native plant species with novel ecological interactions to persist on landscapes, and it remains controversial whether these species alter multiple aspects of communities and ecosystems. We tested whether native and exotic grasslands differ in species diversity, ecosystem services, and an important aspect of functional diversity ( $C_3:C_4$  proportions) by sampling 42 sites along a latitudinal gradient and conducting a controlled experiment. Exotic-dominated grasslands had drastically lower plant diversity and slightly higher tissue N concentrations and forage quality compared to native-dominated sites. Exotic sites were strongly dominated by  $C_4$  species at southern and  $C_3$  species at northern latitudes with a sharp transition at 36-38 degrees, whereas native sites contained  $C_3:C_4$  mixtures. Large differences in  $C_3:C_4$  proportions and temporal niche partitioning were found between native and exotic mixtures in the experiment, implying that differences in  $C_3:C_4$  proportions along the latitudinal gradient are caused partially by species themselves. Our results indicate that the replacement of native- by exotic-dominated grasslands has created a management tradeoff (high diversity versus high levels of certain ecosystem services) and that models of global change impacts and  $C_3/C_4$  distribution should consider effects of exotic species.

## Introduction

Human alteration of ecosystems is causing exotic-dominated plant communities, consisting of recently assembled non-native species with potentially novel ecological interactions, to persist on nearly one-third of Earth's terrestrial surface (Hobbs *et al.* 2006; Kulmatiski 2006; Ellis 2011; Wilsey *et al.* 2011). Community and ecosystem consequences of single exotic species invasions have been well-studied (e.g. Vitousek 1990; Levine *et al.* 2003; Vilà *et al.* 2011; Pyšek *et al.* 2012). For example, Vilà *et al.* (2011) concluded from their meta-analysis that on average, single exotic species invasions result in decreased plant diversity, but that ecosystem processes can increase, decrease, or remain unchanged. In many systems, however, multiple species have been introduced, and they could have greater collective impacts than would be supposed from studying individual species.

The impacts of multiple interacting exotic species on community and ecosystem processes have only recently been considered (e.g. Wilsey *et al.* 2009, 2011; Mascaro *et al.* 2012). Wilsey *et al.* (2009, 2011) and Isbell and Wilsey (2011a) found in common garden experiments that diversity declined to a lower level in exotic-species communities than paired native communities. This decline did not consistently lead to reduced biomass production (Wilsey *et al.* 2011; Isbell and Wilsey 2011b). Multiple species invasions in Hawaii, however, have resulted in more diverse exotic-dominated sites with increased productivity compared to native-dominated sites (Mascaro *et al.* 2012). Thus, over broad spatial scales, where identities and abundances of multiple exotics vary across landscapes, the effects of many exotic species could accumulate to have community and ecosystem effects that are important to biodiversity conservation, ecosystem functioning, and management of ecosystem services.

Functional diversity of plant communities is an important index of ecosystem functioning. For example, plant species with the C<sub>3</sub> versus C<sub>4</sub> mode of photosynthesis differ in many ways, including temperature growth optima, water and nitrogen use efficiency, and carbon uptake, all of which can greatly affect element cycling and seasonal patterns of maximum growth (e.g. Teeri and Stowe 1976; Tieszen *et al.* 1997; Sage and Monson 1999). Having a mixture of C<sub>3</sub> and C<sub>4</sub> species is an important functional aspect of diverse communities that often accounts for increased productivity with richness (Tilman *et al.* 1997). The proportion of C<sub>4</sub> species in the community can alter other community traits such as tissue C:N ratios (Sage and Monson 1999) which influence decomposition rates and the timing of growth to affect higher trophic levels. Information on C<sub>3</sub>:C<sub>4</sub> proportions also is important to predictions of climate change effects on community structure. For example, simulation models based on the correlation between climate and C<sub>3</sub> and C<sub>4</sub> abundances in native-dominated communities predict that warming will favor C<sub>4</sub> over C<sub>3</sub> grass species in rangelands of North and South America (Epstein *et al.* 2002). The proportion of C<sub>3</sub> biomass increases linearly with latitude in native-dominated systems (e.g. Epstein *et al.* 1997), but it is largely unknown how this relationship changes in the now more common situation of exotic species dominance.

Here we extend earlier work to test for differences in diversity, including an important aspect of functional diversity (C<sub>3</sub>:C<sub>4</sub> proportions), and relationships between diversity and ecosystem services between exotic- and native-dominated grasslands. We tested for differences along a latitudinal gradient and in an ongoing companion experiment. The long-term, controlled experiment (see Wilsey *et al.* 2009, 2011) allowed us to test whether any differences in functional diversity could be at least partially due to species themselves rather than to covarying factors. We measured multiple aspects of communities and ecosystems that are considered to be



important to diversity and ecosystem services (“the benefits people receive from ecosystems”) in grasslands and rangelands (e.g., Millennium Ecosystem Assessment 2005; Doll *et al.* 2009; Potts *et al.* 2010; Isbell *et al.* 2011). We sampled plant species richness and diversity, C<sub>3</sub>:C<sub>4</sub> proportions, aboveground net primary productivity (ANPP), multiple aspects of forage quality to herbivores including N concentration, and potential pollinator (bee) abundances, richness, and diversity at all native and exotic sites (e.g., Millennium Ecosystem Assessment 2005; Doll *et al.* 2009; Potts *et al.* 2010). We hereafter refer to plant diversity and all other measures collectively as “diversity and ecosystem services” for simplicity. This is because, although not all of these measures are direct measures of final ecosystem services (e.g., Mace *et al.* 2012), the ability to simultaneously manage all of these measures at high levels would be desirable for meeting goals of biodiversity conservation and provisioning of ecosystem services (Daily 1997; Nelson *et al.* 2009; Isbell *et al.* 2011; Mace *et al.* 2012).

Tradeoffs among management decisions could occur if exotic-dominated systems have lower levels of plant diversity or some ecosystem services but higher levels of others compared to native systems (Tallis *et al.* 2008; Nelson *et al.* 2009). Specifically, we tested whether 1) plant diversity was lower and C<sub>3</sub>:C<sub>4</sub> proportions differed in exotic than native grasslands across the latitudinal gradient, and 2) whether any differences in diversity between native and exotic grasslands were associated with differences in the ecosystem services. We predicted that diversity would be lower in exotic-dominated systems, consistent with the decline in diversity observed in experimental communities of exotic compared to native species (Wilsey *et al.* 2009, 2011; Isbell and Wilsey 2011a), and that diversity declines would be associated with an increase in productivity and, possibly, other ecosystem service levels that could lead to management tradeoffs (Rout and Callaway 2009). For example, most exotic plant species of grasslands were

intentionally introduced by humans, often in order to increase forage production and quality for livestock (Mack and Lonsdale 2001). Consequently, ecosystems may be impacted according to the species traits involved with their introduction (Liao *et al.* 2008; van Kleunen *et al.* 2010). Finally, we used data from experimental communities to test the prediction that exotic species themselves alter functional diversity.

## **Materials and methods**

### *Latitudinal Gradient: Site Selection*

We sampled 21 native- and 21 exotic-dominated (n=21) grasslands of differing species composition from Minnesota to central Texas throughout the tallgrass prairie region, USA in 2010 and 2011. Sites were owned by a variety of private or public organizations. Point-intercept sampling of random locations within each site (described below) confirmed that all selected grasslands were either exotic- or native-dominated (see Results). Native and exotic sites had various land-use histories such as previous haying, grazing, or burning. No exotic sites were purposefully being managed for high exotic proportions to our knowledge. Native sites were never plowed, while exotic sites were previously plowed, previously unplowed but invaded, or owners/managers were uncertain of long-term land-use histories. To minimize impacts of uncontrolled variables on native-exotic comparisons, we used the following criteria for selecting sites. Sites were included if: 1) they were dominated by native or exotic species, 2) for each native site, an exotic-dominated site was in the same geologic region and was located at approximately the same latitude, 3) the site was not plowed within the last seven years (for exotics only, natives were never plowed), 4) sites were not grazed or hayed the year of sampling. Other possible native grassland sites were excluded from our study if we could not locate an

exotic match based on area or other factors mentioned above. Sites were constrained longitudinally to enable comparisons across latitude and one exotic and one native site was sampled per day. Exotic and native sites at a given latitude were separated by 0.6 to 26 km distance (mean 8 km) and sites ranged in size from 1.4 – 17.6 ha (mean 6.5 ha) and 1.1 – 55.8 ha (mean 11 ha), respectively.

#### *Latitudinal Gradient: Diversity and Ecosystem Services*

We measured variables that are important to diversity and ecosystem services in grasslands and rangelands, including plant species richness and diversity (Mace *et al.* 2012), C<sub>3</sub>:C<sub>4</sub> proportions, aboveground productivity, multiple aspects of forage quality, and bee (i.e., potential pollinator) abundances, species richness, and diversity (Millennium Ecosystem Assessment 2005). Sampling took place at peak biomass for native grasslands in the area. Plant species abundances at each site were sampled using a point intercept method, results of which are highly correlated with biomass [mean  $r^2$  across 35 species = 0.89, in Wilsey *et al.* (2011) experiment]. Thus, all relative abundance data are considered to be relative biomass estimates. The method consisted of dropping 1-m long pins perpendicular to the ground at each outside corner of a 20 x 50 cm frame in 25 randomly sampled locations (totaling 100 pin drops per site), and counting the number of pin hits per species. Species were designated as either native or exotic using national references when possible (49% of species) (Flora of North America 1993), and regional references when necessary (Great Plains Flora Association 1986; Gleason and Cronquist 1991; Eilers and Roosa 1994; Diggs *et al.* 1999; Yatskievych 2006). We were unable to identify 3% of species to C<sub>3</sub> or C<sub>4</sub> status and 5% of species to native or exotic status based on the available plant material, and these were removed from proportion C<sub>3</sub> and proportion exotic

variables, respectively. Species within the genera *Carex* and *Melilotus* were combined due to difficulty of verifying species in the field. Proportion of exotic biomass at each site was calculated as the number of exotic species hits divided by total number of hits.

Diversity and ecosystem services were measured at each site and multiple samples were averaged within sites (the unit of observation) to avoid pseudoreplication. Species richness was measured as the total number of species hit by pins at each site, and Simpson's Diversity ( $1/D$ ) was estimated by aggregating all point intercept data into one site-level measurement. Peak aboveground biomass ( $\text{g/m}^2$ ) was sampled by clipping five randomly placed 20 x 50 cm quadrats, drying plant material at 65°C for 48 hours and weighing dried biomass. Live and standing dead material was combined in each quadrat to obtain an estimate of net primary productivity. Forage quality was assessed by measuring C:N ratios, crude protein ( $\text{N} \times 6.25$ ), and two different aspects of digestibility from subsamples of dried, green biomass from clipped plots. Carbon and nitrogen content was measured by homogenizing green biomass from each plot, grinding 2 g of a random subsample from each plot using a Model 2601 Pica Blender Mill (Cianflone Scientific Instruments Corporation, Pittsburgh, PA), weighing 15 – 20 mg of each ground sample using an Orion Cahn® C-33 microbalance (Thermo Electron Corporation, Beverly, MA), and analyzing the subsample of ground material using a Flash EA 1112 Elemental Analyzer (Thermo Finnigan Italia, Rodano, MI, Italy) with atropine as standards. Digestibility was assessed by measuring Acid Detergent Fiber (ADF), which measures relative cellulose and lignin content of plant material (Sensenig *et al.* 2010) and Neutral Detergent Fiber (NDF), which measures ADF plus hemicellulose. Subsamples from two randomly selected biomass plots from each site were sent to AgSource Laboratories, Bonduel, WI, USA, for digestibility analyses. All digestibility estimates are expressed on a 100% dry matter basis. Proportion of  $\text{C}_3$  biomass per

site was assessed by dividing the number of C<sub>3</sub> hits by the total number of hits. Species were designated as C<sub>3</sub> or C<sub>4</sub> using Waller and Lewis (1979) and Sage and Monson (1999).

Bees were sampled at each site using a pan trapping technique, where 8 each of blue, white, and yellow 3.25 oz. Solo brand cups (24 total) were randomly placed in an opening in the canopy 5 meters apart in a 120-m long straight transect in the middle of each site and filled with a mixture of water and Dawn dish soap (Westphal *et al.* 2008). Blue and yellow pan traps were painted with a mixture of blue or yellow fluorescent paint and Silica Flat Paint (Guerra Paint and Pigment, New York, New York, USA). Average and total number of hours pan traps were deployed in native and exotic sites were comparable (mean 6.5 hours in native and 6.7 hours in exotic, total 136 hours in native and 140 hours in exotic). Bees were sampled at one native and one exotic site during each day to eliminate biases associated with weather conditions. All specimens were stored in 70% ethanol, and were then dried, pinned, and identified to the lowest taxonomic level possible using the Discover Life key (Ascher and Pickering 2012) or to morphospecies if species identification was not possible. Some species from the genus *Lassioglossum* were potentially combined within sites if morphospecies could not be identified. All bees captured at a site were combined to generate one site-level estimate. Bee abundances were the number of total bees captured at a site, taxonomic richness was the number of taxa identified at a site, and taxonomic diversity was estimated using Simpson's diversity (1/D) at each site.

#### *Latitudinal Gradient: Soil and Topography Covariates*

Although there were variables such as historical grazing intensity that we were unable to take into account statistically, we did test whether results changed after taking into account soil

variables. Soil variables included percent sand, silt, and clay, soil pH, percent slope, and cation exchange capacity, estimated using the SSURGO Soils database (SSURGO 2012). Each soil variable in our analysis represents an area-weighted average for the site calculated by multiplying values for each soil type (i.e., SSURGO Soil Map Unit, data extracted using Soil Data Viewer 6.0 in ArcGIS 10) by the proportion of the site area occupied by the soil type. These values were then averaged to obtain one site value per soil variable. Data were missing for one site, so 10-cm soil cores were taken in each biomass plot, homogenized, and analyzed for soil texture instead. Because values of some soil variables were correlated, we conducted a Principal Components Analysis (PCA) on all soil variables and used the principal component scores from the first two axes, which explained 74% of the variation in soils, as covariates in analyses. Percent clay and CEC were highly correlated with each other and negatively correlated with percent sand on the first axis, and percent slope and percent silt loaded heavily on the second axis. We tested whether soil variables differed between native and exotic sites by using the first and second principal component axes as covariates in all ANCOVA and MANCOVA analyses.

#### *Latitudinal Gradient: Statistical Analyses*

We used multivariate analysis of covariance (MANCOVA) and ANCOVA to assess whether exotic sites differed from native sites ( $n = 21$ ). Response variables in the MANCOVA included plant diversity, plant species richness, net primary productivity, ADF, NDF, crude protein, and C:N ratios. Explanatory variables included the first and second soil principal component scores as covariates, as well as latitude, origin and latitude by origin interactions. Multivariate data did not deviate from multivariate normal ( $W = 0.98$ ,  $P = 0.59$ ). We then tested

for management tradeoffs by comparing the signs of percent change for measured variables between native and exotic grasslands (Tallis *et al.* 2008). Site area was added as an additional covariate to the univariate test of differences in plant species richness between exotic and native sites. Data were *ln*-transformed when necessary to improve normality (C:N ratios) in both multivariate and univariate analysis. All analyses were conducted in SAS 9.2 using the Proc GLM procedure and Type I SS, and significance was assigned at the  $\alpha = 0.05$  level (Littell *et al.* 2004). Untransformed least-squared means are reported, and percentage change was measured between native and exotic grassland means. Since plant material with higher values of ADF is less digestible, and plant material with higher values of NDF occupies more space in the rumen, thus lowering dry matter intake of cattle, we calculated percent change in digestibility and dry matter intake as percent change in ADF and NDF, respectively, multiplied by -1 in order to represent an increase in those variables in exotic-dominated sites. We fit a linear regression and a logistic, four-parameter regression to the relationship between latitude and proportion of C<sub>3</sub> biomass for native- and exotic-dominated sites, respectively.

Bee variables were analyzed separately due to higher numbers of zero and singleton values and high variability relative to means. Bee variables can exhibit high variability among sampling dates, with lower numbers of bees found early and late in the growing season due to lower floral resources. To account for variability associated with sampling date of pollinators, we included linear and quadratic Julian Day (sampling day) terms as additional covariates in pollinator analyses. We used PROC GENMOD in SAS 9.2 to analyze the bee abundance and richness data with a generalized linear model using a negative binomial error distribution and log-link function (Winfree and Kremen 2009), and the same explanatory variables as the other tests. Simpson's diversity could not be calculated for sites with zero values, which reduced the

number of sites by 12 (exotic  $n = 18$ , native  $n = 12$ ) for analysis of bee diversity. Bee diversity was then analyzed using a generalized linear model in PROC GENMOD with a Poisson distribution and the log-link function. Untransformed least-squared means are reported.

### *Experiment*

Any differences in species and functional diversity or ecosystem services between native and exotic grasslands along the latitudinal gradient could result from variation in environmental factors not accounted for by covariates in analysis, or from variation among species themselves (species identity or interactions). We tested for the potential contributions of species identities/interactions to native-exotic differences in functional diversity ( $C_3:C_4$  proportions) using data from an ongoing experiment with all native and all exotic communities grown under a common environment in Temple, Texas USA (Wilsey *et al.* 2011). We interpret data of no difference in  $C_3:C_4$  proportion between experimental communities of native vs. exotic species as evidence that native/exotic differences in functional diversity along the latitudinal gradient resulted from environmental differences alone. Alternatively, we interpret differences in  $C_3:C_4$  proportion between experimental communities of exotic and native species as evidence that native/exotic differences in functional diversity along the latitudinal gradient resulted partly from species themselves.

We established 64 mixtures by planting 72 equal-sized transplants (biomass  $P > 0.1$  g at planting) of nine species into 1 x 1 m plots in October of 2007 and March of 2008, with statistical blocking on planting date. Plots were assigned to either native or exotic (origin) species and summer irrigation (128 mm from July 15 to August 15 of each year vs. no irrigation) treatments using a factorial treatment arrangement. Random draws of species (4 draws within



each of the two temporal blocks) were used to replicate species composition to increase generality. There were two replicates of each draw per treatment. Native-exotic plots were established so that species were paired phylogenetically and by growth form using a species pool of 18 native and 18 exotic species. Thus, for every native plot, a plot of phylogenetically paired exotic species was established that was initially equivalent in species richness, diversity and proportion of C<sub>3</sub> biomass. This allowed us to test whether native and exotic communities diverged over time, which would be consistent with the hypothesis that species can drive differences. Proportion of C<sub>3</sub> biomass was estimated in June (except 2011) and October of each year using a point intercept method and abundance values were converted to biomass estimates with regression equations. Species diversity was previously found to decline in exotic-dominated experimental communities (Wilsey *et al.* 2009, 2011). See Wilsey *et al.* (2011) for further details.

#### *Experiment: Statistical Analysis*

We analyzed the proportion of C<sub>3</sub> biomass with mixed model repeated measures ANOVA (PROC MIXED in SAS) and Type III SS (Littell *et al.* 2004). Origin and irrigation were fixed effects, and draw was considered a random term nested within block. Repeated measures used an AR(1) covariance structure. Proportion C<sub>3</sub> biomass was logit ( $\log[y/1-y]$ ) transformed before analysis, with the minimum value added to the numerator and denominator.

### **Results**

We found management tradeoffs between plant species diversity and ecosystem services (Tallis *et al.* 2008), wherein exotic-dominated grasslands had much lower levels of diversity but

higher or similar levels of assessed ecosystem services (Fig. 1a, Table 1). Point-intercept sampling confirmed the native-exotic status of grasslands: native sites ranged from 0 – 30% exotic biomass and exotic sites ranged from 68 – 100% exotic biomass. We found that exotic- and native-dominated grasslands differed in diversity and ecosystem services (MANCOVA, origin, Wilk's Lambda ( $W$ ) = 0.16,  $F_{7,30} = 22.05$ ,  $P < 0.001$ ; origin x latitude,  $W = 0.69$ ,  $F_{7,30} = 1.91$ ,  $P = 0.1$ ). Tests for soil differences between exotic and native grasslands confirmed that soil and topography did not differ between grassland types (MANCOVA, origin, Wilk's Lambda ( $W$ ) = 0.95,  $F_{2,37} = 0.89$ ,  $P = 0.42$ ; origin x latitude,  $W = 0.99$ ,  $F_{2,37} = 0.20$ ,  $P = 0.82$ ). Thus, the differences between native and exotic sites were highly significant whether or not soil variables were included as covariates. Overall, a slightly higher level of tissue N concentration and forage quality was countered by drastically lower levels of plant species richness and diversity in exotic grasslands (exotic vs. native means  $\pm 1$  s.e.m.: plant richness  $13.2 \pm 1.4$  vs.  $30.8 \pm 1.4$  species; plant diversity  $2.5 \pm 0.4$  vs.  $6.8 \pm 0.4$ ; crude protein  $7.3 \pm 0.3$  vs.  $6.2 \pm 0.3\%$ ; C:N  $39.6 \pm 2.2$  vs.  $44.8 \pm 2.2$ ; ADF  $38.1 \pm 0.7$  vs.  $40.2 \pm 0.7\%$ ; NDF  $60.7 \pm 1.0$  vs.  $62.8 \pm 1.0$  %) (Fig. 1a). Richness significantly increased with grassland area (Table 1). Exotic species equaling or exceeding 50% relative abundance in an exotic-dominated site include *Bothriochloa ischaemum*, *Bromus inermis*, *Cynodon dactylon*, *Panicum coloratum*, *Poa pratensis*, *Schedonorus arundinaceus*, and *Sorghum halepense* (Online Resource 1). *Andropogon gerardii* and *Schizachyrium scoparium* were the only species exceeding 50% relative abundance in a native-dominated site (Online Resource 2). Aboveground net primary productivity differences between native and exotic dominated systems changed with latitude despite no difference overall (means: NPP exotic  $733.1 \pm 54.9$ , native  $721.7 \pm 54.9$  g m<sup>-2</sup> year<sup>-1</sup>) (Fig. 1a, b, Table 1). No significant differences in potential pollinator variables were detected despite higher averages in native sites

(exotic vs. native means  $\pm 1$  s.e.m., bee abundance  $4.3 \pm 4.1$  vs.  $10.9 \pm 4.1$  bees; richness  $1.9 \pm 0.6$  vs.  $2.4$  species  $\pm 0.6$ ; diversity  $1.9 \pm 0.3$  vs.  $2.2 \pm 0.3$ ) (Fig. 1a; Table 2; Online Resource 3, 4).

We found profoundly different proportions of C<sub>3</sub> and C<sub>4</sub> biomass between native and exotic grasslands across the latitudinal gradient and in the experiment. Although species diversity was related to proportion of C<sub>3</sub> biomass (Online Resource 5), native/exotic differences in proportion of C<sub>3</sub> biomass across the gradient were significant regardless of whether or not linear and quadratic species diversity terms were included as covariates in ANCOVA (latitude x origin  $F_{1,35} = 12.6$ ,  $P = 0.001$  with diversity;  $F_{1,36} = 12.4$ ,  $P = 0.001$  without diversity as covariates). The proportion of C<sub>3</sub> biomass increased linearly with latitude across native sites as expected [ $r^2 = 0.29$ ,  $P = 0.01$ ,  $y = -0.20 + 0.02(\text{latitude})$ ] (Fig. 2). However, exotic sites were better modeled with a logistic rather than linear regression, with strong dominance by C<sub>4</sub> species biomass at southern latitudes, and C<sub>3</sub> species biomass at northern latitudes with a sharp transition at 36-38 degrees [logistic regression,  $P < 0.001$ ,  $y = 0.06 + (0.91 / (1 + (\text{latitude}/37.3)^{-78.8}))$ ] when two C<sub>3</sub> dominated sites at ~33 degrees latitude were removed [ $P < 0.001$ ,  $y = 0.26 + (0.69 / (1 + (\text{latitude}/37.5)^{-108.6}))$ ] when those points were not removed] (Fig. 2). The fit ( $r^2$  values) for exotic sites went from 0.77 with linear regression to 0.95 with logistic regression (0.59 to 0.68 when outliers were not removed).

Proportion of biomass from C<sub>3</sub> species dropped to much lower levels in experimental communities established as all exotic compared to all native species (Fig. 3). The lower proportion of C<sub>3</sub> species biomass in experimental exotic communities is consistent with field results at this latitude (Fig. 2). Native communities had significantly higher proportions of C<sub>3</sub> biomass than exotic communities on all sampling dates (origin,  $F_{1,7} = 68.5$ ,  $P < 0.001$ ,  $n = 32$ ),

and differences were larger during the June sampling periods (Time x Origin  $F_{7,450} = 18.8$ ,  $P < 0.001$ ). Thus, the difference between exotic and native communities was greatest during sampling dates early in the growing season when  $C_3$  species were most prominent. In native communities, there was a seasonal shift between  $C_3$  and  $C_4$  species dominance, indicative of temporal niche partitioning (Fig. 3, also see Wilsey *et al.* 2011). This seasonal  $C_3$ - $C_4$  shift was missing in exotic communities, which showed a decline and then a relatively flat level of  $C_3$  biomass. Irrigation did not significantly alter  $C_3$  proportions (irrig  $F_{1,7} = 1.0$ ,  $P = 0.34$ , irrig x time,  $F_{7,450} = 0.7$ ,  $P = 0.64$ ), nor did it interact with origin (irrig x origin,  $F_{1,7} = 3.4$ ,  $P = 0.11$ , irrig x origin x time,  $F_{7,450} = 0.85$ ,  $P = 0.55$ ).

## Discussion

Ecosystem impacts of novel ecosystems have rarely been assessed at broad scales (but see Mascaro *et al.* 2012). Our results indicate that replacement of native- by exotic-dominated systems in the tallgrass prairie region of the U.S. is associated with a tradeoff in the relationship between species diversity and ecosystem services, with lower diversity but enhanced or similar ecosystem services in exotic sites. The altered latitudinal gradient in  $C_3/C_4$  proportions in exotic grasslands also implies a shift in functional diversity. Although sites had varying legacy effects that are not fully controlled in an observational study, results imply that exotic grasslands did not have consistently lower productivities and had enhanced tissue N and forage quality, presumably qualities for which many exotic species were selected on introduction (Mack and Lonsdale 2001). This occurred despite lower species and functional diversity in exotic grasslands. We were unable to test for differences in attributes of all ecosystem services in the experiment due to small plot sizes (but see Wilsey *et al.* 2011 for diversity-productivity relationships), but when

environmental effects were controlled in the experiment, exotic communities exhibited vastly altered C<sub>3</sub>/C<sub>4</sub> proportions (this study) and reduced complementarity and temporal niche partitioning (Wilsey *et al.* 2009, 2011). Thus, our experimental results are consistent with the view that the differences in C<sub>3</sub>:C<sub>4</sub> proportions observed between native and exotic grasslands are at least partially caused by species themselves (see also Isbell and Wilsey 2011b; Wilsey *et al.* 2011).

Plant species richness and diversity were consistently lower in exotic-dominated sites across the latitudinal gradient, but productivity was not. These results emphasize two important points. First, previous analyses found that adding exotic species increased species diversity at broad scales (e.g. Ellis *et al.* 2012), but even with an additional 40 exotic species, site-level grassland richness and diversity were 57% and 63% lower, respectively, in exotic than native sites. Diversity and richness also were 46% and 35% lower in exotic than native experimental communities at low latitude (Wilsey *et al.* 2011) and richness was 15% lower in exotic communities at high latitudes (Isbell and Wilsey 2011a), consistent with the trend observed along the latitudinal gradient. Highly diverse native grasslands therefore have been replaced by exotic grasslands dominated by fewer species and, hence, with lower species evenness (Wilsey *et al.* 2005). Second, reduced richness in exotic grasslands did not consistently lead to reductions in primary productivity at the spatial scale of measurements (but see Chalcraft *et al.* 2004), contrary to the prediction from biodiversity-ecosystem functioning theory (Hooper *et al.* 2005; Isbell *et al.* 2011; Mascaró *et al.* 2012). If dominant exotic species that are especially productive overyield in species mixtures, then diversity declines due to exotics may not be associated with productivity declines (Wilsey *et al.* 2009).

Our results contrast with those from a study of tropical forests in Hawaii. Mascaro *et al.* (2012) found that both local diversity and primary productivity were higher in exotic-dominated forests. The consistent trend between our study and that of Mascaro *et al.* (2012) is that diversity differed between native and novel systems, albeit in a different direction.

We expected that native-dominated grasslands would have higher abundances and diversity of bee pollinators, but this trend was not significant. We hypothesize that either 1) perennial grasslands in general are important habitats to bees (Morandin *et al.* 2007), 2) bee species turnover is great during a season, which limited our ability to detect differences 3) soils (i.e., covariates) are better predictors of bees than exotic-native status (Table 2), or 4) overwhelming trends in pollinator declines across broad scales in highly fragmented landscapes such as the tallgrass prairie (Samson and Knopf 1994) have limited detectability of differences between native and exotic sites.

Perhaps the most significant finding from this study is that the replacement of native- by exotic-dominated grasslands strongly impacted an important measure of functional diversity – the proportion of C<sub>3</sub> and C<sub>4</sub> species biomass. Our experimental results imply that differences in C<sub>3</sub>:C<sub>4</sub> proportions seen in the latitudinal study could at least partially be caused by exotic species themselves rather than by unaccounted for covarying factors (e.g. disturbances or fertility levels). The large decrease in contribution of C<sub>3</sub> species to experimental exotic communities resulted from strong dominance by C<sub>4</sub> grass species. The strong dominance by either C<sub>3</sub> or C<sub>4</sub> species in exotic grasslands in the latitudinal study implies that, like the experimental communities, exotic communities may lack functional diversity and complementary resource use that underlies diversity-productivity and diversity-stability relationships (Wilsey *et al.* 2009; Isbell *et al.* 2011, Martin *et al.* unpublished data). The extremely strong dominance by a single photosynthetic type

and the sharp transition in dominant photosynthetic mode that we observed in exotic communities has not been previously documented. One explanation for the transition could be that there were large differences in C<sub>3</sub>/C<sub>4</sub> status of species that were introduced at lower versus higher latitudes. Past introductions could have targeted C<sub>3</sub> species in the north, and C<sub>4</sub> species in the south.

Biodiversity loss is sometimes considered a component of global change (Isbell *et al.* 2011; Reich *et al.* 2012). Our results imply that loss of natives and additions of exotics to landscapes has fundamentally altered grassland attributes at regional scales (Wardle *et al.* 2011; Barnosky *et al.* 2012). In particular, additions of multiple exotic species into grassland systems in the Great Plains have resulted in greatly decreased site-level diversity, and altered latitudinal trends in C<sub>3</sub>:C<sub>4</sub> proportions and productivity. Models of C<sub>3</sub>/C<sub>4</sub> distribution (e.g., Epstein *et al.* 1997; Tieszen *et al.* 1997; von Fischer *et al.* 2005; Edwards *et al.* 2010) should be revisited to account for effects associated with exotic species dominance. This will be important when predicting responses of vegetation to climate change, feedbacks to element cycles and climate (e.g. Collatz *et al.* 1998; Sala *et al.* 2000), anthropogenic alteration of <sup>13</sup>C/<sup>12</sup>C ratios in plant and animal remains (Edwards *et al.* 2010; Ellis 2011), and phenology (Wilsey *et al.* 2011; Fridley 2012). Based on this, we maintain that native-exotic status of grasslands should figure prominently in future projections.

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**Table 1.** ANCOVA results (*F/P* values) for tests of differences between native and exotic sites and interactions with latitude for each ecosystem service. PC1 and PC2 covariates are the first and second principal component axes explaining 74% of the variation in soil and topography across sites. Area (in parentheses) was used as a covariate only for plant species richness; all other analyses did not include area as a covariate. Values significant at the  $\alpha \leq 0.05$  level are in bold.

Source	d.f.	NPP		Plant Diversity		Forage Quality		
		ANPP	Simpson's Diversity	Richness	C:N	Crude Protein	ADF	NDF
PC1	1	2.0/0.17	1.5/0.23	2.3/0.14	1.1/0.31	1.1/0.30	1.8/0.19	0.8/0.38
PC2	1	0.1/0.82	<b>4.9/0.03</b>	<b>15.9/&lt;0.01</b>	1.1/0.29	1.7/0.20	2.6/0.11	0.8/0.38
(Area)	(1)			<b>12.7/&lt;0.01</b>				
Latitude	1	0.8/0.39	0.5/0.47	0.17/0.69	0.7/0.42	0.0/0.84	0.3/0.60	<b>8.7/&lt;0.01</b>
Origin	1	0.0/0.9	<b>52.5/&lt;0.01</b>	<b>67.5/&lt;0.01</b>	<b>5.2/0.03</b>	<b>6.1/0.02</b>	<b>4.9/0.03</b>	2.0/0.16
Latitude x Origin	1	<b>4.1/0.05</b>	0.01/0.92	3.1/0.09	2.7/0.11	2.2/0.15	0.3/0.59	<b>4.7/0.04</b>
Error	36 (35*)							

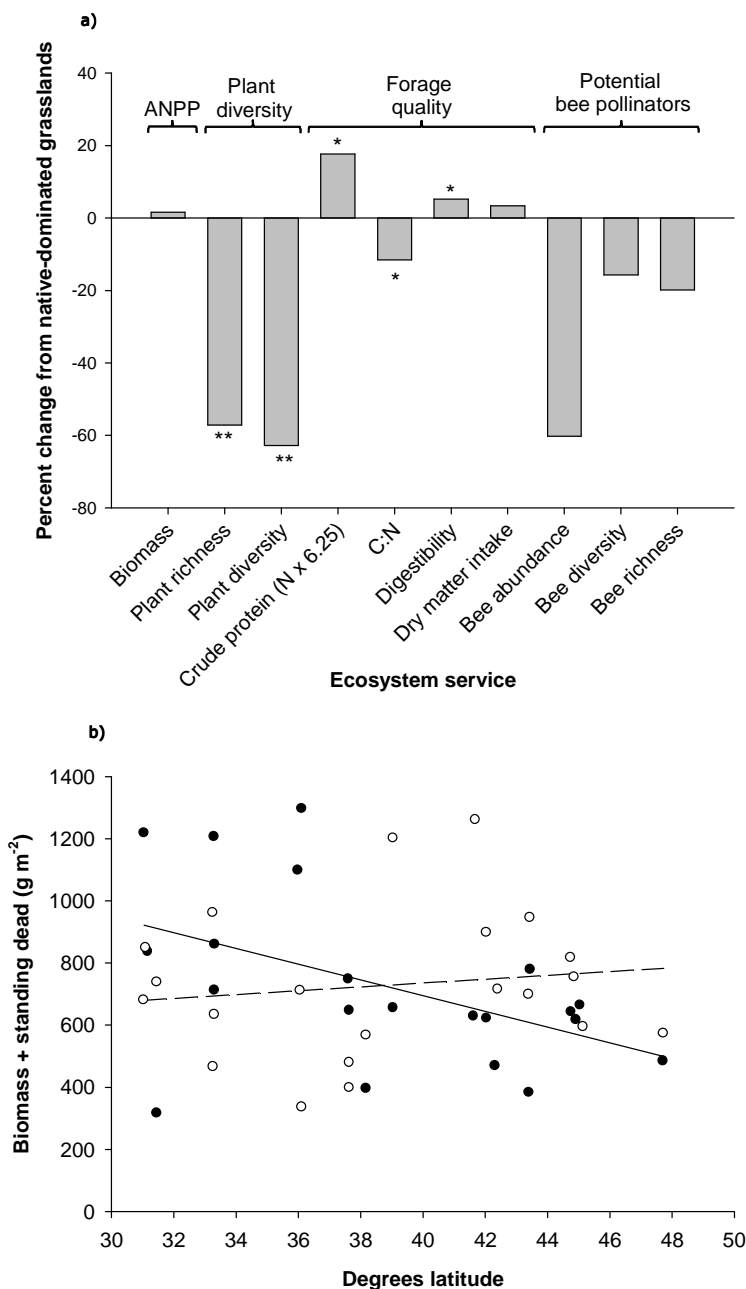
\*Plant richness error d.f. = 35; all other error d.f. = 36.

**Table 2.** Generalized Linear Model results (Chi-Square/*P* values) for tests of differences between native and exotic sites and interactions with latitude for potential pollinator (bee) variables. PC1 and PC2 covariates represent the first and second principal component axes explaining 74% of the variation in soil and topography across sites. Julian Day represents sampling day of the year. Values significant at the  $\alpha \leq 0.05$  level are in bold.

Source	d.f.	Abundance	Simpson's Diversity	Richness
PC1	1	3.1/0.08	3.3/0.07	3.3/0.07
PC2	1	0.5/0.48	<b>5.8/0.02</b>	<b>5.1/0.02</b>
Julian Day	1	0.0/0.98	0.1/0.81	1.2/0.27
Julian Day quadratic	1	<b>7.6/0.01</b>	0.0/0.95	2.7/0.10
Latitude	1	<b>6.1/0.01</b>	0.1/0.82	0.2/0.65
Origin	1	0.1/0.72	0.5/0.49	0.0/0.84
Latitude x Origin	1	2.1/0.15	0.7/0.41	2.4/0.11
Error	34 (22*)			

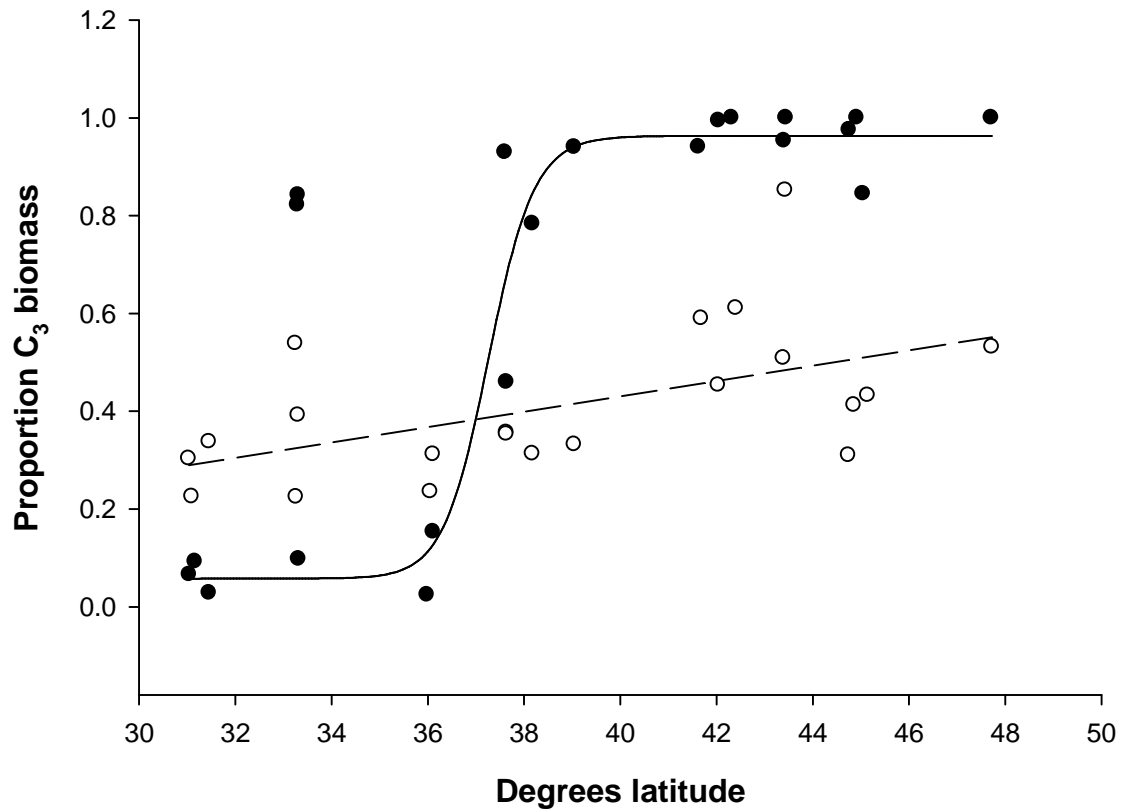
\*Diversity error d.f. = 22; all other error d.f. = 34.

**Fig. 1.** Diversity and ecosystem services in exotic- and native-dominated grasslands. A) Percent change in means of diversity and multiple ecosystem services from native-dominated to exotic-dominated grassland across a latitudinal gradient in the tallgrass prairie region, USA ( $n = 21$ ). The line at zero represents no difference; bars above zero represent an increase and bars below zero represent a decrease in a particular service in exotic- compared to native-dominated grasslands. Significant differences between native and exotic conditions are denoted by asterisks (\*\* $P < 0.001$ , \* $P < 0.05$ ). B) Aboveground net primary productivity (biomass + standing dead) was higher in exotic grasslands at lower latitudes and lower in exotic sites at higher latitudes (exotic closed circles, solid line; native open circles, dashed line) (Origin x Latitude Interaction,  $F_{1,36} = 4.06$ ,  $P = 0.05$ ,  $n = 21$ ).

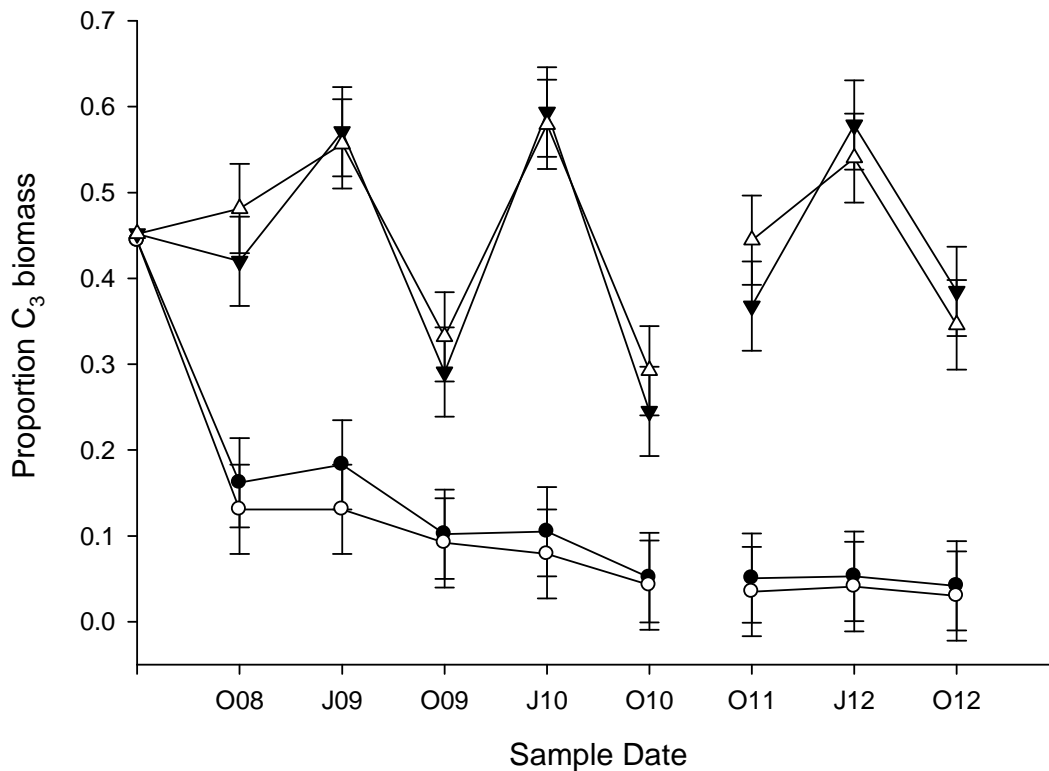




**Fig. 2.** Proportion of aboveground biomass from plants with the C<sub>3</sub> mode of photosynthesis (1 - C<sub>4</sub> biomass) in exotic- (closed circles, solid line) and native-dominated (open circles, dashed line) grasslands across a latitudinal gradient in the tallgrass prairie region, USA. Proportion of C<sub>3</sub> biomass increased linearly across native-dominated sites [ $r^2 = 0.29$ ,  $P = 0.01$ ,  $y = -0.20 + 0.02(\text{latitude})$ ]. Exotic sites exhibited very strong C<sub>4</sub> dominance at southern latitudes, and very strong C<sub>3</sub> dominance at northern latitudes (logistic regression,  $r^2 = 0.95$ ,  $P < 0.001$ ,  $y = 0.06 + (0.91/[1 + (\text{latitude}/37.3)^{-78.8}])$  when two C<sub>3</sub> dominated sites at ~33 degrees latitude were removed;  $r^2 = 0.68$ ,  $P < 0.001$  when those points were not removed).



**Fig. 3.** Proportion of aboveground biomass from plants with the  $C_3$  mode of photosynthesis in experimental plots in central Texas USA over five growing seasons. Sampling was conducted in June (J) and October (O) of each year, but not in June 2011. Plots were planted with equal-sized transplants with either all native or all exotic perennial species using a paired-species approach, and plots were irrigated in the summer (128 mm) to mimic altered climate or were exposed to ambient precipitation (exotic irrigated, closed circles; exotic non-irrigated, open circles; native irrigated, closed triangles; native non-irrigated, open triangles).



**Online Resource 1.** List of species and their relative abundances in exotic-dominated grasslands. Species are only included in the list if they were in the top 90% of species relative abundances, averaged across all exotic-dominated sites. An additional 118 species (not listed here and in the bottom 10% of overall abundance) were found in exotic-dominated sites. Species in bold are exotic.

Species	Latitude (decimal degrees) of each exotic-dominated site										
	31.05	31.17	31.46	33.3	33.31	33.32	35.99	36.12	37.61	37.64	38.18
<i>Bromus inermis</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Poa pratensis</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63	0.14	0.00
<i>Bothriochloa ischaemum</i>	0.38	0.89	0.39	0.00	0.00	0.89	0.00	0.00	0.00	0.00	0.00
<i>Schedonorus arundinaceus</i>	0.00	0.00	0.00	0.74	0.76	0.00	0.00	0.00	0.00	0.29	0.06
<i>Cynodon dactylon</i>	0.23	0.00	0.00	0.12	0.00	0.00	0.84	0.00	0.00	0.00	0.00
<i>Sorghum halepense</i>	0.15	0.01	0.00	0.03	0.01	0.00	0.14	0.85	0.00	0.00	0.00
<i>Panicum coloratum</i>	0.12	0.00	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Daucus carota</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.50
<i>Setaria pumila</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.20	0.16
<i>Melilotus spp.</i>	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.07
<i>Panicum anceps</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.24	0.00
<i>Sporobolus compositus</i>	0.00	0.00	0.01	0.02	0.13	0.00	0.00	0.00	0.00	0.00	0.00
<i>Thinopyrum intermedium</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Andropogon gerardii</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Solidago canadensis</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.10
<i>Phalaris arundinacea</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Nassella leucotricha</i>	0.03	0.00	0.03	0.03	0.03	0.00	0.00	0.00	0.00	0.00	0.00

Online Resource 1 (continued).

Species	Latitude (decimal degrees) of each exotic-dominated site										Cumulative average abundance
	39.05	41.63	42.05	42.32	43.41	43.45	44.76	44.92	45.05	47.72	
<i>Bromus inermis</i>	0.31	0.72	0.73	0.87	0.52	0.49	0.38	0.38	0.18	0.55	0.24
<i>Poa pratensis</i>	0.22	0.13	0.11	0.12	0.25	0.51	0.47	0.30	0.50	0.27	0.42
<i>Bothriochloa ischaemum</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.54
<i>Schedonorus arundinaceus</i>	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.64
<i>Cynodon dactylon</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.70
<i>Sorghum halepense</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75
<i>Panicum coloratum</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.79
<i>Daucus carota</i>	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.81
<i>Setaria pumila</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.83
<i>Melilotus spp.</i>	0.07	0.00	0.00	0.00	0.00	0.00	0.04	0.06	0.00	0.00	0.84
<i>Panicum anceps</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.85
<i>Sporobolus compositus</i>	0.04	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.86
<i>Thinopyrum intermedium</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.87
<i>Andropogon gerardii</i>	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.15	0.00	0.88
<i>Solidago canadensis</i>	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.89
<i>Phalaris arundinacea</i>	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.14	0.00	0.90
<i>Nassella leucotricha</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90

**Online Resource 2.** List of species and their relative abundances in native-dominated grasslands. Species are only included in the list if they were in the top 90% of species relative abundances when abundances were averaged across all exotic-dominated sites. An additional 168 species (not listed here and in the bottom 10% of overall abundances) were found in exotic-dominated sites. Species in bold are exotic.

Species	Latitude (decimal degrees) of each native-dominated site										
	31.04	31.1	31.46	33.26	33.27	33.31	36.06	36.12	37.64	37.64	38.18
<i>Andropogon gerardii</i>	0.03	0.02	0.17	0.04	0.09	0.20	0.35	0.08	0.19	0.19	0.55
<i>Schizachyrium scoparium</i>	0.31	0.58	0.13	0.08	0.12	0.12	0.14	0.40	0.24	0.22	0.08
<i>Sorghastrum nutans</i>	0.02	0.05	0.14	0.02	0.02	0.02	0.06	0.18	0.13	0.11	0.02
<i>Carex spp.</i>	0.00	0.00	0.00	0.02	0.00	0.04	0.02	0.04	0.03	0.02	0.15
<b><i>Poa pratensis</i></b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00
<i>Tripsacum dactyloides</i>	0.00	0.00	0.00	0.25	0.39	0.11	0.00	0.00	0.00	0.00	0.00
<i>Sporobolus heterolepis</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Sporobolus compositus</i>	0.01	0.05	0.03	0.05	0.09	0.16	0.01	0.01	0.02	0.01	0.01
<i>Solidago canadensis</i>	0.06	0.02	0.02	0.08	0.02	0.01	0.00	0.00	0.07	0.01	0.00
<i>Hesperostipa spartea</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Panicum virgatum</i>	0.00	0.00	0.00	0.01	0.00	0.00	0.13	0.00	0.00	0.00	0.02
<i>Symphyotrichum ericoides</i>	0.01	0.02	0.03	0.03	0.01	0.03	0.01	0.00	0.08	0.02	0.00
<b><i>Bromus inermis</i></b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b><i>Sorghum halepense</i></b>	0.21	0.00	0.03	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Dichanthelium acuminatum</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.06	0.16	0.01
<i>Mimosa roemeriana</i>	0.00	0.00	0.00	0.07	0.07	0.09	0.00	0.00	0.00	0.00	0.00
<i>Eriochloa sericea</i>	0.06	0.01	0.13	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00
<i>Nassella leucotricha</i>	0.00	0.01	0.04	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Bouteloua curtipendula</i>	0.03	0.02	0.01	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
<i>Monarda fistulosa</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.01	0.00	0.00
<i>Helianthus maximiliani</i>	0.00	0.05	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00
<i>Salvia azurea</i>	0.01	0.01	0.09	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00
<i>Rubus trivialis</i>	0.05	0.04	0.03	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.00
<i>Ambrosia trifida</i>	0.11	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

**Online Resource 2 (continued).**

Species	Latitude (decimal degrees) of each native-dominated site										
	31.04	31.1	31.46	33.26	33.27	33.31	36.06	36.12	37.64	37.64	38.18
<i>Helianthus pauciflorus</i>	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Acacia angustissima</i>	0.00	0.00	0.03	0.03	0.03	0.04	0.00	0.00	0.00	0.00	0.00
<i>Artemisia ludoviciana</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.01	0.00	0.00	0.00
<i>Ambrosia psilostachya</i>	0.00	0.00	0.00	0.05	0.01	0.00	0.02	0.03	0.00	0.00	0.00
<i>Dichanthelium oligosanthes</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00	0.00	0.00
<i>Amorpha canescens</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.00	0.02
<i>Spartyna pectinata</i>	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Pycnanthemum tenuifolium</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02
<i>Andropogon virginicus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.10	0.00
<i>Muhlenbergia cuspidata</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Zizia aurea</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<b><i>Lespedeza cuneata</i></b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.00
<i>Symphyotrichum lanceolatum</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Helianthus tuberosus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Lespedeza capitata</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.03
<i>Helianthus grossesserratus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Elymus canadensis</i>	0.00	0.01	0.00	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00
<b><i>Schedonorus arundinaceus</i></b>	0.00	0.00	0.00	0.04	0.01	0.00	0.00	0.00	0.00	0.00	0.00
<i>Ruellia humilis</i>	0.00	0.00	0.01	0.00	0.02	0.02	0.00	0.00	0.00	0.00	0.00
<b><i>Bothriochloa ischaemum</i></b>	0.00	0.00	0.00	0.00	0.04	0.00	0.01	0.00	0.00	0.00	0.00

**Online Resource 2 (continued).**

Species	Latitude (decimal degrees) of each native-dominated site										Cumulative average abundance
	39.05	41.69	42.04	42.41	43.4	43.44	44.75	44.86	45.15	47.73	
<i>Andropogon gerardii</i>	0.42	0.12	0.24	0.08	0.40	0.10	0.35	0.18	0.23	0.25	0.20
<i>Schizachyrium scoparium</i>	0.07	0.18	0.08	0.02	0.00	0.00	0.20	0.03	0.00	0.01	0.35
<i>Sorghastrum nutans</i>	0.07	0.02	0.10	0.10	0.04	0.01	0.02	0.10	0.03	0.06	0.41
<i>Carex spp.</i>	0.05	0.01	0.00	0.32	0.06	0.24	0.01	0.08	0.04	0.08	0.47
<b><i>Poa pratensis</i></b>	0.01	0.12	0.07	0.06	0.16	0.17	0.09	0.02	0.27	0.12	0.52
<i>Tripsacum dactyloides</i>	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.56
<i>Sporobolus heterolepis</i>	0.03	0.08	0.05	0.10	0.04	0.04	0.03	0.00	0.30	0.04	0.59
<i>Sporobolus compositus</i>	0.03	0.00	0.00	0.01	0.00	0.00	0.01	0.03	0.00	0.04	0.62
<i>Solidago canadensis</i>	0.03	0.05	0.08	0.01	0.02	0.01	0.00	0.00	0.02	0.02	0.64
<i>Hesperostipa spartea</i>	0.00	0.00	0.02	0.03	0.05	0.00	0.08	0.12	0.02	0.14	0.67
<i>Panicum virgatum</i>	0.01	0.00	0.00	0.02	0.01	0.00	0.01	0.21	0.00	0.00	0.69
<i>Symphotrichum ericoides</i>	0.00	0.03	0.01	0.00	0.00	0.01	0.00	0.03	0.00	0.02	0.70
<b><i>Bromus inermis</i></b>	0.00	0.08	0.01	0.01	0.02	0.13	0.02	0.00	0.00	0.00	0.72
<b><i>Sorghum halepense</i></b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.73
<i>Dichanthelium acuminatum</i>	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.74
<i>Mimosa roemeriana</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.75
<i>Eriochloa sericea</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.76
<i>Nassella leucotricha</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.77
<i>Bouteloua curtipendula</i>	0.00	0.00	0.01	0.00	0.00	0.00	0.07	0.00	0.00	0.00	0.78
<i>Monarda fistulosa</i>	0.00	0.02	0.07	0.00	0.04	0.01	0.00	0.00	0.00	0.00	0.79
<i>Helianthus maximiliani</i>	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.04	0.05	0.02	0.80
<i>Salvia azurea</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.80
<i>Rubus trivialis</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.81
<i>Ambrosia trifida</i>	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.82

**Online Resource 2 (continued).**

Species	Latitude (decimal degrees) of each native-dominated site										Cumulative average abundance
	39.05	41.69	42.04	42.41	43.4	43.44	44.75	44.86	45.15	47.73	
<i>Helianthus pauciflorus</i>	0.00	0.03	0.00	0.01	0.02	0.05	0.00	0.00	0.00	0.01	0.82
<i>Acacia angustissima</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.83
<i>Artemisia ludoviciana</i>	0.00	0.00	0.02	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.84
<i>Ambrosia psilostachya</i>	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.84
<i>Dichanthelium oligosanthes</i>	0.00	0.01	0.00	0.00	0.02	0.03	0.02	0.00	0.00	0.00	0.85
<i>Amorpha canescens</i>	0.00	0.00	0.01	0.00	0.02	0.03	0.00	0.00	0.00	0.01	0.85
<i>Spartina pectinata</i>	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.04	0.00	0.02	0.86
<i>Pycnanthemum tenuifolium</i>	0.08	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.87
<i>Andropogon virginicus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.87
<i>Muhlenbergia cuspidata</i>	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.01	0.05	0.88
<i>Zizea aurea</i>	0.00	0.00	0.00	0.04	0.00	0.01	0.00	0.01	0.00	0.01	0.88
<b><i>Lespedeza cuneata</i></b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.88
<i>Symphotrichum lanceolatum</i>	0.00	0.03	0.00	0.01	0.00	0.01	0.00	0.02	0.00	0.00	0.89
<i>Helianthus tuberosus</i>	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.89
<i>Lespedeza capitata</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.89
<i>Helianthus grossesserratus</i>	0.02	0.00	0.00	0.01	0.00	0.02	0.00	0.00	0.00	0.00	0.89
<i>Elymus canadensis</i>	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90
<b><i>Schedonorus arundinaceus</i></b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90
<i>Ruellia humilis</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90
<b><i>Bothriochloa ischaemum</i></b>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90



**Online Resource 3:** List of bee species and their relative abundances in exotic-dominated grasslands. Species are only included in the list if they were in the top 95% of species relative abundances when abundances were averaged across all exotic-dominated sites.

Species	Latitude (decimal degrees) of each exotic-dominated site										
	31.05	31.17	31.46	33.3	33.31	33.32	35.99	36.12	37.61	37.64	38.18
<i>Lasioglossum sp.</i>	1.00	1.00	0.96	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.00
<i>Augochlorella aurata</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.33	0.71	0.50	1.00	0.50
<i>Halictus ligatus</i>	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.10	0.00	0.00
<i>Melissodes communis</i>	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Melissodes trinodis</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Agapostemon sp.</i>	0.00	0.00	0.00	0.00	0.67	0.00	0.00	0.00	0.00	0.00	0.20
<i>Halictus confusus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Megachile brevis</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.20	0.00	0.00
<i>Agapostemon virescens</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Augochlorella persimilis</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.29	0.00	0.00	0.10
<i>Melissodes comptoides</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Melissodes sp.</i>	0.00	0.00	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.00
<i>Sphecodes sp.</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

**Online Resource 3 (continued).**

	Latitude (decimal degrees) of each exotic-dominated site							Cumulative average abundance
	39.05	41.63	42.05	42.32	43.41	44.92	47.72	
<i>Lasioglossum sp.</i>	0.00	0.17	0.67	0.00	0.50	0.40	0.00	0.29
<i>Augochlorella aurata</i>	1.00	0.00	0.00	0.50	0.50	0.00	0.00	0.57
<i>Halictus ligatus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.63
<i>Melissodes communis</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.69
<i>Melissodes trinodis</i>	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.74
<i>Agapostemon sp.</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.79
<i>Halictus confusus</i>	0.00	0.00	0.00	0.00	0.00	0.60	0.00	0.82
<i>Megachile brevis</i>	0.00	0.17	0.00	0.00	0.00	0.00	0.00	0.85
<i>Agapostemon virescens</i>	0.00	0.00	0.00	0.50	0.00	0.00	0.00	0.88
<i>Augochlorella persimilis</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.90
<i>Melissodes comptoides</i>	0.00	0.33	0.00	0.00	0.00	0.00	0.00	0.92
<i>Melissodes sp.</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.94
<i>Sphecodes sp.</i>	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.96

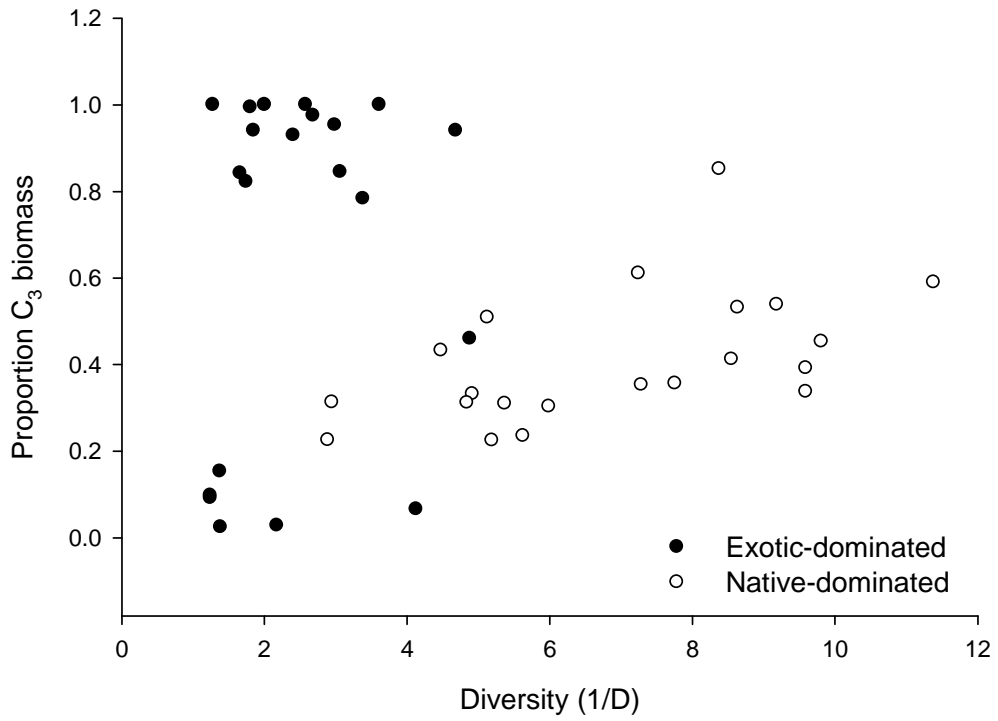
**Online Resource 4.** List of bee species and their relative abundances in native-dominated grasslands. Species are only included in the list if they were in the top 95% of species relative abundances when abundances were averaged across all exotic-dominated sites.

Species	Latitude (decimal degrees) of each native-dominated site						
	31.04	31.46	36.06	36.12	37.64	37.64	38.18
<i>Augochlorella aurata</i>	0.00	0.00	0.43	0.33	0.29	0.25	1.00
<i>Lasioglossum sp.</i>	0.00	0.00	0.57	0.21	0.29	0.00	0.00
<i>Agapostemon virescens</i>	0.00	0.00	0.00	0.00	0.00	0.25	0.00
<i>Ceratina sp.</i>	1.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Melissodes sp.</i>	0.00	1.00	0.00	0.00	0.00	0.00	0.00
<i>Hylaeus affinis</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Augochlorella persimilis</i>	0.00	0.00	0.00	0.44	0.00	0.50	0.00
<i>Sphecodes sp.</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Anthophorula Sp.</i>	0.00	0.00	0.00	0.00	0.43	0.00	0.00
<i>Halictus confusus</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00

**Online Resource 4 (continued).**

Species	Latitude (decimal degrees) of each native-dominated site					Cumulative average abundance
	39.05	41.69	42.04	42.41	43.40	
<i>Augochlorella aurata</i>	1.00	0.00	0.03	0.00	0.00	0.28
<i>Lasioglossum sp.</i>	0.00	0.11	0.03	0.00	0.25	0.40
<i>Agapostemon virescens</i>	0.00	0.05	0.22	0.75	0.00	0.50
<i>Ceratina sp.</i>	0.00	0.00	0.00	0.00	0.00	0.59
<i>Melissodes sp.</i>	0.00	0.00	0.00	0.00	0.00	0.67
<i>Hylaeus affinis</i>	0.00	0.11	0.08	0.25	0.50	0.75
<i>Augochlorella persimilis</i>	0.00	0.00	0.00	0.00	0.00	0.83
<i>Sphecodes sp.</i>	0.00	0.63	0.14	0.00	0.00	0.89
<i>Anthophorula Sp.</i>	0.00	0.00	0.00	0.00	0.00	0.93
<i>Halictus confusus</i>	0.00	0.00	0.06	0.00	0.25	0.95

**Online Resource 5:** The relationship between species diversity and proportion of C<sub>3</sub> species biomass for native and exotic grassland sites sampled across the latitudinal gradient.



**CHAPTER 5: PHENOLOGY AND NICHE PARTITIONING DIFFER BETWEEN  
NOVEL, EXOTIC- AND NATIVE-DOMINATED GRASSLANDS FOR PLANTS, BUT  
NOT FOR POLLINATORS**

A paper to be submitted to an international journal

Leanne M. Martin and Brian J. Wilsey

**Abstract**

The temporal dynamics of ecosystem functioning and services are largely regulated by seasonal patterns, or phenologies, of the Earth's biota. Recent evidence suggests that exotic-dominated systems may exhibit altered phenologies relative to native-dominated systems. However, whether dynamics of plants and pollinators differ between existing novel, exotic-dominated ecosystems and native-dominated systems remains poorly understood. We tested whether exotic- and native-dominated grassland systems differed within a growing season for plant production, species and functional diversity, and pollinator abundances and diversity in the Northern Great Plains tallgrass prairie region, Iowa, USA. We found that niche overlap and some aspects of phenology in exotic-dominated systems were different from native systems for plants, but not for pollinators. Exotic dominated grasslands peaked earlier in biomass production, and exhibited lower levels of species and functional diversity, including higher levels of proportion of C<sub>3</sub> biomass, than native grasslands. These results were related to higher levels of temporal niche overlap in exotic grasslands. Surprisingly, bee pollinator communities did not significantly differ between exotic and native grasslands despite higher forb:grass ratios in native sites, implying that factors other than exotic-native status may be important in structuring pollinator communities in highly fragmented landscapes. Our results imply that phenological differences between species in novel and native grasslands can have important consequences to

diversity and ecosystem functioning, and that exotic or native status of plant species should be considered in future studies of phenology.

### **Introduction**

The temporal dynamics of ecosystem functioning and services across the globe are largely regulated by seasonal patterns of the Earth's biota. Specifically, differences in species' phenologies, or timing of biological events such as growth, reproduction, and senescence, are important for regulating timing of peak primary production, flowering events important for pollinators, and elemental cycling (Morissette et al. 2009, Peñuelas et al. 2009).

The importance of species' phenologies has stimulated increased concern over the impacts of global change on phenological processes. Most of the research on this topic has focused on impacts of global climate change on phenology given the clear link between seasonal temperature and precipitation regimes on the timing of biological events (e.g. Cleland et al. 2006, Cleland et al. 2007, Inouye 2008, Lane et al. 2012). For example, climate warming can induce earlier green-up and flowering dates of plant species (Cleland et al. 2012). Earlier plant phenology in turn should theoretically lead to ecological mismatches between plant species and higher trophic levels, such as their pollinator mutualists, if they do not respond similarly to warming (e.g., Kudo and Ida In Press, but see Hegland et al. 2009, Bartomeus et al. 2011, Rafferty and Ives 2011).

Humans have induced other global changes that may impact timing of biological events by altering over three-quarters of the Earth's ice-free terrestrial surface (Hobbs et al. 2006). One major consequence of anthropogenic alteration is that exotic-dominated ecosystems containing non-native species with potentially novel species interactions now exist on nearly one-third of

the Earth's surface (Kulmatiski 2006, Ellis 2011, Wilsey et al. 2011). Despite the extensive alteration of Earth's biosphere by exotics, however, they have not been considered as a driver of phenology changes until very recently (Willis et al. 2010, Wilsey et al. 2011, Fridley 2012).

Evidence suggesting that exotic-dominated systems may exhibit altered plant phenology relative to native-dominated systems is accumulating. Fridley (2012) found altered phenology between exotic and native species in deciduous forests, where exotic species exhibited extended leaf phenologies compared to native species. Wilsey et al. (2011) found that experimental grassland plots planted with exotic species mixtures greened up two weeks earlier and exhibited weakened and altered inter-correlations among key phenology variables compared to plots planted with native species. Surprisingly, the altered phenology of exotics was consistent across irrigation treatments, which were conducted as a proxy for changes in precipitation associated with climate change (Wilsey et al. 2011). Furthermore, Wilsey et al. (2011) found that higher levels of temporal niche overlap among plant species (i.e., higher levels of species synchrony over time) contributed to diversity declines in experimental exotic compared to native communities. Martin et al. (Chapter 4) also found that strong temporal niche partitioning in an important measure of functional diversity,  $C_3:C_4$  proportions, was lower in experimental exotic compared to native communities. These results suggest that exotic communities lack the temporal functional complementarity that is important in diversity-productivity and diversity-stability relationships (Tilman et al. 1997, Isbell et al. 2011).

Studies of climate change effects on phenology often consider whether mismatches are occurring between mutualistic species from different trophic levels, such as plant and pollinator species (Bartomeus et al. 2011, Rafferty and Ives 2011, Kudo and Ida *In Press*). However, impacts of exotics, which can also exhibit altered phenology, on multiple trophic levels in



existing landscapes remain largely unexplored. In particular, we have a poor understanding of how multiple trophic levels are influenced by temporal dynamics in exotic- versus native-dominated systems (Wolkovich and Cleland 2011). We asked whether systems dominated by exotic versus native species exhibit altered ecosystem and community dynamics of plant and bee pollinator communities over a growing season. We compared plant biomass and aboveground net primary productivity, plant species diversity and richness, and two aspects of plant species functional diversity, including forb:grass ratios and  $C_3:C_4$  proportions, between three exotic and three native dominated grasslands in the Northern Great Plains tallgrass prairie region, Iowa, USA, over a growing season. Proportion of  $C_3/C_4$  species biomass is an important measure of functional diversity that can impact ecosystem functioning (Martin et al. Chapter 4), and higher forb:grass ratios should favor pollinators if they are structured by floral resources associated with forbs. We also compared pollinator (bee) community dynamics, including abundance, richness, diversity, and species composition between grassland types, and further considered whether temporal dynamics of pollinator communities were related to plant community dynamics. Finally, we tested whether temporal niche overlap in species composition of plants differed between exotic- and native-dominated sites, and whether any difference was also apparent in bee communities. We predicted that exotic dominated communities would exhibit altered levels of plant productivity and lower diversity than native-dominated grasslands, and that temporal niche overlap would be higher for plant species in exotic- than native- dominated sites (Wilsey et al. 2011, Martin et al. Chapter 4). Finally, we predicted that if pollinator communities were structured mainly by plant communities within the grasslands and not by other factors (e.g., landscape context), then pollinators would exhibit similar trends as plant diversity and temporal niche overlap that were found in exotic and native dominated grasslands.

## Methods

### *Study Sites*

Three native- and three exotic- dominated grasslands were sampled multiple times during the 2010 growing season in the Northern Great Plains tallgrass prairie region, Iowa, USA, to test whether temporal dynamics of species and functional diversity, niche overlap, and ecosystem services differed between native- and exotic-dominated grasslands. This region is an ideal system to test for differences in temporal dynamics between exotic- and native-dominated systems because less than 0.01% of the original tallgrass prairie remains as unplowed or unconverted remnants in the state of Iowa (Samson and Knopf 1994), and the remaining remnants are dispersed across the state in small fragments (Wilsey et al. 2005) with exotic species prevalent across the landscape. Point intercept sampling (see below) confirmed that sites did not overlap in proportion of exotic species (exotic sites 91 – 100% exotic, mean 95%; native sites 6 – 21% exotic, mean 14% on the last sampling date). Native-dominated sites were all unplowed prairie remnants in the Iowa State Preserve System and included Sheeder Prairie State Preserve (Sheeder, 41°41'N, 94°35'W, 10 ha), Liska-Stanek State Preserve (Liska, 42°24'N, 94°13'W, 7.5 ha), and Richard W. Pohl Memorial Preserve at Ames High Prairie (Pohl, 42°02'N, 93°38'W, 1.5 ha). One (Liska) and one-half (Sheeder) of the native sites were burned in early spring 2010 prior to green-up, so dormant spring burning is confounded with native status for half of the native sites. Both burned and unburned sites represent a common and realistic range of conditions of intact native remnants because they are burned approximately every three years in the spring (Howe 2002). In contrast, exotic sites are not managed by burning. Exotic sites included grasslands within Montieth Wildlife Management Area (Montieth, 41°41'N, 94°35'W, 3.5 ha), Boone Forks Wildlife Management Area (Boone,

42°19'N, 93°56'W, 2 ha), and Iowa State University's Ruminant Nutrition Research Station (ISU, 42°03'N, 93°41'W, 1.5 ha). All exotic-dominated sites were unplowed for at least fifteen years or were never plowed, and had various haying and grazing histories. None of the native or exotic sites were grazed or hayed during the year of the study. Pohl, Liska, ISU and Boone are all located in the Des Moines Lobe geologic region, and Sheeder and Montieth are located in the Southern Iowa Drift Plain. Annual precipitation at all sites has a unimodal distribution, with peak precipitation in May – June, and averages approximately 820 mm year<sup>-1</sup>.

### *Sampling Design and Methods*

We measured temporal dynamics of plants and bees at the site level over one growing season. Plants and bees were sampled four times at each site during the 2010 growing season (May, July, August, and September) such that one exotic and one native site were sampled on the same day (except one native and exotic pollinator sample on the first sample date). The first sample date was used only to calculate bee variables and temporal niche overlap for plants and bees (see below).

Plant species richness, diversity, forb:grass ratios, and proportion of C<sub>3</sub> biomass were estimated at each site using data from a non-destructive point intercept sampling technique, which is highly correlated with biomass (Wilsey et al. 2011). All relative abundance measures using the point-intercept technique are therefore considered to be relative biomass estimates. Species abundances were estimated by dropping 1-m long point intercept pins at each of the four corners of a 20 x 50-cm frame at 25 randomly established 3 x 5 m permanent plots within each site (for 100 total pin drops at each site per time). We counted the number of times pins hit each species, allowing multiple hits per species. Points were raised to contact plants greater than 1-m

tall in some cases. Species richness was measured as the number of species hit by all pins during each sampling time at each site. Species diversity was calculated using Simpson's diversity ( $1/\sum p_i^2$ ) by aggregating abundances of all species within each site for each sampling time, where  $p_i$  is the relative abundance of a species within a site (species hits/total number of hits). Species were designated as either native or exotic (Eilers and Roosa 1994) and the proportion of exotic species abundance was estimated by dividing the number of exotic species hits by total number of hits. Species from the genus *Carex* were combined. Forb:grass ratios were estimated by dividing the number of forb and subshrub hits by the number of grass hits, and are thus expressed as a quotient. Photosynthetic mode was assigned to species using Waller and Lewis (1979) and proportion of C<sub>3</sub> biomass was measured as the number of times pins hit C<sub>3</sub> species divided by the total number of hits at each site.

Aboveground biomass and aboveground net primary productivity (ANPP) were estimated using clipped biomass plots from each site. Above-ground biomass was clipped to 2-cm in a 20 x 50 cm quadrat during each time period in five permanent, randomly selected plots. Biomass was sorted into live (at least some green present) and standing dead (green absent) components, and biomass and standing dead were dried at 65° C for 48 hours to constant mass and weighed. ANPP was estimated using the sum of positive increments in live biomass (Scurlock et al. 2002).

Bees were sampled at each site on each sampling date by randomly placing 8 each of blue, white and yellow (24 total) 3.25 oz. Solo brand cups 5 meters apart in a permanent 120-m transect in the middle of each site. Pan traps were filled with a mixture of water and regular Dawn dish soap (Westphal et al. 2008), and blue and yellow traps were painted with a mixture of blue or yellow fluorescent paint and Silica Flat Paint (Guerra Paint and Pigment, New York, New York, USA). Pan traps were placed in an opening in the canopy to maximize visibility.

Pan traps were deployed for a comparable average number of hours in native and exotic sites per time period (mean 6.3 hours in native and 7.3 hours in exotic per time period). Specimens from all traps within a site and sampling time were combined, and were stored in 70% ethanol until they were dried and pinned. All bee specimens were identified to species when possible using the Discover Life key (Ascher and Pickering 2012), or to the lowest taxa or to morphospecies when species identification was not possible. Some species within the genera *Lassioglossum* and *Sphecodes* were potentially combined due to difficulty separating species. Bee abundances were calculated by summing total number of specimens, bee taxonomic richness was calculated as the total number of taxa, and bee diversity was calculated for taxa using Simpson's Diversity ( $1/D$ ) in each site for each sampling time. We also combined all four samples from each site to generate cumulative bee abundance, richness and diversity estimates for the entire year, and to determine whether species composition for the year differed between grassland types.

We compared temporal niche overlap for plant species composition between exotic and native grasslands by measuring levels of similarity in species composition between sampling dates (three time periods: May to July, July to August, and August to September) within grasslands. We used the Bray-Curtis similarity index,  $BC = 1 - (\sum |p_{ij} - p_{io}|/2)$ , where  $p_{ij}$  is relative abundance ( $p$ ) of a species ( $i$ ) in the initial month ( $j$ ) for that time period and  $p_{io}$  is the relative abundance ( $p$ ) of the same species ( $i$ ) in the next month ( $o$ ) for that time period within the grassland (Wilsey et al. 2011). Therefore, this index calculates and sums across all absolute differences in relative abundances of each species between sampling dates. High similarity values for a particular time period are therefore indicative of high levels of species synchrony, or high temporal niche overlap in species composition (Wilsey et al. 2011). We then conducted the same analysis using bee species composition data.

### *Soil Covariates*

We standardized sampling by choosing an exotic site for every native site that was in the same geologic region and within a reasonable distance such that precipitation and temperature differences would be minimized between native-exotic pairs. To further take soil variation into account, one 10-cm soil core was taken in each biomass plot, and the five cores from each site were homogenized and analyzed for soil texture (percent sand, silt, and clay) to generate one site-level estimate. We then conducted a Principal Components Analysis (PCA) on all soil texture variables and used the principal component scores from the first axis, which explained 85% of the variation in soil texture, as a covariate in all analyses. Percent clay was negatively correlated with percent silt and sand on the first axis.

### *Statistical Analyses*

We analyzed each variable using a mixed model repeated measures ANCOVA (Proc Mixed in SAS) and Type III SS to assess whether temporal trends differed between native- and exotic-dominated grasslands (Littell et al. 2002). Response variables for plants included species richness, diversity, biomass, ANPP, proportion C<sub>3</sub> biomass, forb:grass ratios, or temporal niche partitioning. Response variables for bees included bee abundances, bee taxonomic diversity, bee taxonomic richness (both over time and cumulative estimates), or temporal niche partitioning. Explanatory variables included the first soil principal component as a covariate, as well as origin (i.e., native or exotic), time, and time by origin interactions. Site(origin) and time x site(origin) were included as random terms, and we used the Satterthwaite degrees of freedom approximation. The random site(origin) covariance parameter was estimated to be zero for ANPP; in that case that parameter did not contribute to the degrees of freedom. Site area was

added as an additional covariate to the univariate test of differences in plant species richness between exotic and native sites. Data were *ln*-transformed when necessary to improve normality (biomass, ANPP, and number of bees), and significance was assigned at the  $\alpha = 0.05$  level. Untransformed least-squared means are reported unless otherwise indicated. Finally, we conducted a perMANOVA on species composition of bees to determine whether species composition differed between grassland types. We used the *adonis* function in the *vegan* package 2.0-2 in R version 2.13.2 to conduct the perMANOVA.

## Results

Soils did not differ between grassland types (first principal component scores  $F_{1,4}=3.9$ ,  $P=0.12$ ), indicating that soils did not affect results (Table 1). Aboveground biomass peaked earlier in exotic-dominated grasslands, but exotic sites had 37% lower aboveground net primary production by the end of the growing season (Table 1, Figure 1a). Plant species richness and diversity were consistently (62% and 77%, respectively) lower in exotic-dominated systems (Table 1, Figure 1b, c). Proportion of C<sub>3</sub> biomass was significantly higher in exotic-dominated sites overall. There was a marginally significant difference between grassland types over time, with proportion of C<sub>3</sub> biomass being 54% higher in exotic sites early in the growing season and twice as high in exotic sites by mid-September (Table 1, Figure 1d). Forb:grass ratios were 84% lower in exotic dominated sites ( $P = 0.06$ ), and the difference did not change over time (Table 1, Figure 1e).

We did not detect any differences in bee abundances, richness, or diversity between exotic and native grasslands, either over time or when considering cumulative bee variables for the entire year (Table 2, Figure 2). Bee abundances and richness declined over time in both

exotic and native grasslands (Table 2, Figure 2). Bee species composition did not differ between native and exotic grasslands (PerMANOVA,  $F_{1,3} = 1.4$ ,  $P = 0.68$ ).

Bray-Curtis similarity (temporal niche overlap) differed between exotic and native grasslands for plants, but not for bees. Bray-Curtis similarity for plant species composition between sample dates was consistently high in exotic-dominated sites. However, the difference in temporal niche overlap between exotic- and native-sites was greatest early in the growing season, between May and July, when overlap was 46% higher in exotic than native sites (plants overlap, origin  $F_{1,3}=5.6$ ,  $P=0.10$ , time  $F_{2,8}=3.8$ ,  $P=0.07$ , origin x time  $F_{2,8}=7.1$ ,  $P=0.02$ ) (Figure 3). *Bromus inermis* Leysser comprised the majority of the relative biomass in exotic-dominated sites, with a minimum of 68% (May) and maximum of 77% (September). *Poa pratensis* (L.) was the second-most abundant species in exotic sites, with a maximum of 23% (May) and minimum of 12% (September). Bray-Curtis similarity for bees declined over time in both exotic and native sites, but it did not differ between grassland types (bees overlap, origin  $F_{1,3}=0.11$ ,  $P=0.76$ , time  $F_{2,8}=14.4$ ,  $P<0.01$ , origin x time  $F_{2,8}=0.3$ ,  $P=0.75$ ) (Figure 3).

## Discussion

We found that niche overlap and some aspects of phenology in novel, exotic-dominated systems were different from native systems for plants, but not for pollinator communities. Overall, exotic-dominated grasslands exhibited more constant levels of functional diversity and plant biomass, which peaked earlier, compared to native grasslands. Lower levels of ANPP were associated with consistently lower levels of species richness and diversity, and higher levels of proportion of C<sub>3</sub> biomass in exotic-dominated grasslands. These results suggest that exotic grasslands have lost important levels of functional complementarity in plant communities



compared to native grasslands. Furthermore, although species and functional diversity, including forb:grass ratios, were consistently lower in exotic-dominated grasslands, surprisingly we found no evidence to suggest that pollinator diversity trends differed between grassland types. Consequently, bee communities did not follow plant community dynamics over the growing season. Therefore, although exotic-native status was important to diversity and temporal dynamics of plant production, our results imply that factors other than plant communities could also be important in structuring pollinator community dynamics in these grasslands.

The differences we observed between grasslands in plant production, diversity, and proportion of C<sub>3</sub> species biomass reflected the high plant species synchrony (high temporal niche overlap) in exotic grasslands versus the lower temporal niche overlap in native grasslands. Specifically, the trend in decreasing proportion of C<sub>3</sub> biomass in native grasslands was related to lower niche overlap across time, particularly when species were transitioning from dominance by C<sub>3</sub> to C<sub>4</sub> species biomass. Thus, this niche partitioning was consistent with higher levels of primary production that we did not observe in exotic grasslands. Although one and one-half of our native sites experienced a dormant spring burn, which could increase production, the burned site had the lowest level of ANPP among native grasslands. This suggests burning was not the sole factor driving differences in production. However, future research on burning in exotic and native sites could address whether ANPP would differ with burning between grassland types. Foster et al. (2009) found that when C<sub>4</sub> species were added to C<sub>3</sub> dominated pasture grasslands in Kansas, biomass production increased. Thus, our results are consistent with evidence suggesting that lower temporal niche overlap associated with shifts in C<sub>3</sub>/C<sub>4</sub> species composition over the growing season are important to diversity/productivity relationships (Tilman et al.

1997, Wilsey et al. 2011, Martin et al. Chapter 4). Furthermore, our results are highly consistent with experimental results comparing temporal dynamics of exotic and native communities in a common garden setting (Wilsey et al. 2011, Martin et al. Chapter 4), suggesting that experimental differences translate well to larger scale field conditions. Experimental results indicate that these altered temporal dynamics are at least partially caused by exotic species themselves (Martin et al. Chapter 4).

Exotic-dominated communities with lower niche partitioning may not always have lower productivity, however. For example, low diversity exotic communities that were dominated by  $C_4$  species had reduced temporal niche partitioning, but exhibited higher levels of productivity in the southern tallgrass prairie (Wilsey et al. 2011, Martin et al. Chapter 4). Exotic-dominated sites with high levels of diversity in Hawaii also had higher levels of productivity than native-dominated sites (Mascaro et al. 2012). These results add to the growing body of evidence that exotics are different from native-dominated communities in temporal niche partitioning and diversity-productivity relationships (Wilsey et al. 2009, Wilsey et al. 2011, Isbell and Wilsey 2011, Martin et al. Chapter 4). Understanding when exotics increase or decrease diversity and productivity is an important question for future research.

The altered temporal dynamics of  $C_3/C_4$  proportions that we observed in these existing grasslands indicate that novel and native grasslands differ in functional diversity. Dominant photosynthetic mode is an important aspect of ecosystem functioning because  $C_3$  species differ in multiple, important ways from  $C_4$  species, including season of maximum growth, as well as water, carbon and nitrogen use efficiency (Teeri and Stowe 1976, Tieszen et al. 1997, Sage and Monson 1999). Climate change, therefore, may not be the only factor associated with phenology differences that can impact ecosystem functioning. Exotic-dominated systems also

should be considered when studying altered phenology and ecosystem functioning (Wilsey et al. 2011, Fridley 2012, Martin et al. Chapter 4).

The declines in bee pollinator numbers, richness, and temporal niche partitioning in both exotic and native grasslands over the growing season imply that seasonal trends were more important for pollinator communities than exotic-native status in these grasslands. Surprisingly, this trend occurred despite higher forb:grass ratios in native sites, and it did not appear to be consistent with any of the diversity or temporal niche partitioning trends found in plant communities. If the dormant spring burning impacted pollinator abundances in some of the native sites, we would have expected lower, and not higher, abundances of pollinators immediately after burning with an increase later in the growing season, but this was not the case (Potts et al. 2003). However, burning impacts on bee pollinator communities in both exotic and native systems should be studied in the future to decouple the confounding that can occur with burning native but not exotic grasslands. Understanding bee pollinator community dynamics is challenging due to very high levels of variation across space and time (Williams et al. 2001), so measuring their dynamics across longer time frames and management regimes could be crucial.

Our pollinator results point strongly to the need to test alternative hypotheses determining overall bee pollinator numbers, diversity, and temporal trends in this region in the future (see also Martin et al. Chapter 4). One possible interpretation of our pollinator results, for example, is that exotic-dominated systems have not altered pollinator communities. However, Burkle et al. (2013) found extremely high rates of pollinator species declines in the last 120 years in forest understory communities in Illinois. Although we sampled grasslands and not woodlands, we hypothesize that exceedingly high rates of fragmentation in the tallgrass prairie (Samson and Knopf 1994) may also be associated with high rates of extinction or extirpation of bee species in

the region, which has limited our ability to detect differences between grassland types. Perennial grasslands in general might also provide important habitat (e.g., nesting sites) for remaining pollinator species, which are able to travel to find resources in fragmented landscapes (Morandin et al. 2007). Furthermore, although floral resources, which we did not measure directly, are often a focus of research on pollinator distributions, we know relatively little about utilization of landscape factors by bee communities, or other factors such as disease, that could be important in shaping pollinator communities over a growing season in highly fragmented systems (Hegland et al. 2009). For example, recent evidence also suggests that mismatches between plants and pollinators due to climate warming may be less prominent than previously thought, which implies that pollinator dynamics can also be shaped by other factors such as climate (Hegland et al. 2009, Bartomeus et al. 2011, Rafferty and Ives 2011). The majority of pollinators we found in these grasslands were solitary bees, and their biology as a group is less well studied than honeybees despite their importance to pollination services (Winfree et al. 2007).

Finally, although plants provide pollinators with floral resources, pollinators are also important to plant fitness and plant communities. For example, recent studies found that lower levels of pollinator diversity can be detrimental to not only plant species reproductive success (Albrecht et al. 2012), but also to plant species diversity (Fontaine et al. 2006). If exotic and native grasslands differ in plant diversity but not in pollinator communities or diversity, future studies should consider the implications of these pollinator community trends on varying levels of plant species diversity.

*Conclusions*

Our results have several important implications. First, sampling only once per year to compare exotic and native sites may be problematic when phenology differs as it did here. Exotic sites exhibited earlier peak biomass than native sites in our study. If sites were sampled only once early in the season, results could differ dramatically from results in which sites were sampled only once later in the growing season. Diversity differences, however, may not be as variable. Thus, conclusions on whether exotic or native sites differ from one another for some measures could shift depending on sampling date. We suggest that, when possible, sampling two or more times during the growing season when conducting exotic-native comparisons could generate a more realistic estimate of differences among exotic and native systems. Second, differences between exotic and native grasslands can have important consequences to biodiversity and functional diversity. Our results indicate that under current management scenarios, the replacement of native- by exotic-dominated systems has resulted in loss of plant species and functional diversity and ANPP in the northern tallgrass prairie region. Increases in exotic plant species abundance in novel ecosystems, which are common across the globe, should be considered as a reason phenology and niche partitioning of plant species is changing (see also Wilsey et al. 2011, Fridley 2012). Finally, the surprisingly inconsistent trends of pollinator communities with plant dynamics implies that pollinator communities are not solely structured by local plant communities within fragmented grassland landscapes. Understanding the relative importance of other factors structuring pollinator communities, therefore, could assist with efforts to avoid further loss, and perhaps increase levels, of pollination services.

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**Table 1.** Repeated measures ANCOVA results ( $F$ ;  $P$  values) for tests of differences between native and exotic sites and interactions with time for each ecosystem service. The PC1 covariate is the first principal component axis explaining 85% of the variation in soil texture across sites. Area (in parentheses) was used as a covariate only for plant species richness; all other analyses did not include area as a covariate. Values significant at the  $\alpha \leq 0.05$  level are in bold, and values significant at the  $\alpha \leq 0.10$  level are indicated with an asterisk.

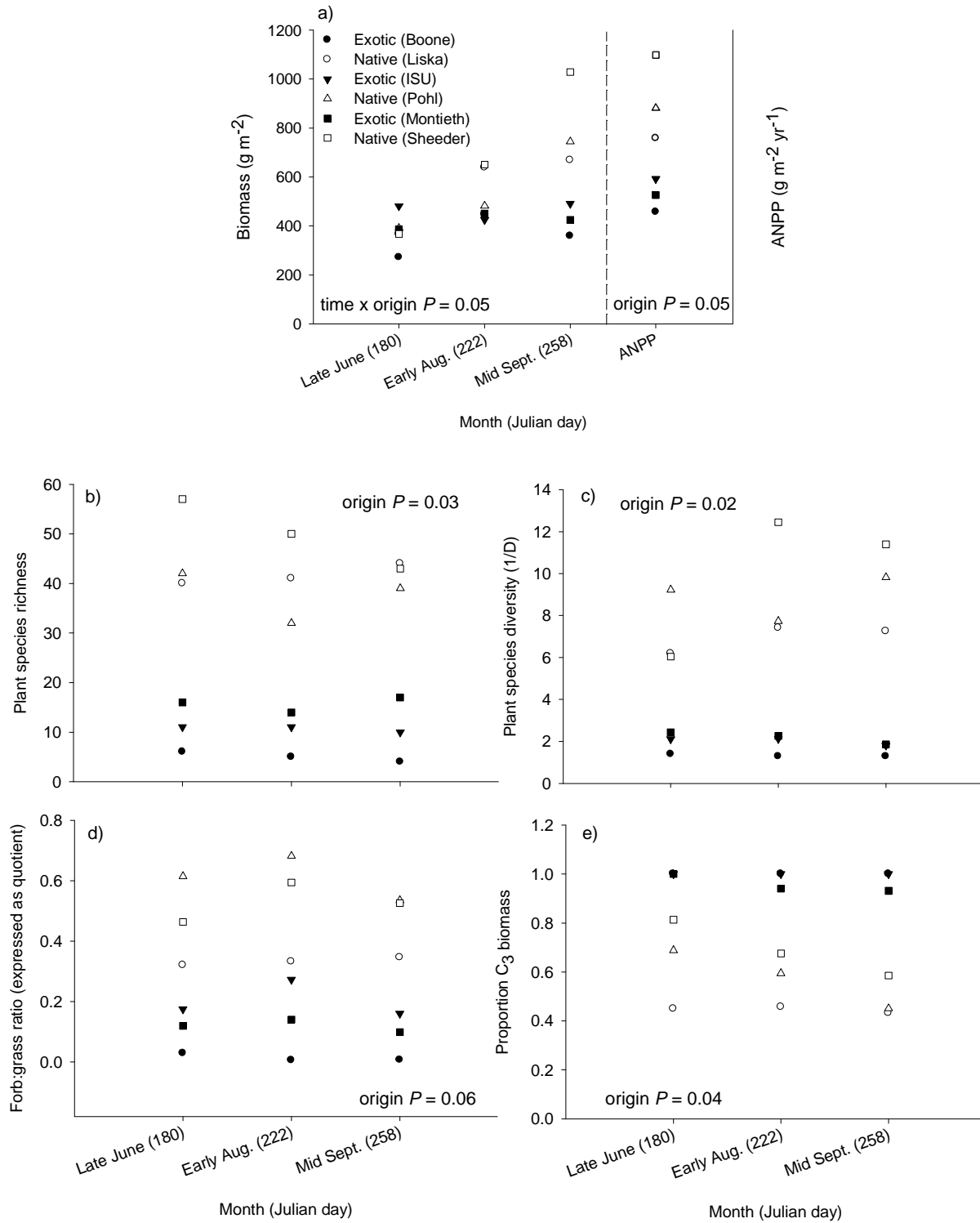
Source	d.f.	Species Diversity			Functional Diversity	
		Biomass	Simpson's Diversity	Richness	Proportion C <sub>3</sub> biomass	Forb:Grass ratio
PC1	1	1.2; 0.35	0.1; 0.81	1.7; 0.32	0.0; 0.99	0.3; 0.99
(Area)	(1)			0.0; 0.89		
Origin	1	1.4; 0.32	19.4; <b>0.02</b>	28.7; <b>0.03</b>	12.6; <b>0.04</b>	8.4; 0.06*
Error	3(2)					
Time	2	9.3; <b>&lt;0.01</b>	0.9; 0.44	1.1; 0.37	5.6; <b>0.03</b>	3.3; 0.09*
Time x Origin	2	4.6; <b>0.05</b>	1.4; 0.30	0.6; 0.59	3.2; 0.10*	0.3; 0.76
Error	8					

Plant richness main error d.f. = 2; all other main error d.f. = 3.

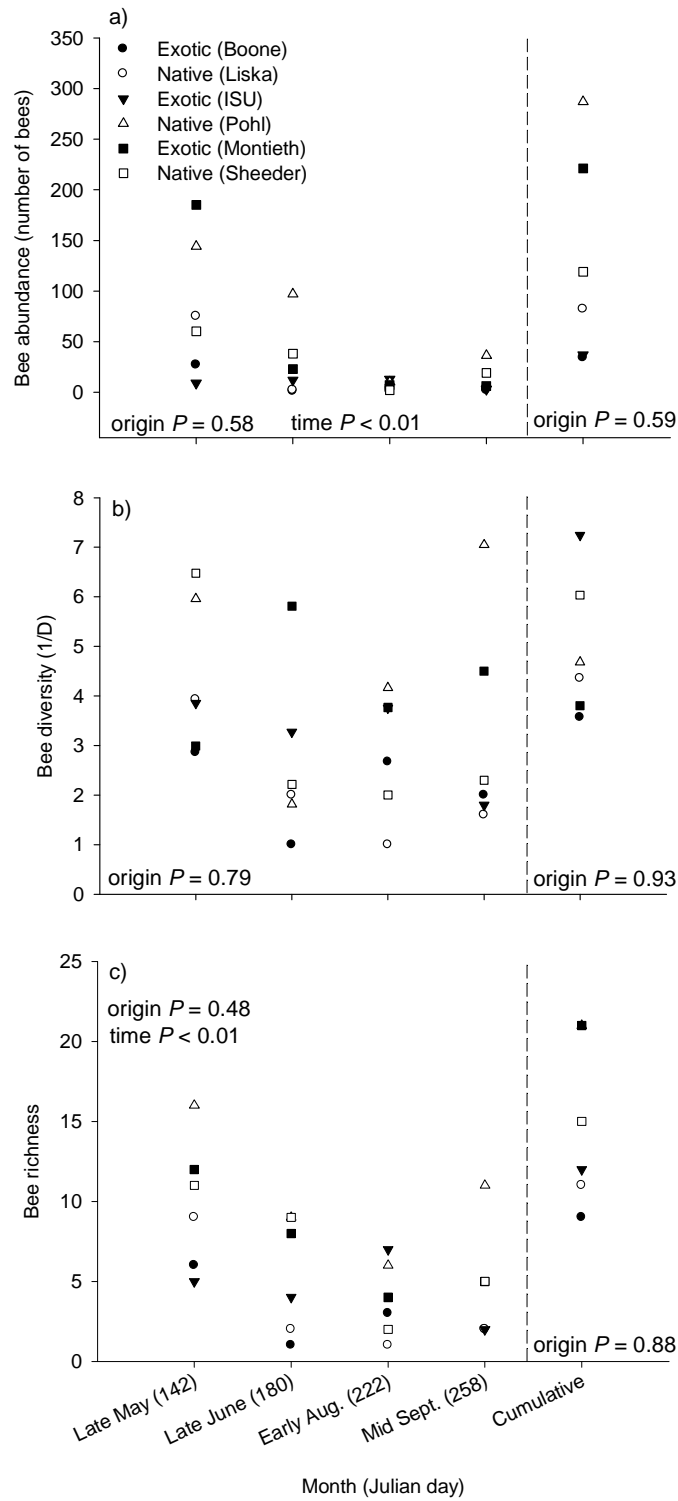
**Table 2.** Repeated measures ANCOVA results (*F*; *P* values) for tests of differences between native and exotic sites and interactions with time, as well tests for differences when data across the season was combined, for bee pollinator variables. The PC1 covariate is the first principal component axis explaining 85% of the variation in soil texture across sites. Values significant at the  $\alpha \leq 0.05$  level are in bold.

Source	d.f.	Bee Abundance	Bee Diversity	Bee Richness	d.f.	Cumulative Bee Abundance	Cumulative Bee Diversity	Cumulative Bee Richness
PC1	1	0.0; 0.85	0.1; 0.81	0.2; 0.72	1	0.0; 0.85	0.0; 0.98	0.0; 0.93
Origin	1	0.4; 0.58	0.1; 0.79	0.7; 0.48	1	0.4; 0.59	0.0; 0.93	0.0; 0.88
Error	3				3			
Time	3	9.3; <b>&lt;0.01</b>	1.9; 0.19	11.1; <b>&lt;0.01</b>				
Time x Origin	3	2.2; 0.14	2.4; 0.12	2.5; 0.11				
Error	12							

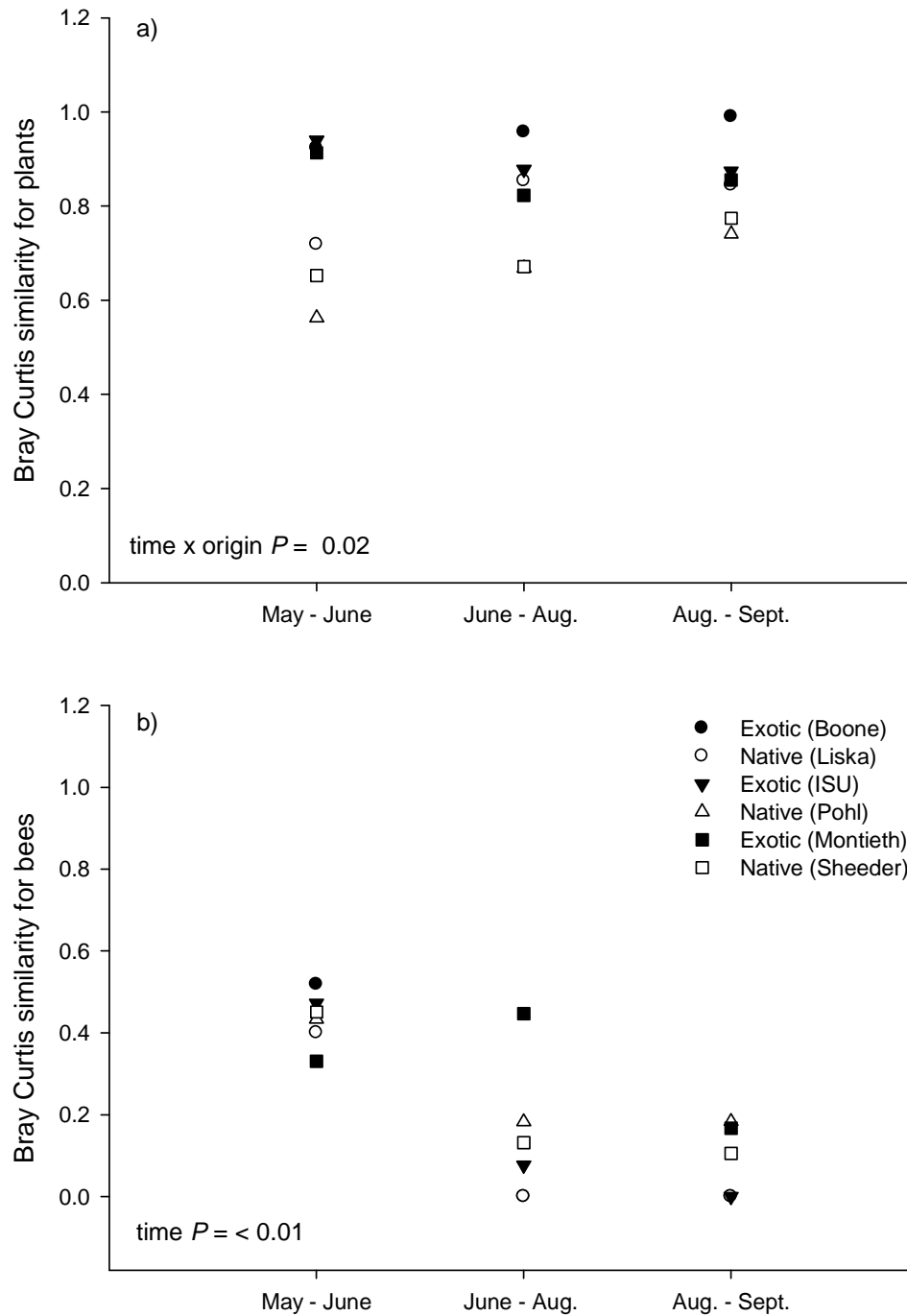
**Figure 1.** Differences in plant biomass and ANPP (a), species richness (b), diversity (c), forb:grass ratios (d) and proportion of C<sub>3</sub> biomass (e) between exotic- and native-dominated grasslands in the tallgrass prairie region, Iowa, USA.



**Figure 2.** Differences in bee abundance (a), and bee richness (b), and diversity (c), between exotic- and native-dominated grasslands in the tallgrass prairie region, Iowa, USA.



**Figure 3.** Temporal niche overlap for plant (a) and bee (b) species, where higher levels of Bray-Curtis similarity indicate higher levels of niche overlap (higher synchrony), between sampling dates in exotic- and native-dominated grasslands in the tallgrass prairie region, Iowa, USA.



## CHAPTER 6: GENERAL CONCLUSIONS

### Summary

Work from this dissertation examined how native species assemble after disturbance in an exotic-dominated context, and considered whether communities dominated by native versus exotic species exhibit differences in multiple community and ecosystem measures. We found that when community assembly history of native seeds was altered in a landscape with high exotic species propagule pressure, differences in timing and priority effects determined whether a more diverse, native community or an exotic-dominated community developed (Chapter 2). Specifically, more diverse, native-dominated communities developed when a 30-species seed mix was seeded in the spring, without priority effects, but low diversity, exotic-dominated communities developed when the seed mix was added in summer, or after an early-establishing species was seeded first (with priority effects). Differences in these plant communities resulted in differences in fire temperatures, fuel mass, and aboveground net primary productivity. This suggests that community assembly processes can contribute to changes in ecosystem effects. Furthermore, the alternate exotic and native states that developed were persistent in the face of a native species seed addition (Chapter 3). When we added native seeds to alternate states six and seven years after the communities developed, the seed addition did not shift the low diversity exotic state to a state with higher levels of native abundance or diversity. This occurred despite adding the native seeds to exotic states in this spring, which was when native states developed initially, and despite adding them to burned plots with an open canopy, which was expected to maximize recruitment. Thus, our results indicate that communities that appear random and idiosyncratic in field conditions may instead result from predictable community assembly processes (Drake 1991). Furthermore, exotic and native states can be persistent in the face of

native seed pressure. Our results suggest that restorations should maximize efforts to generate diverse, native plant communities during the earliest phases of restoration.

Alternate exotic versus native states also exist on landscapes, and they could differ in multiple community and ecosystem measures. We tested for differences between exotic and native-dominated grasslands along a latitudinal gradient in the tallgrass prairie region, and in an ongoing, controlled field experiment. We found that exotic- versus native-dominated states exhibit management tradeoffs, where exotic-dominated ecosystems had lower levels of species diversity and richness, but slightly higher levels of forage quality, including tissue N concentrations (Chapter 4). Furthermore, we found important differences in functional diversity. Specifically, native grasslands exhibited a linear increase in proportion of C<sub>3</sub> biomass with increasing latitude, which is consistent with other studies (e.g., Epstein et al. 1997). In contrast, exotic-dominated grasslands were strongly dominated by C<sub>4</sub> species at lower latitudes and by C<sub>3</sub> species at higher latitudes, with a sharp transition at around 34 – 36 degrees. The pattern we found in exotic-dominated grasslands has never been documented before. Evidence from our experimental results indicated that differences in functional diversity could be at least partially due to exotic species themselves rather than to covarying factors.

Finally, we found that niche overlap and some aspects of phenology differed between exotic and native grasslands for plant communities, but not for bee pollinator communities in central Iowa, USA (Chapter 5). Exotic-dominated grasslands had earlier peak biomass and lower levels of ANPP and species and functional diversity than native grasslands. The lower ANPP and higher levels of proportion of C<sub>3</sub> biomass in exotic grasslands overall, and particularly later in the growing season, reflected the high levels of temporal niche overlap in exotic compared to native grasslands. Surprisingly, pollinator communities did not differ



between grassland types, and did not appear to be associated with temporal dynamics in plant communities. These results suggest that bee pollinator communities might be influenced by additional factors other than the local plant communities in these highly fragmented grasslands.

### **Future Research**

The work presented here elucidated several areas for future research. First, although evidence is mounting that assembly history processes are important in determining variation in species composition and diversity (Körner et al. 2008, Chase 2010, Fukami et al. 2010, Martin and Wilsey 2012) we need a better understanding of the repeatability of this process in field settings. Specifically, replicating community assembly history experiments over multiple years will advance our understanding of the variation associated with community assembly history (Chase 2003). This will allow us to ask whether the assembly history process takes place in a consistent manner across years, or if the increasing amount of variation in temperature and precipitation associated with climate change will produce more variable results over many years. Furthermore, conducting community assembly history experiments in the context of exotic-dominated ecosystems containing species that were different from those that were in our system will be important. Our study was conducted in a landscape that had high levels of perennial  $C_3$  species propagule pressure. At southern latitudes, however, where perennial exotic species are  $C_4$  dominated (Wilsey et al. 2011, Martin et al. Chapter 4), will spring seeding promote both exotic and native dominated states of  $C_4$  species? Overall, conducting community assembly history studies over multiple times and places would allow us to more completely understand how restoration projects can be enhanced early during the restoration process.

Although community assembly history processes resulted in high levels of beta diversity in our study, the beta diversity consisted of alternate states of native versus exotic communities. A question still unanswered that is important to ecological restoration of native species, therefore, is how to simultaneously increase high levels of alpha and beta diversity of native, and not exotic, species. Planting species in finer-sized patches holds promise for increasing invasion resistance in restored prairie communities (Yurkonis et al. 2011). An additional possibility for increasing alpha diversity of native species includes attempting to seed native plant species during times when they would naturally disperse. This would mimic a more “natural” community assembly history pattern. For example, Frischie and Rowe (2012) found that seeding early-maturing species right away when they would naturally disperse, rather than during a dormant season, increased establishment and diversity of restored prairie communities. Although this method may be time consuming during the initial restoration process, if this process is one key to producing diverse restorations, it could result in less effort later in the restoration process. Therefore, perhaps planting species in finer-sized patches, and when they would more naturally disperse based on their phenology, could increase local alpha diversity. Planting different mixes across locations in this manner may also increase beta diversity. Testing hypotheses such as these simultaneously could be a fruitful area of research.

The differences we found between native- and exotic-dominated landscapes implies that exotic ecosystems are providing higher levels of some ecosystem services, such as forage quality, but lower levels of diversity. Recent evidence suggests that low diversity, exotic-dominated ecosystems may also be more susceptible to a catastrophic shift to an alternate state (MacDougall et al. 2013). In cases where exotic-dominated systems exhibit lower diversity but provide higher levels of ecosystem services, however, managing for high levels of forage quality,

and thus low diversity, may be more desirable when managing cattle. Therefore, a logical direction for future research is to ask how to mitigate management tradeoffs associated with native versus exotic-dominated systems (Tallis et al. 2008). Specifically, can we manage systems that are used for haying or grazing purposes to simultaneously have high levels of species diversity and high levels of forage quality (Doll et al. 2009)? If native species maintain higher levels of species diversity than exotic species (Wilsey et al. 2009, 2011), then understanding how we can manage grasslands that contain a diversity of native species, and that have high forage quality, may be an important avenue for future research.

When comparing species diversity between exotic and native grasslands, we considered site-level diversity only, and found that species diversity was considerably lower in exotic-dominated grasslands (Chapter 4). However, whether beta diversity differs between exotic and native grasslands across the gradient will be an important question for future research. Specifically, does similarity in species composition decline more rapidly with distance across the gradient in exotic- than native-dominated grasslands, or vice-versa? Global biotic homogenization and the impact on biodiversity and ecosystems is an increasing concern (Olden et al. 2004). Studies testing questions of biotic homogenization have used floristic data (e.g., Qian and Ricklefs 2007), but we have the opportunity to test this hypothesis across a latitudinal gradient with species abundance data.

Finally, it is clear that we need a much better understanding of the factors that shape bee pollinator communities in highly fragmented landscapes such as the tallgrass prairie (Samson and Knopf 1994). Although plant-pollinator relationships may be more closely linked when looking at single species of pollinators, we found no differences in relationships between plant and pollinator communities in exotic and native grasslands, despite higher forb:grass ratios in

native sites. Assessing whether landscape factors surrounding exotic and native grassland sites are impacting pollinator communities in these fragmented landscapes could be an important first step. For example, if exotic grasslands are near areas with higher levels of forbs, such as in roadside ditches and anthropogenic dwellings, perhaps even exotic grasslands provide suitable nesting habitat for bees, which could travel to find suitable floral resources nearby (Morandin et al. 2007).

### **Conclusions**

Overall, results from this work imply that in situations when exotic-dominated, novel ecosystems are present, their effects should be considered when testing ecological theories in the field and when considering impacts of species on communities and ecosystems. Specifically, we found that testing community assembly history theory by altering timing and priority effects of native species seeds in a landscape with high exotic species propagule pressure altered beta diversity. However, rather than resulting in beta diversity among native species, we found that assembly history generated alternate exotic versus native states that were persistent over a long time frame that is relevant to restoration, even with native seed additions. Furthermore, we found that exotic-dominated grasslands differed in species and functional diversity, temporal niche partitioning, and in ecosystem measures, from the native-dominated systems that they have replaced. Rather than ignoring effects of multiple exotic species, they should be considered as an important component of future research on 1) the mechanisms generating variation in species composition and diversity across space and time, and 2) the ecosystem consequences of this variation.

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