Seasonal Shifts in Diversity and Composition of a Tallgrass Prairie Restoration Have Implications for Sampling Time

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ABSTRACT

Restorations change across the growing season. Because of this, the point in the season that a restoration is sampled may affect the conclusions reached based on the sample. In this study, we explore seasonal changes in a prairie restoration experiment in eastern Kansas and investigate how these changes affect observed composition, biodiversity, and the effects of seeding density treatment on the plant community based on when, and how completely, vegetation is sampled. Free State Prairie was established in 2014 to test the effects of forb seeding density on forb establishment, diversity, and restoration success. We compared absolute cover data collected in early June and early September 2019 to each other and to combined data. We found changes in both composition and biodiversity from early-to-late in the season. Sown forbs decreased in cover and richness, while sown grasses increased in cover and richness. Nonsown species did not change in cover but decreased in richness. Neither individual sample fully represented the overall composition or biodiversity of the community. We detected a significant negative effect of forb seeding density on diversity in June, and with combined data, but not in September. As sampling time can affect both broad patterns of composition and diversity and observed results of establishment and management techniques, sampling multiple times in a year will provide the fullest and most accurate picture of the community. When multiple samples are impractical, sampling time should be selected carefully based on the phenology of the restoration and the variables of interest.

Keywords: biodiversity, grassland, phenology, seeding density

Restoration Recap

- Regular vegetation monitoring is critical for evaluating restoration success and informing management, but many restoration projects are not sampled often enough to fully evaluate outcomes—even restorations that are monitored annually are often sampled only once per year.
- Because restorations change across the growing season, results of sampling are likely to change depending on when during the growing season a restoration is sampled.
- Ideally, restorations should be sampled multiple times in a year to most accurately assess restoration quality and the effectiveness of establishment and management techniques.
- If restorations are only sampled at one point in the year, the sampling time should be selected carefully based on the characteristics of the restoration and the questions being asked.

Though restoration often improves the diversity and function of degraded and destroyed ecosystems, restorations have rarely been documented to achieve levels of diversity comparable to intact reference ecosystems (Lockwood and Pimm 1999, Benayas et al. 2009), and restoration outcomes vary (Brudvig 2011). Given this, it is important to monitor restorations to accurately assess biodiversity and composition and determine the efficacy of establishment and management techniques. Unfortunately, restorations are often under-monitored or entirely un-monitored due to the time, cost, and knowledge of plant identification required to conduct vegetation surveys (Suding 2011, Dickens and Suding 2013, Barak et al. 2021). A survey of prairie restoration managers found that

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while most consider monitoring important for assessing whether restoration objectives are being met and informing management and seed mix design, they are not able to devote as much time to monitoring their restorations as they would like (Barak et al. 2021). Monitoring programs are often underfunded (Dickens and Suding 2013), and researchers are frequently working on multiple projects and must divide time and resources among them. Even when projects are monitored annually, they are often only sampled once per year, potentially limiting inferences that can be made from the data.

Accurately assessing diversity and composition is especially difficult in tallgrass prairie restorations as growing seasons are long and plant communities change continuously across the growing season. For example, C_3 species, such as most forbs and many non-native grasses, tend to green up and reach peak biomass earlier than C_4 species, such as most native prairie grasses, and many invasives tend to green up earlier than native species (Wilsey et al. 2011). Many factors, biotic and abiotic, contribute to restoration seasonality. Temperature, moisture, and sunlight, as well as insects and mycorrhizae, all change seasonally and affect the composition of plant communities (Buisson et al. 2017, Mandyam and Jumpponen 2008). Tallgrass prairies are strongly shaped by seasonal disturbance, both naturally occurring and as part of deliberate management. Since disturbances such as mowing, burning, and grazing occur at particular times in the season, niches in restorations are opened up seasonally. Seasonality in restorations is also affected by the choice of which species to seed, and many seed mixes specifically lack spring blooming species (Havens and Vitt 2016, Barak et al. 2021). Data collected at a single point in time are unlikely to fully represent the biodiversity and composition of a site. If the factors being studied disproportionately affect early or late-developing species, the overall results of the study could even be exaggerated or obscured. All of this makes it essential to understand how sampling time may influence observed biodiversity, composition, and capacity to detect the effects of experimental treatments or management interventions.

In this study we present results from Free State Prairie, a long-term tallgrass prairie restoration experiment in eastern Kansas established in 2014 to test the effects of forb seeding density on forb establishment, species composition and diversity, and restoration success. Tallgrass prairies are an especially diverse ecosystem, but tallgrass prairie restorations often have considerably lower biodiversity than prairie remnants, especially with respect to forbs, which make up most of the above-ground biodiversity in native prairies (Kindscher and Tieszen 1998, Polley et al. 2005, Martin et al. 2005). One possible explanation, among many, for the lack of forb diversity is seed limitation. Forbs tend to be seeded at much lower densities than grasses, both because forb seeds tend to be more expensive and because grasses are often prioritized for their capacity to grow and spread more quickly and to prevent soil erosion, improve soil quality, and prevent invasion by non-native species (Dickson and Busby 2009). If forbs are limited by seeding rate, the typical restoration seeding density may be too low for them to establish and persist, particularly in competition with more abundant grasses (Dickson and Busby 2009, Carter and Blair 2012, Goldblum et al. 2013, Jaksetic et al. 2018). In contrast, forbs may be limited by the availability of suitable microsites or niche space, in which case adding a higher density of seed would be ineffective.

Since the planting year of 2014, Free State Prairie has been sampled once every year in late August/early September. At this point in the season, most C_4 grasses are close to their peak, and many forb species are past their peak. In the study reported here, we explored the implications of sampling time by conducting a vegetation survey in early June 2019, before most native prairie forbs reach their peak abundance, in addition to the normal late-season survey. We address three main questions: 1) How do biodiversity and species composition change from early to late in the growing season? 2) What proportion of overall diversity is captured by early sampling vs late sampling? 3) Does sampling time influence our ability to detect the effects of manipulating forb seeding density on the community? The results provide information on how composition and biodiversity shift seasonally and evaluate which sampling times are most accurate for assessing composition and biodiversity of tallgrass prairie restorations.

Methods

Study Site and Experimental Design

Free State Prairie was established spring 2014 as an experimental tallgrass prairie restoration at Free State High School, Lawrence, Kansas, U.S. (38°58′39.6″ N, 95°18′28.1″ W) in a 112 m × 50 m (0.567 ha) abandoned football practice field. Annual extreme temperatures for the area range from below 0°F (−18°C) to above 100°F (38°C). Monthly average temperature ranges from a low of 20°F (−7°C) in January to just above 90°F (32°C) in July. The average rainfall is 1,014 mm (39.9 in), most of which occurs April through September (during the growing season) (National Centers for Environmental Information 2015). The restoration is surrounded by mowed areas of non-native cool season grasses, with nearby wooded areas on three sides. The soil type of the area is clay loam consisting of sand and silt residuum weathered from shale and limestone on top of a hardpan of bedrock (USDA 2015). However, before the site was made into a practice football field, soil from an unknown location was added to level the site (D. Hirmas 2014, University of Kansas, personal communication). While in use as a football practice field, the site was occasionally top dressed with black soil and sand (Lubin et al. 2019). Shortly prior to restoration establishment, the site was dominated by non-native cool season grasses. In October 2013, the site was treated with glyphosate herbicide to remove cool season grasses.

The restoration experiment consists of 18 13.7 m × 16.3 m plots arranged in three rows of six plots each separated by 2-m wide mowed aisles (Supplemental Material, Figure S1). Each plot contains four 1 m × 1 m sampling subplots. Subplots are located in the four corners of each plot, 3 m in from the long edges and 3.5 m in from the short edges of the plots to avoid edge effects. Ten native prairie grass species (Supplemental Material, Table S1) were seeded by drill at the same density (~8894.839 g PLS/ha) across all plots. Thirty-one native forb species (Supplemental Material, Table S1) were hand sown at seeding densities that varied by treatment: no forbs, a standard density of forbs for restoration (~2648.5 g PLS/ha), or twice this standard density (~5297.14 g PLS/ha). We refer to these treatments as "0xForb", "1xForb", and "2xForb" respectively. Further details on establishment of the site can be found in Jaksetic et al. (2018). The site has been managed with yearly spring burns through 2019, and selective removal of woody and invasive species.

Data Collection and Analyses

To evaluate species composition, we conducted cover surveys in early June and early September 2019 in each of the 72 1-m² subplots. We visually estimated absolute percent cover of each species based on the percent of ground covered by that species' canopy. Total percent cover often exceeds 100% due to overlapping canopies. While we sampled all treatments, we only included treatments where forbs were sown (1xForb and 2xForb) in the analyses reported here. We averaged percent cover data across the four 1–m² subplots for each plot.

To examine differences in overall species composition between treatments (1xForb, 2xForb), and seasons (early, late), we performed multivariate analyses using permutational multivariate analysis of variance (PERMANOVA) in PRIMER (v6, Quest Research Limited, Auckland, New Zealand). In order to evaluate community responses largely driven by species abundances we conducted a PERMANOVA using Bray-Curtis similarity. We performed a second PERMANOVA based on presence/absence of species using Sorensen similarity. For the former analysis, we relativized and square root transformed species cover data to allow representation of less abundant species prior to calculating the resemblance matrix. We used a within-subjects PERMANOVA design that modeled Season as a within-subjects fixed effect, seeding Treatment as a between-subjects fixed effect, and Block and Plot as random effects. We used PERMDISP to test homogeneity of multivariate dispersions. We used principal coordinates analysis to visualize compositional differences between seasons and treatments. We used similarity percentage (SIMPER) analysis to determine which species contributed most to the differences found between early and late-season samples by PERMANOVA. To evaluate the extent to which individual species contributions to seasonal differences in composition were explained by their abundances, we conducted Spearman rank correlation in R (version 4.0.2, The R Foundation, Vienna, Austria) to determine the relationship between species' Bray-Curtis SIMPER values and their average site-level abundance.

We conducted univariate analyses in R using lmer (Bates et al. 2015) to build linear mixed models and lmerTest (Kuznetsova et al. 2017) to obtain ANOVA tables for these models. These analyses included three levels of season: early, late, and combined. Combined cover ('peak cover') was obtained for each species in a plot by taking the higher of the two cover values (June or September) as an estimate of peak-season abundance. Peak cover was treated as a third seasonal category for comparison in univariate analyses to compare measures from each season to overall values. Combined richness ('total richness') and combined diversity ('total diversity') were calculated based on peak cover data, which includes all species only present in one sample or the other, as estimates of total richness and diversity across the growing season. We performed univariate analyses on cover and richness for species grouped as sown forbs, sown grasses, and nonsown species. Non-native and native nonsown species were grouped together for analysis because of their overlap in characteristics and because site management involves removal of the most aggressive invasive species. Separate analyses on non-native and native nonsown species can be found in the supplemental materials, but should be interpreted with caution (Supplemental Materials, Table S3 and Figure S3). We also performed univariate analyses on diversity metrics for the community as a whole, including community richness, Pielou's evenness, and exponent of Shannon diversity (e^H). All metrics were based on cover data averaged across the four 1-m² quadrats for each plot. Linear mixed models included Season and Treatment as fixed effects, and Block and Plot as random effects. We tested assumptions by inspection of residuals plots. Plot was removed from the model for evenness to correct for singular fit. We calculated diversity indices in R using the BiodiversityR package, which includes vegan (Kindt and Coe 2005). We performed Tukey-Kramer tests when Season or Season × Treatment were significant to determine pairwise relationships.

Results

Species Composition

PERMANOVA and principal coordinates analyses with Bray-Curtis similarity show significant differences in species composition between cover surveys conducted early and late in the growing season (Pseudo F = 19.22, p = 0.001), and significant differences across blocks (Pseudo F

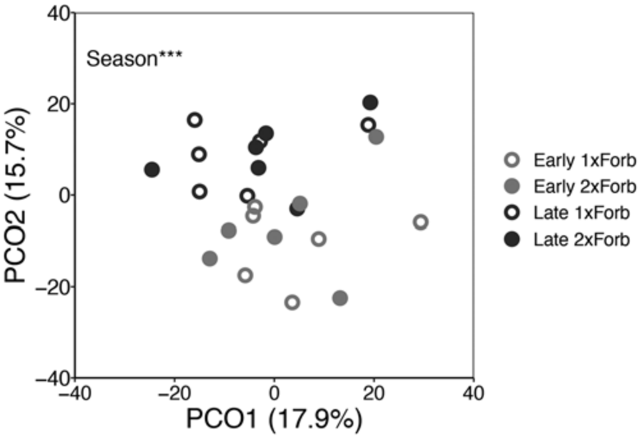

Figure 1. Bray-Curtis principal coordinates analysis. Colors indicate season, circle fill indicates treatment. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

= 2.18, $p = 0.003$) and plots (Pseudo F = 11.49, $p = 0.001$), but no significant effect of forb seeding density treatment (Pseudo F = 1.4, $p = 0.15$) or interactions between treatment and season (Pseudo F = 2.29, $p = 0.08$) (Figure 1). Analyses based on species presence-absence showed similar results. SIMPER analyses conducted with both Bray-Curtis and presence-absence data reveal that these compositional differences between early and late cover surveys are both based on changes in the abundances of the most abundant species and based on species turnover of low abundance species (Table 1, Table S2). Bray-Curtis SIMPER ranks are highly positively correlated with cover ranks ($r = 0.985$, $p < 0.001$) (Table 1). Sown and nonsown species of the same growth form contribute approximately equally to the compositional differences between seasons, regardless of whether they differ in cover (Table 2). Sown and nonsown forbs contribute the most to both the compositional differences and total cover, though sown forbs are just over twice as abundant on average as nonsown forbs, while they contribute similarly to the compositional differences. Sown grasses, nonsown graminoids, and woody species contribute to the compositional differences largely in proportion to their contribution to total cover.

Cover of sown forb species was significantly affected by season, decreasing 20% from early to late season ($p < 0.001$), and was significantly higher in the 2×Forb treatment than in the 1×Forb treatment in all seasons ($F_{1,5} = 9.88$, $p = 0.03$). There was no interaction between season and treatment ($F_{2,20} = 2.84$, $p = 0.08$) (Figure 2A). Sown forb cover was significantly lower than peak in both early ($p = 0.03$) and late samples ($p < 0.001$), though early season sampling captured a greater proportion of peak sown forb cover. Early sampling captured 89% of peak sown forb cover, compared to only 71% with late sampling (Figure 2A).

Cover of sown grasses was significantly affected by season, increasing 59% from early to late season ($p < 0.001$) and was not significantly affected by treatment ($F_{1,5} = 0.64$, $p = 0.46$) (Figure 2B). Sown grass cover was significantly lower than peak in the early season sample ($p < 0.001$), but not in the late season sample ($p = 0.79$). Only 61% of peak sown grass cover was captured with early sampling, compared to 96% with late sampling (Figure 2B).

Cover of nonsown species did not change significantly from early to late season ($p = 0.89$) and was not significantly affected by treatment ($F_{1,10} = 1.99$, $p = 0.19$) (Figure 2C).

Table 1. The 15 species that contribute the most to the compositional differences between early and late-season surveys based on Bray-Curtis SIMPER analysis, their common names, functional groups (based on sowing and growth form), origin (native or nonnative), percent contributions to the compositional differences between seasons (% Cont.), abundance ranks based on average percent cover across both surveys (Cover Rank), and which season they are most abundant (Season). *Symphyotrichum refers to a mix of *S. pilosum* (frost aster) and *S. ericoides* (heath aster).**

Genus and Species	Common Name	Functional Group	Origin	% Cont.	Cover Rank	Season
*Symphyotrichum**	Aster	Nonsown forb	Native	5.20	7	Late
Poa pratensis	Kentucky bluegrass	Nonsown grass	Nonnative	5.15	4	Late
Bromus inermis	Smooth brome	Nonsown grass	Nonnative	5.03	5	Early
Torilis arvensis	Spreading hedgeparsley	Nonsown forb	Nonnative	4.90	11	Early
Tripsacum dactyloides	Eastern gammagrass	Sown grass	Native	4.09	10	Late
Monarda fistulosa	Wild bergamot	Sown forb	Native	3.59	1	Early
Andropogon gerardii	Big bluestem	Sown grass	Native	3.59	2	Late
Bromus tectorum	Cheatgrass	Nonsown grass	Nonnative	3.57	16	Early
Helianthus grosseserratus	Sawtooth sunflower	Sown forb	Native	3.38	8	Late
Sorghastrum nutans	Indian grass	Sown grass	Native	3.15	9	Late
Penstemon digitalis	Smooth beardtongue	Sown forb	Native	2.68	3	Early
Ratibida pinnata	Grey-head prairie coneflower	Sown forb	Native	2.65	6	Late
Vernonia baldwinii	Western ironweed	Sown forb	Native	2.58	13	Early
Symphyotrichum novae-angliae	New England aster	Sown forb	Native	2.55	15	Late
Eupatorium altissimum	Tall boneset	Nonsown forb	Native	2.35	14	Late

Table 2. Percent contributions to the compositional differences between early and late samples based on SIMPER analysis with Bray-Curtis similarity, and percent of total cover (normalized to 100%) of each functional group (based on sowing and growth form).

Functional Group	% Contribution	% of Total Cover
Sown forb	34%	43%
Sown grass	16%	18%
Nonsown forb	31%	21%
Nonsown graminoid	16%	14%
Nonsown woody	4%	3%

Nonsown cover was significantly lower than peak in both early ($p < 0.001$) and late samples ($p < 0.001$), and both captured about the same proportion of peak nonsown cover: early sampling captured 67% of peak nonsown cover, while 69% was captured with late sampling (Figure 2C). Of the total peak nonsown cover, 35% was of native species and 65% was of non-native species.

Biodiversity

Species richness (including all sown and nonsown species) was significantly affected by season, decreasing 16% (5.25 species) from early to late season ($p < 0.001$) but was not significantly affected by treatment ($F_{1,5} = 2.86$, $p = 0.15$) (Figure 3A). Compared to total values, species richness was significantly lower in both early ($p < 0.001$) and late season samples ($p < 0.001$), though early season sampling captured a greater proportion of total richness. 88% of total species richness was captured with early sampling, while only 74% was captured with late sampling (Figure 3A). Evenness did not change significantly from early to late season ($p = 0.16$). Evenness was significantly higher in the 1×Forb treatment than in the 2×Forb treatment ($F_{1,25} = 12.18$, $p = 0.002$) (Figure 3B). Species diversity (e^H) did not change significantly from early to late season ($p = 0.99$). Diversity was significantly higher in the 1×Forb treatment than in the 2×Forb treatment in the early season and total samples (both $p = 0.04$), but not in the late sample ($p = 0.35$) (Figure 3C). Diversity was significantly lower than total in both early ($p < 0.001$) and late season ($p < 0.001$) samples, and both captured about the same proportion of total diversity. 84% of diversity was captured with early sampling, and 83% was captured by late sampling (Figure 3C).

Sown forb richness was significantly affected by season, decreasing 14% (1.66 sp.) from early to late season ($p = 0.01$) (Figure 3D). Sown forb richness did not differ significantly from the total value in the early season sample ($p = 0.35$) but was significantly lower in the late season sample ($p < 0.001$). 95% of sown forb richness was captured with early sampling, compared to only 81% with late sampling (Figure 3D).

Sown grass richness did not change significantly from early to late season ($p = 0.28$) (Figure 3E). Sown grass richness was slightly but significantly lower than total in the early season sample ($p = 0.01$), but not in the late season sample ($p = 0.28$). 84% of sown grass richness was captured with early sampling, compared to 92% with late sampling (Figure 3E).

Nonsown species richness was significantly affected by season, experiencing the most dramatic decline of 22% (3.91 sp.) from early to late season ($p < 0.001$) and was greater in the 1×Forb treatment than in the 2×Forb treatment ($F_{1,5} = 8.67$, $p = 0.03$) (Figure 3F). Nonsown richness was significantly lower than total in both early ($p < 0.001$) and late season samples ($p < 0.001$), though early season sampling captured a greater proportion of total nonsown richness. 85% of nonsown species richness was captured with early season sampling, while only 67% was captured with late season sampling (Figure 3F). Of the 64 total nonsown species found at the site across both surveys, 34 were native, and 30 were non-native.

Discussion

Tallgrass prairie restorations change considerably across the growing season. This study explores such changes in a long-term prairie restoration in eastern Kansas and

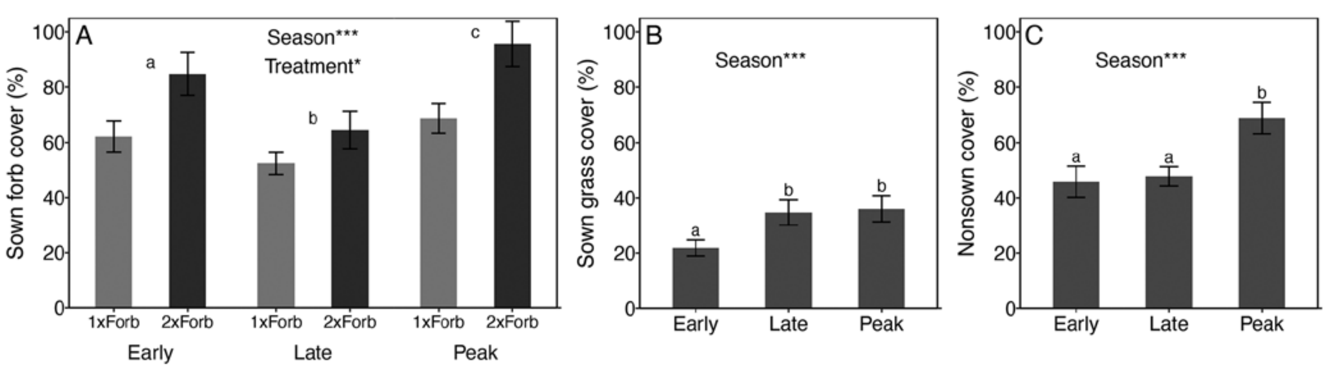

Figure 2. Percent cover (mean ± 1 SE) of A) sown forbs, B) sown grasses, and C) nonsown species. Cover can exceed 100% due to overlap. Seasons that are significantly different ($p < 0.05$) are indicated with different letters. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

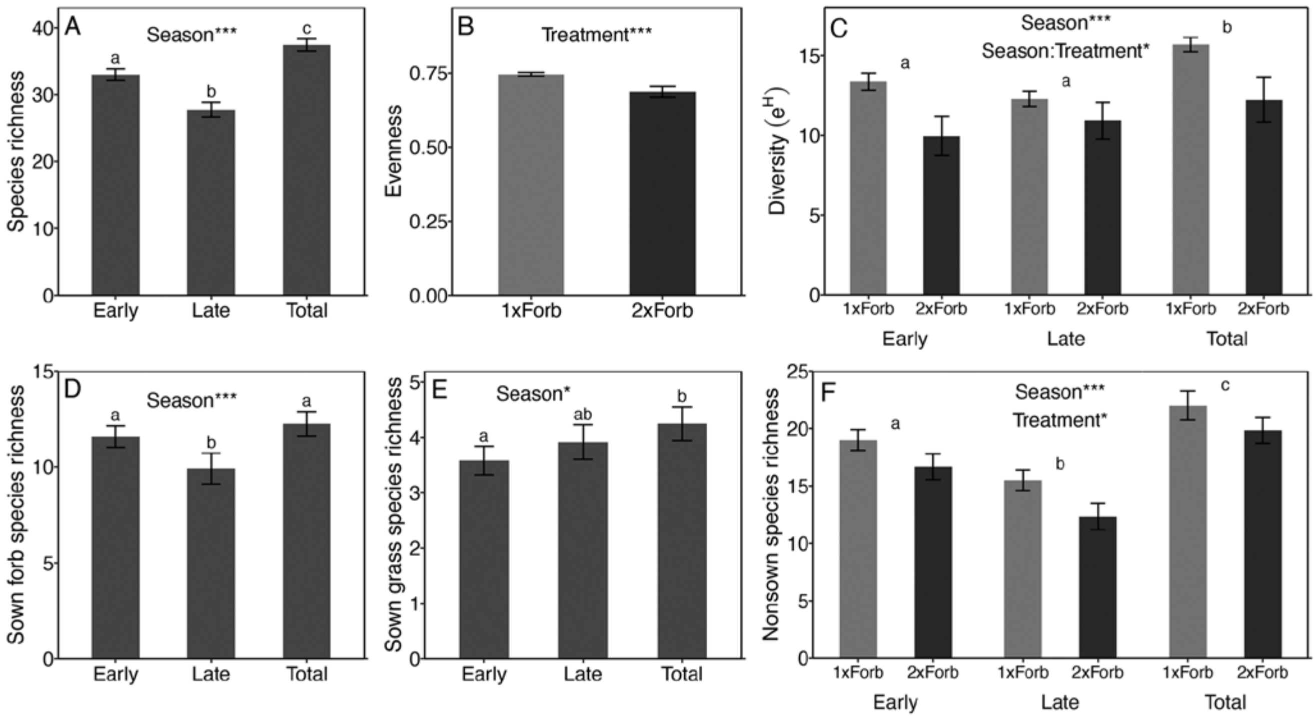

Figure 3. A) Average (mean ± 1 SE) species richness, B) evenness, and C) exponent of Shannon diversity. Species richness (mean ± 1 SE) of D) sown forbs, E) sown grasses, and F) nonsown species. Diversity metrics are at the 4-m² scale. Seasons that are significantly different (*p* < 0.05) are indicated with different letters. * *p* < 0.05, ** *p* < 0.01, * *p* < 0.001.**

examines how these changes may affect inferences made regarding observed species composition, biodiversity, and effects of management interventions based on when and how completely vegetation is sampled. Based on cover surveys conducted at Free State Prairie in early June and early September of 2019, we found that sampling time influences observed composition, biodiversity, and detection of some effects of the forb seeding density treatment. Sampling only once underestimates the overall diversity of the community. Early sampling tends to better represent the overall abundance and richness of sown forbs, while late sampling tends to better represent the overall abundance and richness of sown grasses. Early sampling revealed significant negative effects of increased forb seeding density on Shannon diversity that were not detectable in the late-season sample. These results reveal considerable phenological changes in this tallgrass prairie community and indicate that sampling time and frequency should be considered carefully when evaluating the efficacy of restoration establishment and management.

At our site, both biodiversity and species composition changed markedly from early to late in the growing season. Total community richness decreased from June to September due to a decrease in the number of sown forb species and nonsown species (Figure 3). The shifts in composition (Figure 1) involve shifts in the abundance of relatively abundant species that were present in both June and September (Table 1), as well as seasonal species turnover, particularly in the lower-abundance nonsown species (Supplemental Material, Figure S2 and Table S2). In general, composition shifted seasonally from strongly forb-dominant to moderately forb-dominant, with cover of forbs decreasing and cover of grasses increasing (Figure 2). This pattern of vegetative phenology is broadly similar to patterns of flowering phenology found nearby at Konza Prairie, a native tallgrass prairie in northeastern Kansas (Craine et al. 2012), despite our study site being a restoration and having dramatically lower richness. This broad similarity is not entirely surprising, as flowering phenology and timing of maximum height, a form of vegetative phenology, have been found to be highly correlated in many herbaceous grassland species (Sun and Frelich 2011). At both Free State Prairie and Konza Prairie, C_3 forbs tend to have earlier phenology than C_4 grasses, with the nonnative species (at Konza) and the largely weedy or nonnative nonsown species (at Free State) having the earliest phenology (Craine et al. 2012). This suggests that the effects of sampling time on observed composition may be similar across different sites within a region.

In addition, as would be expected in a community with high phenological diversity, neither early nor late sampling alone fully captures overall diversity or richness at our site, though early sampling captures a larger portion of overall species richness than late sampling (Figure 3A). This is due to early sampling capturing a larger portion of sown forb richness than late sampling (Figure 3D) and a considerably larger portion of nonsown species richness than late sampling (Figure 3F). However, because of seasonal species turnover, each sample alone captures significantly fewer nonsown species than both samples together. Late season sampling captured a larger portion of sown grass richness (Figure 3E), though only because of the patchier distribution of sown grasses in the spring. The seasonality of any given restoration will be influenced by a wide range of factors. The local climate (Craine et al. 2012), the phenology of the seed mix (Havens and Vitt 2016), management practices such as burn timing (Howe 1994), the phenology of nonsown species, and many other factors will all likely affect which sampling time is ideal for a particular site. As climate change affects phenology, diversity, and composition, it is also likely to alter the patterns observed on particular dates (Wolkovich et al. 2013, Whittington et al. 2015, Li et al. 2019). Effects of seasonality on community composition are not limited to prairies, restorations, grasslands, or even plant communities, and similar results have been found in aquatic macroinvertebrate communities in freshwater streams (Reinholdt Jensen et al. 2021).

We also found that sampling time influences our ability to detect the effects of the forb seeding density manipulation on the community. Based on the early season and combined data, increasing forb seeding density decreases diversity. Based on the late season data, seeding density does not significantly affect diversity (Figure 3C). Goldblum et al. (2013) also found that some effects of a seeding density manipulation (of a mix of forbs and grasses) were only detectable in certain points in the growing season. In their study, an increase in total plant cover and decrease in common ragweed cover were seen in July and August, but not in June (Goldblum et al. 2013). This indicates that sampling only once can lead to an inability to detect effects of certain restoration treatments or management interventions on the plant community depending on the growth phenology of the species involved in the response relative to the time of sampling. As a result, it is essential to choose sampling time carefully, especially if it is only possible to sample once during the growing season.

A decrease in diversity with increased forb seeding density would seem contrary to expectations that diversity in restorations should generally be seed limited. The decline in diversity observed in our study in response to forb seed addition was likely due to the competitive suppression of other species, particularly nonsown species, by several sown forbs that established well and became particularly abundant in the 2×Forb seeding density treatment, namely *Monarda fistulosa* (wild bergamot) and to a lesser extent *Penstemon digitalis* (foxglove beardtongue) (Supplemental Material, Figure S4). The seasonality of this effect on diversity may be explained by the abundant sown forbs favored by the 2×Forb treatment also achieving greater abundance early in the season. Sown forbs were highly abundant at our site in general, even in the 1×Forb treatment where a standard density of forb seed was sown, with average sown forb cover of around 62% in June, and 52% in September (Figure 2A). In contrast, sown grasses were relatively low in abundance, with average sown grass cover of about 22% in June and 35% in September (Figure 2B). Our unusually high sown forb cover likely allows them to reach sufficiently high abundance early in the 2×Forb treatment, where their average cover is about 85% (Figure 2A), to suppress other species and reduce diversity.

Several effects of increasing forb seeding density at Free State Prairie remained the same regardless of sampling time. Increased forb seeding density increased sown forb cover (Figure 2A) and led to the decrease in richness of nonsown species (Figure 3F) in both June and September 2019. Because of this decrease in nonsown species richness, forbs made up a greater proportion of overall species richness in the 2×Forb treatment (37% of species early and 39% of species late) than in the 1×Forb treatment (33% of species early and late). Increased forb seeding density did not increase absolute sown forb richness at either sampling time (Figure 3D). This indicates that at this site in 2019 the richness of sown forbs was not limited by initial seeding density and may be limited instead by some other factor such as competition for microsites, but that sown forb cover in aggregate is limited by initial seeding density. Enhancement of overall sown forb cover, but not forb richness, likely reflect responses of a few relatively abundant sown forbs such as *M. fistulosa*, *P. digitalis*, and *Symphyotrichum novae-angliae* (New England aster) that benefitted from the higher seeding rate at the expense of other sown and nonsown species (Supplemental Material, Figure S4). One possible mechanism for this apparent advantage is temporal priority, a phenomenon where species may gain initial competitive advantage via resource preemption by arriving to a site earlier than competitors (Young et al. 2001, Weidlich et al. 2020). Increasing seeding density within a set area could allow sown forbs initial access to a greater number of microsites. Another possible mechanism is that increased seeding density could help competitive species overcome a competition/colonization tradeoff, where more competitive species are typically limited by their inability to spread as quickly or as far as less competitive species which are better colonizers (Tilman 1994). Self-thinning may also have contributed, as adding a greater density of seed would have created greater density-dependent interactions early on that may have favored more aggressive species.

Because plant communities change seasonally, and that seasonal change has real biological relevance and implications for conservation, ideally, restorations should be sampled at multiple points throughout the season to fully capture diversity, composition, and any treatment effects. When data are collected at multiple times during the season, the data should be analyzed by individual seasons as well as in aggregate, as this will provide information on the phenological structure of the community, and treatment or management effects may emerge only in one of the seasons, or in the combined data. If sampling multiple times in a year is not possible, the sampling time should be chosen carefully to avoid underestimating diversity or missing treatment effects. Our results suggest that for our site, if only one survey can be conducted, a single sample early in the season is preferable to a sample late in the season. Early sampling better represented the richness and cover of sown forbs (the chief object under study), revealed more treatment effects, and captured a greater portion of overall diversity. Early season sampling also had the benefit of sampling more forbs when they were blooming, making some species easier to identify than after they started to senesce. In this study we were limited to two sampling points, and we may have found different results if we had sampled three or more times. The ideal sampling time for a given restoration will depend on the type of restoration, management techniques, treatments, seeded species, variables of interest, and the location of the restoration. Fortunately, as shown by the results here and in previous research at this site (Jaksetic et al. 2018, Lubin et al. 2019), even a single late-season sample can still reveal a considerable amount about the community and treatment effects.

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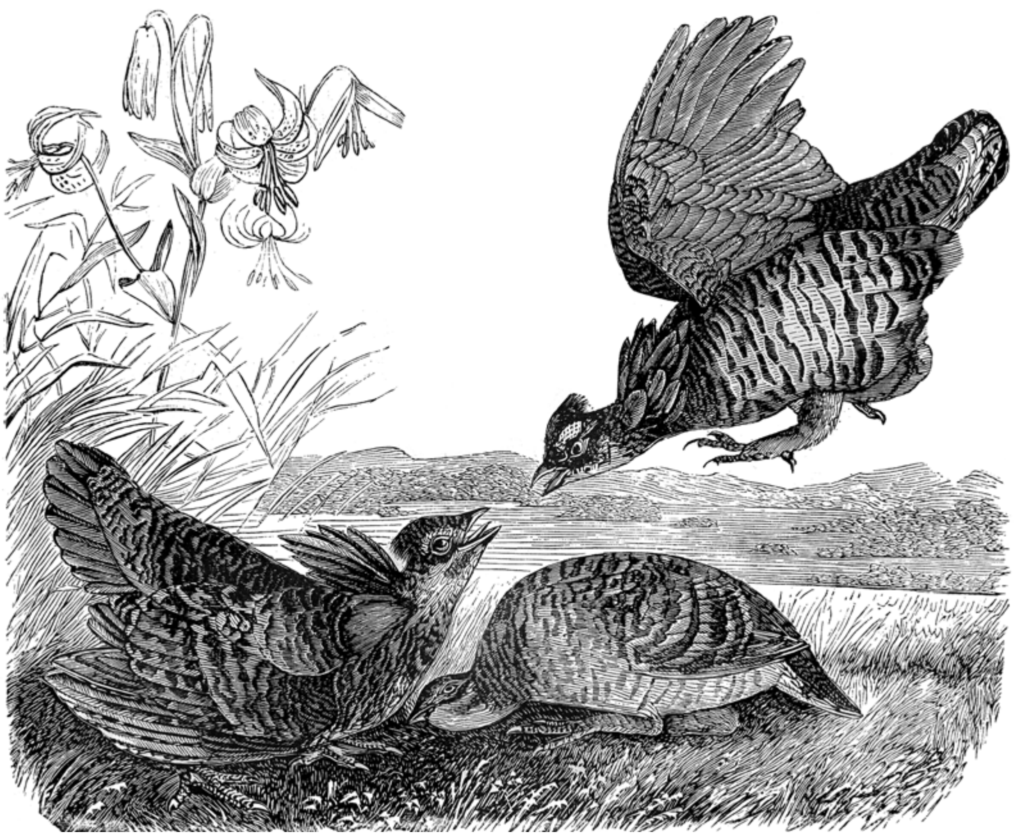

Prairie hens. Source: Goodrich, S.G. Goodrich. 1859. *Animal Kingdom Illustrated*. Vol 2 (New York, NY: Derby & Jackson), The Florida Center for Instructional Technology, College of Education, University of South Florida, fcit.usf.edu.