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Seed and microsite limitations mediate stochastic recruitment in a low-diversity prairie restoration

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Abstract Recruitment of species into plant communities requires the arrival of viable propagules to coincide with the availability of suitable microsites for establishment. Accordingly, the rarity of recruitment events due to seed and/or microsite limitations may constrain the establishment, diversity, and spatial distribution of species in plant communities, thus potentially mediating stochastic recruitment—herein described as probabilistic and unpredictable patterns of species establishment over space that can emerge in the absence or in spite of environmental heterogeneity. To examine this premise, we applied a gradient of propagule pressure, using 37 native forb species, to plots subjected to disturbances of varying intensity in a low-diversity grassland restoration in Eastern Kansas, USA. We monitored establishment for three years, assessing the effects of propagule pressure and disturbance on sown species stem density, richness, composition, and community dissimilarity. Seed limitation was the primary constraint on species richness in this grassland, but both propagule pressure and disturbance had positive, interactive effects on stem density. Increased propagule pressure enhanced recruitment and reduced community dissimilarity among disturbance treatment replicates, thus tempering stochastic recruitment. High propagule pressure led to compositional divergence among disturbance treatments, indicative of deterministic species sorting. These results suggest that seed limitation and stochastic recruitment have important implications for beta diversity and spatial structuring of plant community species compositions, acting to (1) generate and maintain beta diversity by producing stochastic spatial variation in species composition among environmentally similar localities; while concurrently (2) limiting beta diversity by constraining the expression of niche-

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based species sorting in response to environmental heterogeneity.

Keywords Beta diversity · Disturbance · Microsite limitation · Propagule pressure · Seed addition · Stochastic

Introduction

Understanding processes that govern the recruitment of individuals and species into communities is of fundamental importance to numerous areas in basic and applied ecology, including biotic invasions; succession; community assembly; species coexistence; rare species conservation; and ecological restoration (Tilman 1997; Jutila and Grace 2002; Frances et al. 2010). Constraints on recruitment in plant communities have frequently been characterized by the dichotomy of seed versus microsite limitation (Turnbull et al. 2000; Myers and Harms 2009). Seed limitation refers to constraints on species establishment that result from limited dispersal and propagule arrival into suitable microsites. The term microsite limitation encompasses a variety of local barriers to recruitment, which may include limited physical space for establishment, abiotic constraints, or various forms of biotic resistance such as resource competition, seed predation, pathogens, or a lack of mutualists (Eriksson and Ehrlén 1992). Seed addition experiments have shown that species establishment, species distributions, and the alpha diversity of plant communities may often be constrained both by seed and microsite limitations (Xiong et al. 2003; Myers and Harms 2009). These two sources of recruitment limitation are not mutually exclusive, so an important goal is to better understand their relative influences in governing species distributions and structuring communities.

Another key aspect of species recruitment is its unpredictability or stochasticity over space. Successful recruitment of a species requires the dispersal of viable propagules to suitable microsites in sufficient numbers to insure the establishment of adults. If we envision a community composed of numerous local microsites, spatial variation in species recruitment among microsites may in part reflect deterministic and selective filters on recruitment associated with environmental heterogeneity (i.e., species-environment sorting, sensu Leibold et al. 2004). However, spatial variation in recruitment may also reflect the interplay of stochastic processes that act wholly or partially independent of the selective effects of environmental heterogeneity. Here, we define stochastic recruitment as the probabilistic and unpredictable pattern of species establishment over space that can emerge in the absence or in spite of environmental heterogeneity. Two processes may contribute to such spatial recruitment stochasticity: (1) unpredictable patterns of dispersal and seed rain across a community; and (2) the probabilistic and stochastic nature of establishment from available seed within plant communities. In the latter case, the probability of a single seed germinating and surviving to maturity is typically very small, even for species that are well adapted to compete and flourish in a particular habitat as an adult. According to Tilman's stochastic niche hypothesis (2004), per-seed recruitment probabilities in such communities would be low due to intense resource competition with established resident species. When recruitment does occur it would be dependent on the availability of resources not utilized by resident species and would thus tend to be rare, probabilistic, and spatially unpredictable. Here, recruitment is envisioned as a resource-dependent yet spatially stochastic process.

The inherent stochasticity associated with recruitment has important implications for community structure (Tilman 2004). For example, stochastic recruitment may constrain local species richness (alpha richness) by limiting rates of colonization into a locality even if propagules are available. Additionally, stochastic recruitment may limit species' spatial distributions within and among communities by preventing species from occupying and competing in all microsites to which they are well suited, thus leading to spatial variation and unpredictability in species composition. Stochastic recruitment therefore has important implications for beta diversity (Chase 2010), herein defined as spatial turnover of species among localities (compositional dissimilarity). Beta diversity traditionally refers to species-turnover in response to environmental differences among communities, herein defined as deterministic beta diversity. However, species-turnover may also occur among environmentally similar patches within a community, herein defined as stochastic beta diversity.

As predicted by stochastic niche theory (Tilman 2004), the effects of recruitment stochasticity on species establishment and community structure may be mediated through increased propagule pressure, which increases the number of opportunities to overcome low individual odds of establishment in a locality, and/or by disturbances that increase resource abundance and microsite availability for arriving propagules, thus increasing the probability that an individual propagule may successfully establish. Accordingly, experimental increases in the availability of species and their propagules (reduced seed limitation) and/or the application of moderate disturbance (increased microsite availability) are predicted to overwhelm the inherent stochasticity of recruitment and (1) increase local plant density and species richness; and (2) increase the predictability of species establishment among environmentally similar sites and thus decrease community dissimilarity among localities (reduce beta diversity). Interestingly, because increased propagule availability should increase the frequency with which species consistently reach and successfully establish in microsites to which they are well adapted, it follows that enhanced propagule input would increase opportunities for deterministic species sorting and community differentiation in response to underlying niche heterogeneity—leading to a prediction that increased propagule pressure would (3) increase compositional dissimilarity (increase beta diversity) among patches/communities with dissimilar environments. This is consistent with metacommunity theory, which predicts that the contribution of deterministic species sorting to metacommunity differentiation along environmental gradients will decrease as habitat connectivity and dispersal rates among localities decline from modest to low levels (Leibold et al. 2004).

In this study, we experimentally evaluate the interplay of seed limitation, microsite limitation, and recruitment stochasticity in regulating the establishment and diversity of native forb species in a well-established but low-diversity tallgrass prairie restoration. In doing so we (1) explore implications of recruitment limitation for regulating species composition, diversity, and spatial structure in plant communities; (2) evaluate predictions extending from stochastic niche and metacommunity theories regarding the consequences of stochastic recruitment for alpha and beta diversity; and (3) explore potential applications for enhancing forb diversity in prairie restorations. Restoration is a necessary compliment to conservation of tallgrass prairie, which has experienced more habitat loss than any major North American ecosystem (Samson and Knopf 1994). However, restored prairies seldom approach the botanical diversity of remnants, largely due to poor establishment and persistence of native forbs (Kindscher and Tieszen 1998; Martin et al. 2005; Polley et al. 2005), which constitute the largest component of species richness in remnant prairies (Weaver and Fitzpatrick 1934). The “forb problem” is well exemplified by the Rockefeller Experimental Tract in eastern Kansas where the present experiment was conducted. This site is a former non-native pasture planted in 1957 with a mixture of four native warm-season grasses. Although the site is located near two high-diversity remnant prairies, one located 0.3 km to the north and another 0.6 km to the west, few native forb species colonized the site from these potential seed sources over the course of 50 years (Kettle et al. 2000; Long 2010). It is unknown whether poor forb recruitment and diversity at the site reflect an inhospitable local abiotic or biotic environment for establishment (microsite limitation) or constraints on the capacity of propagules to reach the site or arrive in sufficient numbers to overcome stochastic recruitment bottlenecks (seed limitation).

We strongly suspect that many forb species are absent from the site simply due to seed limitation, but that others are limited more by various forms of abiotic and/or biotic resistance. For example, competition from the dominant grasses planted in 1957 likely represents a significant biotic barrier to establishment. Forb colonization in restored prairies can be inhibited by competition with highly productive warm-season grasses (Collins 1992; Kindscher and Tieszen 1998; Copeland et al. 2002) and by reduced light availability due to accumulated grass litter (Knapp and Seastedt 1986; Facelli and Facelli 1993). Because of strong preemptive competition by native grasses, restoration of native forb diversity may require disturbances that free resources to create establishment microsites. In this study, we sow seed mixtures at incremental rates of propagule pressure and employ disturbance of varying intensity to explore whether persistent barriers to recruitment can be mediated by increasing propagule pressure and/or by increasing microsite availability. We address the following questions:

(1) What are the relative roles of seed versus microsite limitation in constraining prairie forb recruitment and diversity?
(2) How are the effects of stochastic recruitment mediated by propagule pressure and disturbance intensity?
(3) What is the potential role of stochastic recruitment in regulating beta diversity among environmentally similar and dissimilar localities?

Methods

Study site and experimental procedure

We conducted this study in the Rockefeller Experimental Tract at the University of Kansas Field Station in northeastern Kansas (39°N, 91°W), within a 5.6 hectare native warm-season grass planting that was converted from non-native pasture in 1957 and is dominated today by *Andropogon gerardii* (big bluestem)*, Sorghastrum nutans* (Indian grass), and *Panicum virgatum* (switchgrass).The specific location of our experiment within this area was selected to minimize environmental heterogeneity among plots and is approximately level, occurs within one soil type, and has a uniform management history.

The experiment has a factorial split-plot design with disturbance (3 levels) as the whole-plot factor and propagule pressure (4 levels) as the subplot factor, with five replicates of each disturbance x propagule pressure combination for a total of 60 subplots. In mid-April 2005, we established 4 m × 4 m whole-plots ($n = 15$) separated by 3 m buffers. We randomly assigned whole-plots to receive one of three disturbance treatments ($n = 5$) intended to create a gradient of microsite availability, while using techniques suitable for application to restoration practice: no disturbance (undisturbed), summer burning (summer burn), or broad-spectrum herbicide application followed by summer burning (herbicide). Although applying herbicide to native grasses may seem counterintuitive for restoration, this treatment emulates conditions of prairie plantings in which resident vegetation is nearly absent at the time of seeding due to prior use of glyphosate resistant row-crops. Furthermore, if resident vegetation in highly productive grasslands severely inhibits recruitment, then spraying and seeding small patches throughout a landscape could be a viable strategy for enhancing forb establishment. Broad-spectrum herbicide (glyphosate) was applied in early-July 2005, causing mortality of resident vegetation prior to burning. Prescribed burns were conducted in mid-August 2005.

Each 4 m × 4 m whole-plot contained four 2 m × 2 m subplots, each sown at one of four levels of propagule pressure (none, low, mid, and high). Centered within subplots were 1 m^2 quadrats in which stems of each sown species were independently counted during mid-June each year from 2005 through 2008, using nomenclature from the PLANTS Database (USDA-NRCS 2013). Sown species seed mixes comprised equal numbers of viable seed per species for 37 native prairie forbs (online resource 1) purchased from vendors of locally sourced native seed. To account for variation in viability, we only purchased seeds from single-source accessions with pure live seed (PLS) tests. Species were selected because they are characteristic of the regional tallgrass prairie species pool and are present in nearby remnant prairies, but had not colonized our site prior to the experiment. Seed mixes were designed so that each species was sown at an equivalent rate of 0 (none), 16 (low), 42 (mid), or 110 (high) seeds per m^2. We weighed 100 seeds of each species to calculate average seed mass per species, then weighted that value by PLS estimates to create seed mixes with approximately equal numbers of viable propagules per species. Seeds were mixed with sawdust to facilitate uniform distribution and were hand-broadcast in late-December 2005.

Photosynthetically Active Radiation (PAR; μmols/m^2/s) was measured to determine the effect of disturbance on light availability, which is an important limiting resource in restored tallgrass prairies (Camill et al. 2004), and to indicate the extent to which prescribed disturbances increased microsite availability. We measured PAR in mid-July from 2005 to 2007 using a ceptometer (AccuPAR LP-80, Decagon Devices, Washington, USA). For each 4 m × 4 m whole-plot, we calculated four PAR interception values (one per subplot) based on paired measurements approximately 50 cm above the canopy and at the soil surface, inserting the ceptometer probe beneath the litter layer when necessary. Light measurements were recorded within one hour of solar noon under clear sky conditions. Light penetration is the percentage of PAR above the canopy to reach the soil surface.

Statistical analyses

To examine inter-annual changes in light availability in response to disturbance treatments, we conducted repeated-measures analysis of variance (ANOVA) and a posteriori multiple comparisons among treatments within each sampling year using Tukey's HSD test. We used sown species stem count data to calculate total stem density (the cumulative stem count per 1 m^2 quadrat) and sown species richness, examining data from the final sampling period, three years after seed addition (2008), to best approximate enduring effects on sown species composition. We used split-plot ANOVA to examine effects of propagule pressure and disturbance on stem density and sown species richness, as data complied with assumptions of sphericity and normality. Because a modest number of sown species established in the unsown subplots, likely due to inadvertent seed movement from sown to unsown subplots by wind or surface water flow, we included the unsown subplots in our analyses to take advantage of a fortuitously expanded propagule pressure gradient. Here, we view the unsown subplots as representing an extremely low level of propagule pressure, while acknowledging that the identity and abundance of propagules dispersed into unsown subplots cannot be quantified. Thus, failure of a sown species to establish in an unsown subplot may result simply because no propagules were dispersed into the unsown subplot, a severe form of dispersal limitation not experienced by other levels of propagule pressure. To confirm that results were not contingent upon unsown subplots, the ANOVAs were repeated with data from unsown subplots excluded, as reported in online resource 2. The preceding analyses were conducted in SPSS 17.0 (2008). To assess whether variation in richness among treatments resulted from increasing total stem density independent of species identity, we conducted individual-based rarefaction, which estimates the richness that would occur in a given treatment at lower density, using the null model software program EcoSim (Gotelli and Entsminger 2009). The split-plot ANOVA was repeated using species richness values generated by rarefaction curves.

We quantified community dissimilarity among sampling quadrats by calculating a matrix of Bray–Curtis dissimilarity values based on relative abundance of sown species stem counts (hereafter, relative abundance) using PC-ORD (McCune and Mefford 1999). Bray–Curtis dissimilarity ranges from 0 to 1, with higher scores indicating that communities under comparison have different species compositions and/or abundances, and lower scores indicating a similar composition and abundance. We performed Nonmetric Multidimensional Scaling (NMDS) in PC-ORD with 500 iterations of relative abundance data using Bray–Curtis dissimilarity. NMDS ordinations were conducted to depict variation in sown species composition and dispersion in response to propagule pressure at each level of disturbance and in response to disturbance at each sown level of propagule pressure. Sown species occurred sporadically in unsown plots, and were entirely absent from some unsown and undisturbed plots, thus yielding a weakly structured dissimilarity matrix that prohibited ordination of disturbance treatment groups within the unsown propagule pressure level.

We used Permutational Analysis of Variance (PERMANOVA) and Permutational Analysis of Multivariate Dispersions (PERMDISP; Anderson 2006; Anderson et al. 2008) to evaluate treatment effects on sown species composition. When PERMANOVA and PERMDISP are employed in tandem and in combination with the inspection of ordination diagrams, it is possible to interpret complex treatment effects on community composition that result from some combination of shifts in multivariate data cloud location (centroid shifts) and changes in multivariate dispersion (changes in mean distance to centroid; a measure of beta diversity; Anderson et al. 2008). When multivariate dispersions are homogeneous among disturbance treatments, a significant term in PERMANOVA would indicate a *location effect* only, indicative of species sorting among disturbance treatment groups (deterministic beta diversity). However, if dispersions are heterogeneous, a significant term in PERMANOVA could reflect a *dispersion effect* or both a *dispersion* and *location effect*. In addition, in factorial designs, measures of dispersion for main effects can reflect shifts in location in response to other main effects. As a result, using PERMANOVA and PERMDISP in tandem is essential for interpreting the results of such designs.

We use PERMDISP to explicitly evaluate effects of propagule pressure and disturbance on within-treatment spatial variability of recruitment (stochastic beta diversity). Treatments exhibiting greater dispersion (community dissimilarity) in sown species composition among replicates would be taken as evidence of greater recruitment stochasticity, provided that environmental heterogeneity was similar among replicates within all treatment combinations. To examine effects of propagule pressure, disturbance, and their interaction on community dissimilarity, we performed factorial PERMDISP. To evaluate potential *location effects* on species composition in response to treatments, we used split-plot factorial PERMANOVA in combination with PERMDISP, accompanied by inspection of ordination diagrams as recommended by Anderson et al. (2008). Given the sporadic dispersal of sown species into unsown plots, it is expected that unsown plots would exhibit greater within-group dissimilarity and compositional differences relative to other treatment groups. However, we repeated these analyses with unsown plots excluded to confirm that results were not contingent upon inclusion of unsown plots (online resource 2). We found no significant interaction terms in factorial PERMDISP and PERMANOVA analyses. However, indications of an interaction in ordination diagrams led us to perform one-way PERMDISP and PERMANOVA analyses to explore effects of propagule pressure at each level of disturbance and disturbance at each sown level of propagule pressure. Such an approach is warranted given limited sample sizes and the inherent difficulties in evaluating interactions in factorial designs where both location and dispersion effects are evident (Anderson et al. 2008). PERMDISP were performed using a freely available FORTRAN program (Anderson 2004). PERMANOVA was performed in PERMANOVA + for PRIMER 6 (Anderson et al. 2008). P values for PERMDISP and PERMANOVA were calculated using 9,999 randomly generated permutations of the Bray–Curtis dissimilarity matrix, without correcting for multiple comparisons.

Lastly, to identify species underlying compositional differences among disturbance treatments at the low, mid, and high levels of propagule pressure, we performed Indicator Species Analysis (ISA; Dufrene and Legendre 1997) in PC-ORD. For each species, ISA assigns an indicator value (*IV*) for each disturbance treatment that can range from 0 to 100 % of perfect indication, based on species presence and stem density. Significance of the observed maximum *IV* with respect to treatment was calculated for each species using a Monte Carlo test with 9,999 iterations.

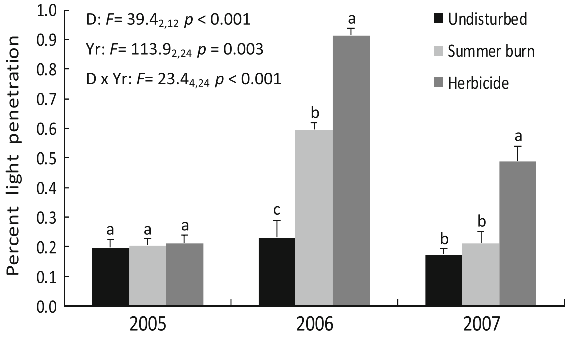

Fig. 1 Mean light penetration (+1 SE) for disturbance treatments (2005–2007) illustrates differences in light availability among disturbance treatments. Significant effects of Disturbance (D), Year (Y), and Disturbance × Year (D × Y) interactions are reported for repeated-measures ANOVA. Significant differences ($p < 0.05$) among treatments within years are denoted by the absence of a common letter

Results

In post-treatment year 2006, PAR penetration was significantly increased by disturbance ($F = 44.6_{2,12}$ $p < 0.001$; Fig. 1), with the greatest increase in response to herbicide. By 2007, PAR penetration was still significantly influenced by disturbance ($F = 23.6_{2,12}$ $p < 0.001$) and elevated in response to herbicide, but was no longer significantly higher in summer burned whole-plots than in undisturbed whole-plots (Fig. 1).

Stem density varied significantly with the main effect of propagule pressure, marginally significantly with the main effect of disturbance, and significantly with the propagule x disturbance interactions (Fig. 2a). Both propagule pressure and disturbance increased stem density, but the magnitudes of these effects were contingent upon each other. This interaction illustrates that the magnitude of the positive effect of disturbance on stem density was reduced by increasing propagule pressure. Similarly, the magnitude of the positive effect of propagule pressure on stem density was reduced when accompanied by disturbance. Propagule pressure had a significant positive effect on richness, while the positive trend of disturbance on richness was slightly non-significant (Fig. 2b). Rarefied species richness was not significantly influenced by propagule pressure or disturbance ($p > 0.05$).

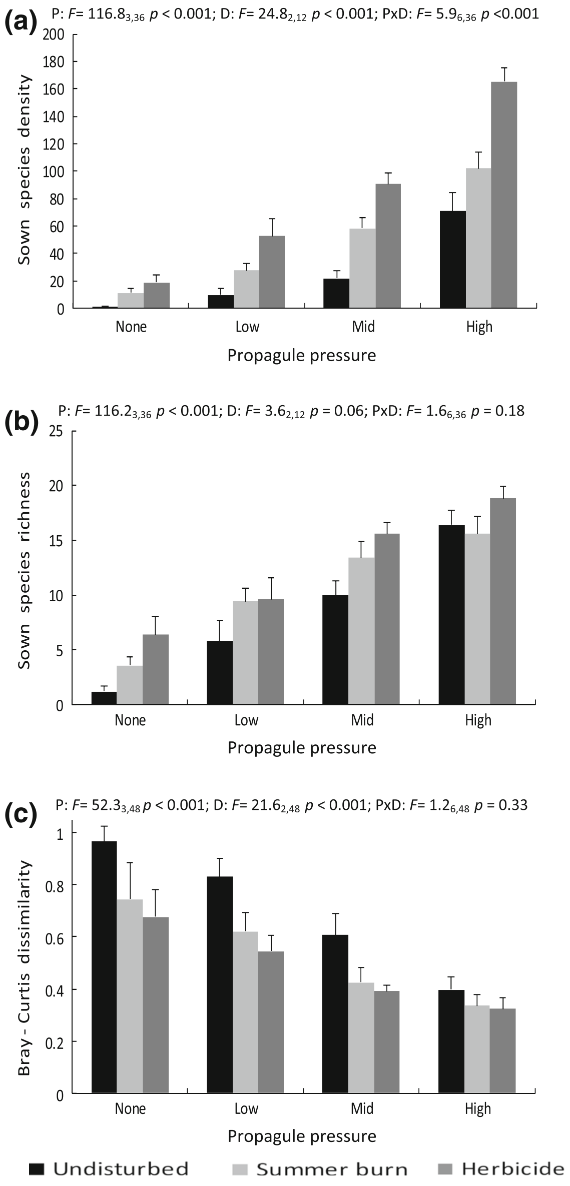

Fig. 2 Mean density (**a**), richness (**b**), and Bray–Curtis dissimilarity (**c**) of sown species for disturbance treatments across each level of propagule pressure. Mean Bray–Curtis dissimilarity (**c**) is the average of all pairwise comparisons of dissimilarity for quadrats within each factorial treatment group. *Error bars* represent +1 SE (**a**–**b**) and 95 % confidence intervals (**c**). Results of split-plot ANOVA (**a**–**b**) and PERMDISP (**c**) indicate significance of Propagule Pressure (P), Disturbance (D), and Propagule × Disturbance interactions (P × D)

Compositional dissimilarity, assessed using PERMDISP, was significantly decreased by the main effects of propagule pressure and disturbance (Fig. 2c), indicating reductions in stochastic beta diversity. The interaction term was not significant. Patterns observed in the ordination diagrams indicated complex effects of propagule pressure and disturbance on sown species composition (Fig. 3), including apparent effects on data cloud dispersion (community dissimilarity) and data cloud location (centroid shifts). As with PERMDISP, PERMANOVA revealed significant main effects of propagule pressure ($F = 3.6_{3,36}$ $p < 0.001$) and disturbance ($F = 5.3_{2,12}$ $p < 0.001$) on species composition, but with no interaction ($F = 1.0_{6,36}$ $p = 0.051$). However, it was unclear the extent to which the significant PERMANOVA terms in the full factorial analysis resulted from effects on data cloud location versus effects on dispersion. In addition, patterns observed in the ordinations suggested the presence of contingent or interactive effects of propagule pressure and disturbance not reflected as significant interaction terms in the factorial PERMANOVA analysis. Because of these complexities, we conducted a series of one-way PERMDISP and PERMANOVA analyses to better understand effects of propagule pressure at each level of disturbance and effects of disturbance at each sown level of propagule pressure.

One-way PERMDISP and ordination diagrams revealed significant reductions in community dissimilarity in response to increased propagule pressure at all levels of disturbance (Fig. 3a, b, c). One-way PERMANOVA demonstrated that propagule pressure only had a significant effect on community composition in the undisturbed treatment, though the ordination and highly significant PERMDISP (Fig. 3a) indicate that this significance resulted primarily from dispersion effects rather than centroid shifts. Furthermore, with unsown plots excluded from one-way analyses PERMANOVA are not significant, while PERMDISP remain highly significant, at all levels of disturbance (online resource 2). These results suggest that propagule pressure effects in the full factorial PERMANOVA largely reflect dispersion rather than shifts in data cloud location, thus compositional differences in response to propagule pressure within a given level of disturbance are due to reduced community dissimilarity rather than species sorting.

One-way PERMANOVA illustrated significant effects of disturbance at all levels of propagule pressure, which became progressively more significant as propagule pressure increased (Fig. 3d, e, f). As previously discussed, significant differences among groups found using PERMANOVA can result purely from differences in dispersion if such differences are

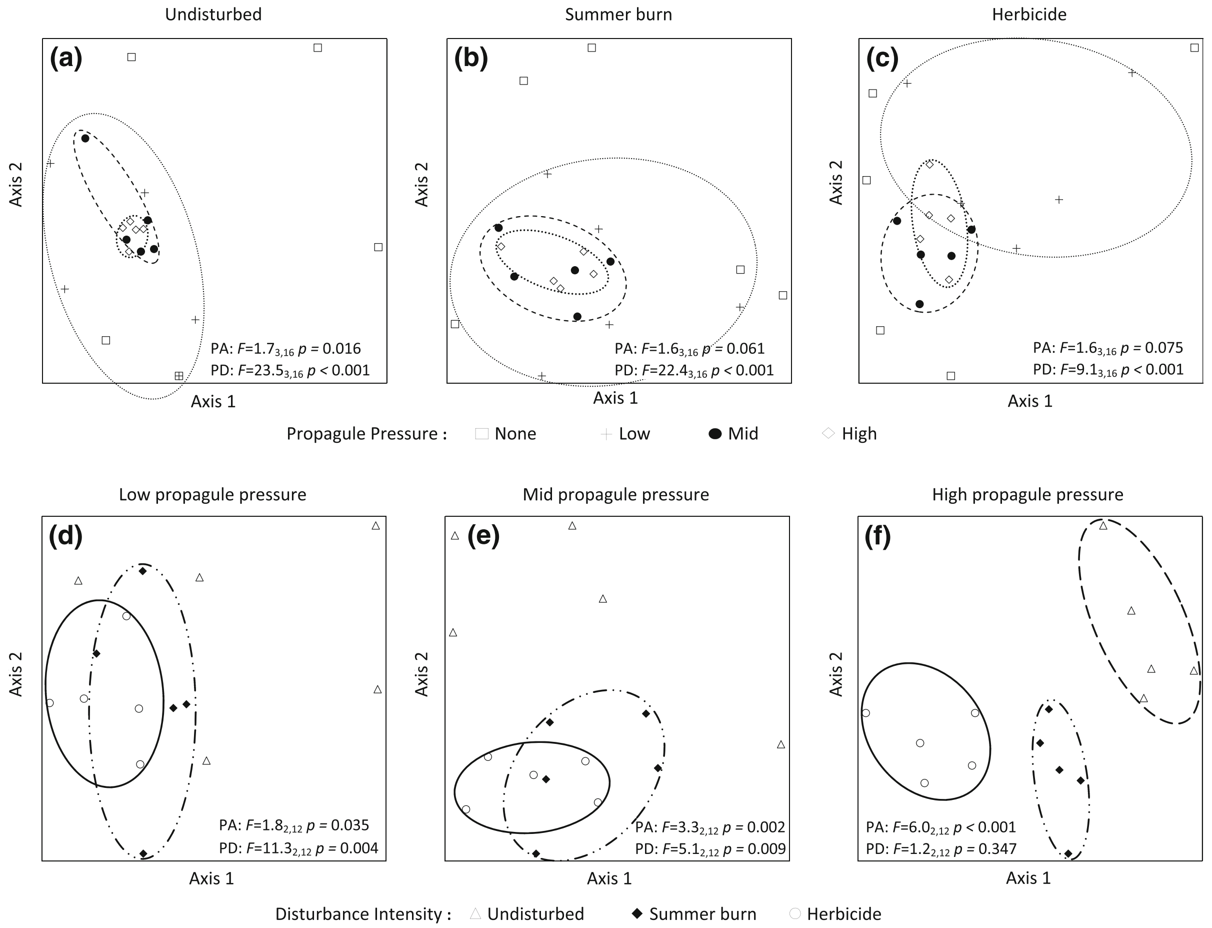

Fig. 3 Nonmetric multidimensional scaling (NMDS) illustrates sown species compositions for propagule pressure levels within each disturbance treatment (**a**–**c**) and for disturbance treatments at low (**d**), mid (**e**), and high (**f**) propagule pressure. The Euclidean distance between points illustrates Bray–Curtis dissimilarity between communities. One-way PERMANOVA (PA) and PERMDISP (PD) indicate differences among groups in composition and within treatment group dispersion, respectively

large (Anderson 2001). At low and mid propagule pressure, one-way PERMDISP are highly significant and data clouds overlap among treatment groups (Fig. 3d, e), suggesting that disturbance effects on community composition are largely due to dispersion. However, at high propagule pressure, there are no effects on dispersion (non-significant one-way PERMDISP) and clear differences in centroids without overlapping data clouds (Fig. 3f), an indication of species sorting and deterministic community differentiation among the different disturbance types (deterministic beta diversity). These results suggest that there were highly significant effects of disturbance on community composition at all levels of propagule pressure, but that they shifted largely from dispersion effects to centroid location effects as propagule pressure increased. Such complex, interactive effects on composition cannot be disentangled from dispersion in the factorial PERMANOVA, which likely explains the lack of an interaction.

Further evidence of non-random species sorting among disturbance treatments in response to increased propagule pressure is provided by Indicator Species Analysis. At high propagule pressure, the undisturbed and herbicide treatments were each significantly associated with five indicator species, three of which remained as significant indicators of these two treatments at mid propagule pressure, while there were no significant indicator species for either treatment at low or no propagule pressure (online resource 1). One species, *Echinacea pallida*, was a consistently significant indicator of the summer burning treatment at low, mid, and high propagule pressures.

Discussion

Our study provides evidence that recruitment of some native forb species from the regional species pool is constrained by both propagule and microsite availability in this low-diversity grassland, with propagule availability being substantially more limiting than microsite availability. In addition, stochastic recruitment resulting from seed and microsite limitation was moderated by increased propagule pressure and disturbance intensity. Our findings implicate the importance of seed limitation and the inherent stochasticity of recruitment in generating spatial variation in community composition among environmentally similar patches (stochastic beta diversity), while concurrently constraining the expression of niche-based species sorting and community differentiation over space in response to environmental heterogeneity (deterministic beta diversity). Previous studies have asserted that the influence of stochasticity on community composition is inversely related to community size and density (Tilman 2004; Gravel et al. 2006; Orrock and Watling 2010). Our study extends this premise to illustrate contrasting implications of seed limitation for beta diversity within and among communities.

The highly significant positive effects of both propagule pressure and disturbance on sown species stem density clearly illustrate the presence of both seed and microsite limitations on individual establishment. The significant interaction between these variables suggests that microsite availability imposes a lesser constraint on establishment when propagules are highly abundant, while high levels of propagule pressure are less critical for establishment when microsites are plentiful. The lack of an interaction between propagule pressure and disturbance on richness indicates that the highly significant effect of propagule pressure and the marginally non-significant effect ($p = 0.06$) of disturbance on richness were additive. Seed limitation of species richness in this restored grassland appears to be much stronger than, and independent of, microsite limitation, at least with respect to recruitment in the third growing season after seed addition. The non-significance of disturbance or a disturbance x propagule pressure interaction for species richness may be indicative of species pool exhaustion (all sown species well suited to a particular treatment had established), suggesting that significant effects on richness might have emerged if additional species had been included in seed addition mixes. The highly significant effects of increased propagule pressure on richness were eliminated by rarefaction, indicating that increased richness was simply due to reduced recruitment stochasticity and thus greater probability of individual plant establishment, independent of species identity. Consistent with the predictions of Stochastic Niche Theory (Tilman 2004), this suggests that forb recruitment and species richness are limited by the arrival of seed in sufficient numbers to overcome recruitment stochasticity.

The role of propagule pressure and microsite availability in mediating recruitment stochasticity has pronounced implications for beta diversity among and within communities. Community dispersion (compositional dissimilarity among replicates) decreased in response to increased propagule pressure for all disturbance treatments (Fig. 3a, b, c), revealing the importance of seed limitation in regulating stochastic recruitment. The unsown subplots illustrate pronounced dispersion (Fig. 3a, b, c) in response to severe limitations on both the number and identity of arriving propagules. Decreased community dispersion in response to increased disturbance intensity, observed at low and moderate levels of propagule pressure (Fig. 3d, e), illustrates the role of microsite limitation in regulating stochastic recruitment. Alternatively, if disturbance had reduced environmental heterogeneity among replicates, then reduced dispersion could also be interpreted as a deterministic response to environmental homogenization of microsites available for recruitment. However, for this alternative explanation to be supported, disturbance should have also reduced dispersion at high propagule pressure, which was not observed (Fig. 3f). Collectively, these results are consistent with Stochastic Niche Theory (Tilman 2004), while also illustrating the potential contribution of stochastic recruitment to beta diversity.

While propagule pressure mediates the relative influence of stochastic processes on structuring patterns of recruitment (Orrock and Fletcher 2005; Orrock and Watling 2010), further interpretation requires consideration of how local and regional dynamics influence recruitment (Chase 2003; Tilman 2004; Chase 2005). Successful colonization events are dependent upon an increase in resource availability concurrent with the arrival of propagules (Davis et al. 2000; Tilman 2004), and previous studies have demonstrated that resource limitations in highly productive communities can negate the ability of seed addition to enhance recruitment (Foster 2001; Stevens et al. 2004). In our experiment, propagule pressure was the most significant determinant of increased stem density, and resultant decreases in stochastic beta diversity, suggesting that limited propagule pressure, rather than resource availability, is the primary constraint on recruitment in this community (Foster 2001; Tilman 2004). However, more severe disturbance did enhance light availability and augment the density of sown species at each propagule pressure level, congruent with previous findings that both microsite and seed limitations can mediate recruitment in grassland plant communities (Mouquet et al. 2004).

Increased disturbance intensity caused a fluctuation in available light and created suitable microsites for sown propagules (Fig. 1). Light availability in grasslands is often suppressed by thatch accumulation, and other experiments have found that, as in this study, colonization success declines with reduced light (Tilman 1993; Davis and Pelsor 2001). Light availability is an important limiting resource in disturbance-dependent grasslands (MacDougall and Turkington 2007) and is often a greater constraint than nutrient availability on species establishment in restored prairies (Camill et al. 2004) such as the site in which this experiment was conducted. In response to prescribed fire, canopy light penetration was enhanced for only one growing season before returning to approximate pre-treatment levels (Fig. 1), demonstrating that even short-term resource fluctuations can increase community invasibility (Davis and Pelsor 2001). In addition to altering light, prescribed fires can also produce temporary nutrient fluxes (Wan et al. 2001), which may have further facilitated species establishment. Herbicide application created a more enduring change in light availability and almost certainly increased the availability of soil resources that would have otherwise been consumed by resident vegetation. This severe disturbance further reduced compositional dissimilarity following seed addition, providing an extreme example of Tilman's (2004) assertion that resource consumption by resident vegetation creates a stochastic filter on recruitment.

Our results also indicate that seed limitation and stochastic recruitment may act to constrain niche-based species sorting and deterministic beta diversity in response to environmentally heterogeneity among localities. Evidence for this is provided by strong community differentiation among disturbance treatments at high propagule pressure, as illustrated by clear differences in centroids with no overlap in data clouds among disturbance treatments (Fig. 3f). Additional evidence of niche-based species sorting is provided by indicator species analysis, which depicts non-random affinities of several sown species (11) with a particular disturbance treatment at high propagule pressure (online resource 1). Furthermore, the number of species exhibiting significant affinities with a particular disturbance treatment increased with propagule pressure, demonstrating that the expression of species sorting depends upon increased seed availability to overcome recruitment stochasticity. These findings are congruent with several models of community assembly (Leibold et al. 2004; Orrock and Watling 2010), which predict that altered connectivity, dispersal, and propagule pressure will modify the relative contribution of stochastic and niche-based processes to community assembly. In the case of metacommunity theory, the importance of niche-based species sorting along environmental gradients can be either strengthened or weakened by increases in connectivity and dispersal. Increases in connectivity from low to moderate levels, such as in our study, are expected to increase species sorting by assuring adequate access of species to their preferred environments. However, if connectivity and dispersal are too great, source-sink dynamics and mass effects may overwhelm the importance of species sorting, thus homogenizing communities.

Understanding the influence of propagule pressure and disturbance on recruitment has important implications for ecological restoration. Our study demonstrates that seed limitation constrains richness and recruitment of prairie forbs from the regional species pool. Although richness was significantly enhanced by seed addition even without disturbance in this grassland restoration, the importance of disturbance for enhancing native forb establishment and richness likely increases in restoration sites where microsite availability is more severely limited due to greater productivity and/or warm-season grass dominance. Increased propagule pressure reduced beta diversity among environmentally similar patches, suggesting that excessive seeding rates may inadvertently limit the diversity of restoration sites that, due to uniform management and/or abiotic (e.g., edaphic, topographic) uniformity, exhibit little environmental heterogeneity. On the other hand, species sorting among environmentally dissimilar patches suggests that restored diversity could be maximized by prescribing heterogeneous disturbance regimes concurrently with high seeding rates. Furthermore, if the specific environmental preferences of a given species are known, then high individual seeding rates may effectively promote establishment of a species in its optimal environment, which is particularly relevant given the considerable cost and/or effort of procuring seed for native forb species. These findings highlight the importance of understanding interactions between propagule pressure, prescribed disturbances, and the environmental affinities of sown species in order to enhance diversity in grassland restorations.

Conclusions

This experiment demonstrates that both seed and microsite limitation constrain recruitment of native prairie forbs, but that propagule availability is the primary constraint on species richness in this low-diversity restored grassland. As limitations on individual recruitment are overcome, increased propagule pressure and disturbance intensity act to moderate stochasticity of the recruitment process. Interestingly, this moderation of stochastic recruitment has contrasting effects on beta diversity. Increased propagule pressure acts to reduce stochastic beta diversity among environmentally similar localities, while concurrently increasing deterministic beta diversity and species sorting in response to environmental heterogeneity.

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