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Abstract

The relative importance of seed availability vs. biotic interactions that affect early life-stages in limiting plant population sizes and determining composition of plant communities is a central debate in plant ecology. We conducted a seed addition experiment in restored tallgrass prairie in central Kansas to determine 1) whether addition of seed of 18 native forb species produced persistent (3 growing seasons) increases in the species' population sizes and plant species richness, 2) what properties of recipient communities best explained spatial variation in added species' establishment and 3) whether seed size explained inter-specific patterns in establishment success. Adding seed led to persistent increases in the number of added species present and in plant species richness at 1 of 3 sites. Increased species richness at the one site where community composition was structured by seed availability largely resulted from greater densities of four species. Seed size did not predict species' establishment success. Pre-existing plant species richness was correlated with added species' establishment success, but the direction of the relationship (positive vs. negative) varied among sites. Living aboveground plant biomass in experimental plots in the year of seed addition was negatively correlated with the number of added species established three years later. Our results provide further evidence for large spatial variation in seed limitation of plant community composition. Surprisingly, mean light availability and heterogeneity in light, both important parameters in conceptual models of grassland plant coexistence, did not predict the response of the recipient plant community to seed addition as well as pre-existing plant species richness and living aboveground biomass. (257 words)

Keywords: spatial heterogeneity, ecosystem productivity, seed mass, over-seeding

Introduction

      Improved ability to predict the ecological circumstances under which plant population sizes and community composition are determined by seed availability will yield significant insights for both basic and applied community ecology. For example, herbivores that reduce host plant seed production will only affect population sizes of their host plants if the sizes of those populations are limited by seed availability (Crawley 1989, Louda and Potvin 1995). Further, the applicability of models of species coexistence that hypothesize a trade-off between colonization and competitive abilities depends upon seed limitation of populations of competitively dominant species (Tilman 1994, Turnbull et al. 1999). In an applied context, the ability to predict where adding seed of locally-rare species will produce persistent populations is central to effective ecological restoration.

      Grassland and savanna plant communities, especially successional ones, often may be invasible and/or unsaturated with species (Tilman 1997, Foster et al. 2004, Zeiter et al. 2006). Community "invasibility" means that species whose propagules are added to the community establish populations. By contrast, the degree of "species saturation" of an ecological community reflects the extent to which adding propagules of novel species increases species richness, meaning that added species establish populations without excluding pre-existing species from the community. Rigorous evaluations of seed limitation of plant population size, community invasibility and saturation are rare because few seed addition studies have been of sufficient duration to determine whether adding seed increases numbers of reproductive individuals of the added species (reviewed in Turnbull et al. 2000). However, several long-term studies have documented sustained

population increases and community responses to seed addition in grasslands and savannas (Foster and Tilman 2003, Foster et al. 2004, Zeiter et al. 2006). One of the striking and poorly explained results of seed addition experiments in herbaceous plant communities is the large variation among sites in the magnitude of population and community response to increased density and diversity of the seed rain.

Establishment success of species that are added to a plant community as seed likely will depend upon characteristics of both the recipient community and of the colonizing species. Differences between sites in mean resource availability or in the amount of spatial heterogeneity in resource availability may be proximate determinants of community invasibility and saturation (Grace 1999, Bakker et al. 2003). In mesic grasslands, sites with high ecosystem productivity may not respond to seed additions either because insufficient light penetration through the canopy prevents adult recruitment (Grime 1973, Foster et al. 2004) or because few species are adapted to grow under conditions of high soil resource availability (Taylor et al. 1990, Safford et al. 2001). Spatial homogeneity in resource levels also may limit community responses to added seed. Theory predicts that in the absence of spatial variation in the identities or ratios of limiting resources, a single species that is most efficient at using the limiting resource will exclude other competitors (Tilman 1988, Tilman and Pacala 1993).

While site-to-site variation in patterns of abiotic resource availability may be the proximate causes of variation in community invasibility and saturation, several influential hypotheses to explain variation in seed limitation focus upon characteristics of the plant community itself, specifically plant standing crop and pre-existing plant species diversity. The influence of seed limitation on plant species richness often is hypothesized to

decrease with increasing community biomass (Huston 1999, Foster et al. 2004). In restored tallgrass prairie in northeastern Kansas, Foster et al. (2004) demonstrated a shift from seed limitation to microsite limitation of plant species richness along gradients of increasing community biomass and ecosystem productivity. The Empty Niche Space Hypothesis suggests that high species diversity in the recipient community, by more completely occupying niche space, will leave fewer resources needed by colonizing species to establish and will limit invasibility (Hutchinson 1957, Tilman 1997, Mwangi et al. 2007). The availability of niche space to particular invader species is likely to depend upon the similarity in life-form of the current community occupants and the invader (Tilman 1997, Mwangi et al. 2007). Here we test the ability of variation in 1) mean resource availability, 2) spatial heterogeneity in resource levels, 3) living plant community biomass and 4) pre-existing species richness in the recipient community to explain spatial variation in establishment of added species and in changes in species richness in response to seed addition in restored grasslands of south-central Kansas, USA.

The relationship between a plant species' competitive ability and the degree of seed limitation of the species' populations is central to conceptual models of plant species coexistence. Large-seeded species often out-compete small-seeded species when they colonize the same microsite (Rees 1995, Turnbull et al. 1999). However, small-seeded species may persist in the community if they better disperse to empty microsites than do large-seeded species (Ehrlen and Eriksson 2000, Kalamees and Zobel 2002). If this colonization / competitive ability hypothesis is generally applicable then large-seeded species should show the greatest response to seed addition because their limited dispersal ability leaves many suitable microsites occupied only by small-seeded species. For

ecological restoration, relationships between easily quantifiable species' traits, such as seed size, and establishment success can provide species-specific guidelines for restoring populations of locally rare species.

Here we asked 1) to what extent seed addition of 18 forbs into mid-successional, restored grasslands increased the added species' population sizes and total species richness in the recipient community at the seedling and flowering life-stages of the added species?, 2) whether variation in mean light penetration, spatial heterogeneity in light, living plant community biomass or pre-existing species richness best explained short and long-term responses of the recipient community to seed addition?, and 3) whether seed size predicted which added species increased most in density after seed addition?

Methods

Study Sites

This research was conducted in floodplain tallgrass prairie at the Ninnescah Experimental Tract (37.53ºN, 97.7ºW) and Pawnee Prairie Park (37.66ºN, 97.43ºW) in Sedgwick County, Kansas USA (Table 1). At both prairies, soils at the lowest elevations are silt-loams. At slightly higher elevations, soils are silt-clay-loams at Ninnescah and loams at Pawnee (USDA 1979). These slightly higher elevations, which we refer to as "uplands," are within the 100-year floodplains (meaning flood at least once a century) of the Ninnescah River (Ninnescah) and Cowskin Creek (Pawnee). Sizes of the restored prairies were 40 ha at Ninnescah and 22 ha at Pawnee. Restoration of both prairies began in the mid-1980's after decades of row crop agriculture. The prairies are dominated by *Andropogon gerardii*, *Sorghastrum nutans*, *Panicum virgatum* and *Bouteloua*

curtipendula. At low elevations, *Tripsacum dactyloides* occurs. Mean annual

precipitation in Sedgwick County is 74 cm (USDA 1979). Historically, south-central

Kansas was at the western edge of tallgrass prairie (Great Plains Flora Association 1986).

Experimental Design

1.5 m X 1.5 m experimental plots were randomly located in low and higher

elevations at each prairie. At Ninnescah, we established 10 plots at low elevations and 10

plots at higher elevations. The smaller size of the prairie at Pawnee allowed only 6 plots

at low and 6 plots at higher elevations. At Ninnescah, mean (± s.e.) distance between

neighbor plots was 21.2 m (±3.0) at low elevations and 18.7 m (±3.1) at high elevations.

At Pawnee, mean distance between neighbor plots was 26.8 m (±9.2) at low elevations

and 23.6 m (±6.3) at high elevations. Upland and lowland plots were separated by > 400

m at Ninnescah and > 200 m at Pawnee. Within each 1.5 m X 1.5 m plot, two 50 cm X

25 cm sub-plots were located 20 cm from diagonally opposite corners. In each plot, one

sub-plot received added seed and the other sub-plot served as a no-seed-addition control.

Seeds of 18 native forb species (Table 2) were sown in seed addition sub-plots at

Ninnescah on February 25 2005 and at Pawnee on March 19 2005. All added species,

except *Liatris pycnostachya*, occurred naturally in both prairies. Sedgwick County KS is

at the western range limit of *L. pycnostachya* (Great Plains Flora Association 1986). We

bought seed of 16 species in winter 2005 from commercial vendors in southern Nebraska

and southern Minnesota. *Solidago missouriensis* and *Argemone polyanthemos* seeds

were collected in autumn 2004 in southern Nebraska. Tetrazolium tests conducted by the

seed companies indicated that seed viability of the purchased species was >70%. High

germination rates in peat pots for all species except *Argemone polyanthemos* confirmed high rates of seed viability. The seed mixture included equal representation of species from productive lowland prairie and species from less productive higher sites. Each seed addition plot received 40 seeds of each species, for a total of 720 seeds. Seeds were sprinkled from a height of 20 cm amid the existing vegetation.

Measurements of Species Richness, Species Abundances and Aboveground Biomass

We made species lists for each 50 cm X 25 cm sub-plot in mid-July 2005-2007. For each species, we noted whether it occurred in the sub-plot as seedlings only, non-seedlings only or both as seedlings and non-seedlings. Seedlings were identified by dried or, in some cases, live cotyledons. For each sub-plot, we used the number of species with non-seedling individuals as an estimate of pre-existing (before seed addition) plant species richness. Individuals of each added species were counted in sub-plots in May 2005 (cotyledon life-stage), October 2005 (end of the first growing season) and October 2007 (when some individuals of most added species had flowered). Flowering plants were counted by species in each sub-plot for the 18 added species in July and October 2006 and monthly May - October 2007. Because some added species produce multi-stemmed individuals, we counted stems that entered the soil close to one another and at an angle that suggested convergence below ground as a single plant. We marked flowering plants with cable ties to avoid re-counting them.

To estimate aboveground biomass in experimental plots, living plant tissue produced during 2005 was harvested in two 25 cm X 25 cm frames per experimental plot August 31-September 2 2005 at Pawnee and September 2-15 2005 at Ninnescah.

Aboveground herbaceous tissue dies each winter in these prairies and new woody growth was readily distinguished from previous year's growth, so we were confident that the harvested biomass was produced in 2005. Litter was not collected. Biomass was harvested in the corners of the 1.5 m X 1.5 m plots that were not occupied by seed addition and control sub-plots. Biomass was dried for 3 days at 60ºC and weighed.

Light Measurements

Light penetration to the soil surface was quantified as the quotient of photosynthetic photon flux density at the soil surface divided by photosynthetic photon flux density above the canopy. We measured photon flux density using an AccuPAR L-80 ceptometer (Decagon Devices). The AccuPAR L-80 ceptometer has an 80 cm probe with 80 light sensors evenly-spaced along its length. For each sub-plot, we took two measurements of soil surface PPFD by inserting the ceptometer beneath the litter layer along either 50 cm edge of the sub-plot. After each soil surface measurement, we took an above canopy measurement. Light measurements were taken between 1000 and 1400 hours. We made early-season light measurements June 19-20 2005 (the end of the time when many seedlings had live cotyledons) and late-season measurements August 29 2005 at Pawnee and September 16 2005 at Ninnescah (the time of peak standing crop). For mean penetration, readings from the 80 sensors were averaged. To quantify small-scale, horizontal spatial heterogeneity in light penetration to the soil surface, photon-flux densities for each 10 cm segment along the ceptometer probe were recorded separately. Light heterogeneity was expressed as the standard deviation of these 10 cm segment means for the two measurements made on either edge of a sub-plot.

Statistical Analyses

For all analyses, we pooled upland and lowland plots at Pawnee because the topographic gradient was slight and, with the loss of one plot that was overtaken by poison ivy (*Toxidendron rhus*), the sample size at Pawnee Upland was small. We used one-way ANOVA with the Tukey-Kramer method for post-hoc comparisons to compare 2005 aboveground plant biomass, light penetration in June and August 2005 and standard deviation in light penetration in June and August 2005 among Ninnescah Upland, Ninnescah Lowland and Pawnee. We used Kruskal-Wallis tests to compare plant species richness and forb richness among the three sites. If the Kruskal-Wallis test was significant then we compared pairs of sites with Wilcoxon two-sample tests.

In all statistical analyses of community and population responses to seed addition, 1.5 m X 1.5 m plot, rather than 50 cm X 25 cm sub-plot, was the unit of observation. The dependent variables analyzed were the differences in the number of added species present, in plant species richness or in densities of individual species between the seed addition sub-plot minus the control sub-plot within a plot. We analyzed the differences between paired seed addition and control sub-plots because added species were almost entirely absent from control sub-plots in some censuses. The overwhelming number of zeros for control sub-plots made using split-plot analyses or even non-parametric analyses to directly compare counts of added species or individual species' densities between seed addition and control sub-plots problematic. To analyze effects of seed addition on plant species richness and population sizes, we used 29 plots. We abandoned three plots that were overgrown by poison ivy. We used 27 plots to analyze relationships

between properties of the recipient plant community and community response to seed addition because one set of light measurements was missing for two plots.

To evaluate whether seed addition resulted in more of the added species present, greater plant species richness and greater densities for each added species, we used Wilcoxon signed rank tests to compare the differences between seed addition and control sub-plots with zero. For differences in the number of added species present and in densities of individual plant species between seed addition and control sub-plots, we used separate Wilcoxon tests to evaluate effects of seed addition in May 2005, October 2005 and in October 2007. For differences in plant species richness between seed addition and control sub-plots, we used separate Wilcoxon tests to evaluate effects of seed addition in July 2005 and July 2007. We examined effects of seed addition for Ninnescah Upland, Ninnescah Lowland and Pawnee, separately.

We used log-linear models (PROC GENMOD; SAS 9.1; SAS Institute) with a poisson error distribution and a log-link function to examine whether properties of the recipient plant communities explained variation in the extent to which seed addition increased the number of added species present, reflecting community invasibility, and species richness, reflecting community saturation. Properties of recipient plant communities that we tested in these models included mean living aboveground plant biomass per plot, mean light penetration in June and August 2005, standard deviation in light penetration in June and August 2005, mean pre-existing plant species richness per plot and mean pre-existing forb species richness per plot. If the difference in a response variable between a seed addition sub-plot and a control sub-plot was negative then we added an integer to all differences for that response variable to ensure values ≥ 0.

For log-linear models of the number of added species that were present, each model included site, life-stage (end of first growing season vs. beginning of flowering) and one property of the recipient plant community plus all interaction terms. Plot was a repeated measurement in these models. We used an unstructured correlation matrix. Values of the Quasilikelihood under the Independence Model Criterion (QIC) were used to compare the fits of models that contained different plant community properties as explanatory variables (Pan 2001). The deviance could not be used to evaluate the fit of these models because repeated measurements on plots required that parameters be estimated using generalized estimating equations, which are based on quasilikelihood rather than maximum likelihood. Log-linear models of plant species richness contained site and one property of the recipient plant community plus the interaction term. We modeled plant species richness in July 2007 only because 2005 species lists were made before August light measurements. Because repeated measurements on plots were not analyzed for species richness, we used deviance values to evaluate the fit of these models.

We examined the relationship between individual species' establishment success and mean seed mass using repeated-measures ANCOVA. Each species was a separate unit of observation. We quantified individual species' establishment success as the mean difference, averaged across experimental plots, between the number of individuals of that species in the seed-addition sub-plot minus the control sub-plot. The explanatory variables were seed mass, site, life-stage plus the interactions.

Results

Differences among sites

Living aboveground plant biomass ($F_{2,24}$ = 7.71, p = 0.003), standard deviation in light penetration in June ($F_{2,24}$ = 3.98, p = 0.032) and pre-existing forb species richness (χ^2_2 = 6.38, p = 0.041) differed significantly among the three sites (Table 1). Post-hoc comparisons showed that Ninnescah Upland had less aboveground plant biomass than Pawnee (t = -3.87, p = 0.002) and lower pre-existing forb species richness than Ninnescah Lowland (Z (Wilcoxon two-sample test statistic) = 2.27, p = 0.023). Ninnescah Lowland had less spatial heterogeneity in June light penetration in than did Pawnee (t = -2.57, p = 0.043).

Population- and Community-Level Responses to Seed Addition

There was substantial variation among sites in the plant community response to seed addition. Seed addition significantly increased the number of added species in experimental plots in May 2005 at Ninnescah Upland (S (Wilcoxon sign-rank test statistic) = 27.5, p = 0.002), Ninnescah Lowland (S = 14, p = 0.0156) and Pawnee (S = 27.5, p = 0.002) (Fig. 1). In October 2005, the number of added species present was greater in seed addition sub-plots at Ninnescah Upland (S = 27.5, p = 0.002) and Pawnee (S = 27.5, p = 0.002). By October 2007, the number of added species present was significantly greater in seed addition sub-plots only at Ninnescah Upland (S = 27.5, p = 0.002). Similarly, seed addition resulted in a significant increase in the number of added species that had flowered by October 2007 at Ninnescah Upland (S = 14, p = 0.0156) only (Fig. 2). Plant species richness in seed addition sub-plots was greater than in control

sub-plots at Ninnescah Upland (S = 22.5, p = 0.0039) and Pawnee (S = 20.5, p = 0.0156) in July 2005. By July 2007, plant species richness was greater in seed addition sub-plots only at Ninnescah Upland (S = 27.5, p = 0.002; Fig 3).

Where seed addition produced persistent community responses, these patterns were driven by increased density of 4, or at most 5, species. Initially, most species showed a transient density increase after seed addition. Eleven species had significantly higher densities (all $p < 0.05$) in seed addition sub-plots than in control sub-plots in May 2005. In October 2005, four added species, *Amorpha cansescens*, *Lespedeza capitata*, *Monarda fistulosa* and *Salvia azurea*, had significantly higher densities in seed addition sub-plots. In October 2007, four added species, *A. canescens*, *Helianthus maximilliani*, *L. capitata* and *S. azurea*, had significantly higher densities where seeds were added. *Monarda fistulosa* was marginally more dense in seed addition sub-plots in October 2007. We never observed *Argemone polyanthemos*, *Oenothera rhombipetala*, *Rumex altissimus*, *Solidago missouriensis* and *Coreopsis tinctoria* in seed addition sub-plots.

Eight added species had flowered in seed addition sub-plots by October 2007. These species were *Achillea millefolium*, *Dalea candida*, *H. maximilliani*, *L. capitata*, *M. fistulosa*, *Rudbeckia hirta*, *S. azurea* and *T. bracteata*. Only *A. millefolium* flowered in control sub-plots. Density of flowering individuals in 2007 was significantly higher in seed addition sub-plots for *L. capitata* (S = 10.5, p = 0.0313) and marginally significantly higher for *H. maximilliani* (S = 7.5, p = 0.0625) only.

Recipient Community Characteristics Associated with Establishment of Added Species

Pre-existing plant species richness and living aboveground plant biomass appear to contribute to determining plant community invasibility (Table 3). Log-linear models that included pre-existing plant species richness explained slightly more of the variation in establishment of the added species than did models that included living aboveground plant biomass. However, both of these explanatory variables provided a much better fit than did the third-best model. The relationship between pre-existing plant species richness and invasibility differed among sites (pre-existing species richness X site interaction: $\chi^2_2 = 9.76$, p = 0.008; Fig. 4). Community invasibility was marginally significantly related to pre-existing species richness at Ninnescah Upland ($\chi^2_1 = 3.08$, p = 0.051) and Pawnee ($\chi^2_1 = 3.05$, p = 0.081), but at Ninnescah Upland the relationship was positive whereas at Pawnee it was negative. Effects of aboveground living plant biomass on community invasibility depended upon the life-stage of the added species (biomass X life-stage interaction: $\chi^2_1 = 4.32$, p = 0.038; Fig. 5). The difference between seed addition and control sub-plots in the number of added species present was negatively related to 2005 plant biomass ($\chi^2_1 = 4.91$, p = 0.027) only at the end of the third growing season.

None of the recipient plant community parameters that we measured explained spatial variation in the effect of seed addition on plant species richness. The log-linear model that included mean light penetration in June had the lowest deviance, but neither light penetration in June ($F_{1,21} = 0.79$, p = 0.374) nor its interaction with site ($F_{2,21} = 1.43$, p = 0.262) approached significance.

Relationship Between Seed Mass and Species' Establishment Success

A species' seed mass was not significantly related to its establishment success in seed addition sub-plots at the cotyledon life-stage, at the end of the first growing season or at the end of the third growing season. This result was unaffected by whether the analysis was conducted using all 18 added species or only the 13 species that we found in at least one sub-plot.

Discussion

Large spatial variation in invasibility and species saturation of floodplain tallgrass prairie plant communities is consistent with the pronounced site-to-site variability found in other experiments that have addressed seed limitation of herbaceous plant community composition at multiple sites (Ehrlen and Eriksson 2000, Zeiter et al. 2006). Although we were unable to identify parameters of recipient plant communities that explain this spatial variation in species saturation, our results do suggest that either pre-existing plant species richness or aboveground plant biomass or, perhaps, both contribute to variation in plant community invasibility.

Our data raise the possibility that the direction of the relationship between pre-existing plant species richness and added species' establishment may differ among sites. The trend toward a negative relationship between pre-existing species richness and establishment of added species at Ninnescah Upland supports the "empty niche hypothesis." At Pawnee, there was a trend for the few species that did establish to establish in plots that were already species rich. Positive relationships between invasibility and pre-existing species richness can result if both high pre-existing species

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richness and added species' establishment occur in benign microsites (Shea and Chesson 2002). In floodplain prairies, topographically depressed microsites that hold standing water for long periods after rains may be especially difficult conditions for seedling establishment. Casual observations indicate that depressed microsites at Pawnee held pools of water longer than at either Ninnescah Lowland or Ninnescah Upland.

Both log-linear analysis and between-site comparisons (e.g. only Ninnescah Upland, the site with significantly less living aboveground biomass, showed persistent effects of seed addition) support a negative correlation between aboveground plant biomass and community invasibility. In our study, differences in invasibility that are correlated with aboveground plant biomass cannot be attributed to a paucity of species in the seed rain that are adapted to productive ecosystems (Taylor et al. 1990, Safford et al. 2001). Species that are characteristic of productive, lowland prairies and less productive, upland sites were equally represented in our pool of added species. Further, effects of aboveground biomass do not appear to result from reduced light penetration to the soil surface, as is often hypothesized (Grace 1999, Foster et al. 2004). At our study sites, light availability at the soil surface, which is a function of both living plants and litter, may be less important in driving spatial variation in effects of seed addition than is light availability once young plants extend their first adult leaves above the litter. Strong effects of neighbor plant biomass on recruitment of added seed after a young plant has grown above the litter layer is consistent with the appearance after the third growing season of a significant relationship between living aboveground plant biomass and number of added species that established. Experiments that independently manipulate

aboveground plant biomass and plant species richness are needed to tease apart their contributions to determining invasibility.

Spatial heterogeneity in resource availability is a critical mechanism of species coexistence in conceptual models of plant community structure (Tilman 1988, Tilman and Pacala 1993), yet resource heterogeneity's importance in explaining variation in community composition in nature rarely has been evaluated (but see Stevens and Carson 2002, Bakker et al. 2003, Reynolds et al. 2007). In theory, spatial heterogeneity in resource levels can enhance plant species richness by providing opportunities for niche differentiation, thereby allowing a higher proportion of colonizing species to avoid competitive exclusion. The empirical evidence that does exist for the importance of spatial heterogeneity in light availability in determining grassland plant community composition, however, is mixed. Bakker et al. (2003) provided correlative evidence that increased heterogeneity in light penetration underlies the positive effect of moderate grazing on plant species richness in tallgrass prairies. By contrast, Stevens and Carson (2002) experimentally demonstrated that in an old-field plant community mean light penetration to the soil surface better explained spatial variation in plant species richness than did heterogeneity in light. Our results also suggest that spatial heterogeneity in light penetration is not a primary factor in establishment of colonizing species. Perhaps the role of spatial heterogeneity in resources in structuring restored plant communities is less than in native communities. The amount of resource variability in restored communities may be much less than in communities that retain natural disturbance regimes and complete guilds of disturbance-producing animals (MacDougall and Wilson 2007).

Where we observed an increase in the number of added species present and in plant species richness in response to seed addition, these community-level effects largely were driven by four, or possibly five, added species. For 20%-25% of the species added in this study, population size may be limited by seed availability. Contrary to our expectations, seed mass did not predict which species' densities increased after seed addition. The lack of correlation between seed mass and population response to seed addition is not consistent with models of species coexistence that hypothesize a competitive- / dispersal-ability trade-off. Seed mass may be less important to recruitment success in relatively unproductive grasslands because high light availability in the understory makes stored nutrients less critical (Tilman 1997, Kalamees and Zobel 2002). However, this hypothesis does not convincingly explain our results because aboveground plant biomass at our sites (Table 1) was comparable to European floodplain grasslands (200 g/m^2 – 800 g/m^2) where seed size and seed-limitation of population size were positively related (Bissels et al. 2006).

Population increases by large-seeded species after seed addition could be muted if large seeds increase vulnerability to cotyledon herbivory or granivory. In other North American restored grasslands, rodent granivores preferentially consume large seeds (Howe and Brown 2001). We did not quantify differences in seed removal rates or cotyledon damage between species. However, we frequently observed cotyledon herbivory that removed large amounts tissue.

Consistent with mounting empirical evidence, our results show that seed limitation can be an important process determining plant community composition, but that spatial variation in its influence is large. Gradients in living plant biomass and pre-

existing plant species richness are likely to be important axes for organizing a conceptual

framework for where and when propagule limitation will contribute to plant community

structure. For further progress in resolving the conditions under which seed availability

structures plant communities and populations, long-term seed addition studies are

essential. Responses at the seedling life-stage can be fleeting and relationships between

community properties and responses to seed addition may not emerge until after multiple

growing seasons.

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Table 1. Properties of the pre-existing plant communities and patterns of light availability at Ninnescah Upland, Ninnescah Lowland and Pawnee. Mean values (± s.e.) are shown. Sites where the mean value for the variable named at the top of the column differed significantly, as indicated by post-hoc comparisons, are labeled with different superscript letters (e.g. "a" vs. "b"). For biomass and measures of light penetration and heterogeneity, post-hoc comparisons were made with Tukey-Kramer tests. For plant and forb species richness, post-hoc comparisons were made with Wilcoxon ranked sum tests with Bonferroni-adjusted significance levels. Live aboveground plant biomass is in grams / m^2. Upland and lowland plots at Pawnee were pooled for analysis as explained in the Methods.

Site	Abvgrnd biomass	Light Pen, June	Light Pen, August	Light Hetero, June	Light Hetero, August	Spp. Rich.	Forb Spp. Rich.
Ninnescah Upland	187.1 ± 25.6 [a]	0.093 ± 0.014	0.09 ± 0.014	0.069 ± 0.009 [a, b]	0.091 ± 0.014	3.75 ± 0.29	1.05 ± 0.26 [a]
Ninnescah Lowland	333.1 ± 117.6 [a, b]	0.08 ± 0.009	0.112 ± 0.014	0.059 ± 0.008 [a]	0.062 ± 0.012	4.21 ± 0.24	2.28 ± 0.37 [b]
Pawnee	633.4 ± 103.2 [b]	0.12 ± 0.017	0.091 ± 0.011	0.102 ± 0.014 [b]	0.07 ± 0.008	4.9 ± 0.43	2.0 ± 0.36 [a, b]

Table 2. Species added as seed. Habitat descriptions are from Great Plains Flora

Association (1986). Species with bolded names are characteristic of productive sites. All

species are perennials, except for the annual/ biennials *Coreopsis tinctoria* and *Oenothera*

rhombipetala. Species that occurred in control sub-plots are indicated in the fourth

column.

Species	Family	Habitat	In Control Sub-Plots?	Mean Seed Mass (mg)
Asclepias incarnata	Asclepiadaceae	Floodplains, wet prairie	N	5.12
Achillea millefolium	Asteraceae	Grasslands, areas of mild disturbance	Y	0.12
Coreopsis tinctoria	Asteraceae	Seasonally damp, disturbed sites	N	0.22
Helianthus maximilliani	Asteraceae	Dry or damp prairie	Y	1.52
Liatris pycnostachya	Asteraceae	Damp prairie	N	2.52
Ratibida columnifera	Asteraceae	Prairies, disturbed sites	N	0.85
Rudbeckia hirta	Asteraceae	Disturbed prairies	Y	0.15
Solidago missouriensis	Asteraceae	Prairies, sparsely wooded areas	N	0.1
Tradescantia bracteata	Commelinaceae	Moist prairies, disturbed sites	N	2.7
Amorpha canescens	Fabaceae	Prairies, hillsides	N	3.5
Dalea candida	Fabaceae	Prairies, open woodlands	N	1.17
Glycyrrhiza lepidota	Fabaceae	Prairie ravines, moist areas	N	8.6
Lespedeza capitata	Fabaceae	Upland prairies, old fields	Y	3.7
Salvia azurea	Lamiaceae	Upland prairies	Y	3.27
Monarda fistulosa	Lamiaceae	Prairie hillsides	N	0.325
Argemone polyanthemos	Papaveraceae	Sandy prairies, floodplains	N	4.22
Rumex altissimus	Polygonaceae	Open wet places	N	1.92
Oenothera rhombipetala	Onagraceae	Sand prairies	N	0.2

Table 3. Quasilikelihood under the Independence Model Criterion (QIC) values to compare the fit of log-linear models that contained different properties of the recipient plant community to the observed variation in (number of added species that occurred in the seed addition sub-plot – number of added species that occurred in the control sub-plot) among plots. All models included the same number of explanatory variables: site, life-stage of the added plant species (seedling vs. flowering), one recipient plant community property and all interaction terms. Lower QIC values indicate better fit to the data.

Recipient Plant Community Property	QIC
Pre-existing number of plant species	-856.13
Mean aboveground plant biomass	-852.26
Pre-existing number of forb species	-822.64
Standard deviation light penetration, June	-819.70
Mean light penetration, June	-788.35
Standard deviation light penetration, August	-711.89
Mean light penetration, August	-709.49

Fig. 1. Number of added plant species per 50 cm X 25 cm sub-plot for the three sites. Seed of 18 species was added in winter 2005. Bars are standard errors.

Fig. 2. Number of species that were added as seed to seed addition sub-plots in Feb-March 2005 that had flowered by October 2007 for the three sites.

Fig. 3. Total number of plant species per 50 cm X 25 cm sub-plot for the three sites. Seed of 18 species was added in winter 2005. Bars are standard errors.

Fig 4. Effects of pre-existing plant species richness on community invasibility varies among study sites. (Number of added species in seed addition sub-plots in Oct 2007 – number of added species in control sub-plots in Oct 2007) vs. number of plant species present per plot before adding seed. Marginally significant ($0.05 < p < 0.1$) relationships at Ninnescah Upland and Pawnee are indicated by solid lines. Non-signficant relationship ($p > 0.1$) at Ninnescah Lowland is indicated by a dashed line.

Fig. 5. Effects of living aboveground plant biomass on community invasibility varies with life-stage of the added species. (Number of added species in seed addition – number of added species in control sub-plots) vs. biomass of the recipient community. Regression line indicates significant relationship between 2005 living plant biomass per plot vs. added species' presence in 2007 when members of the cohort began flowering.
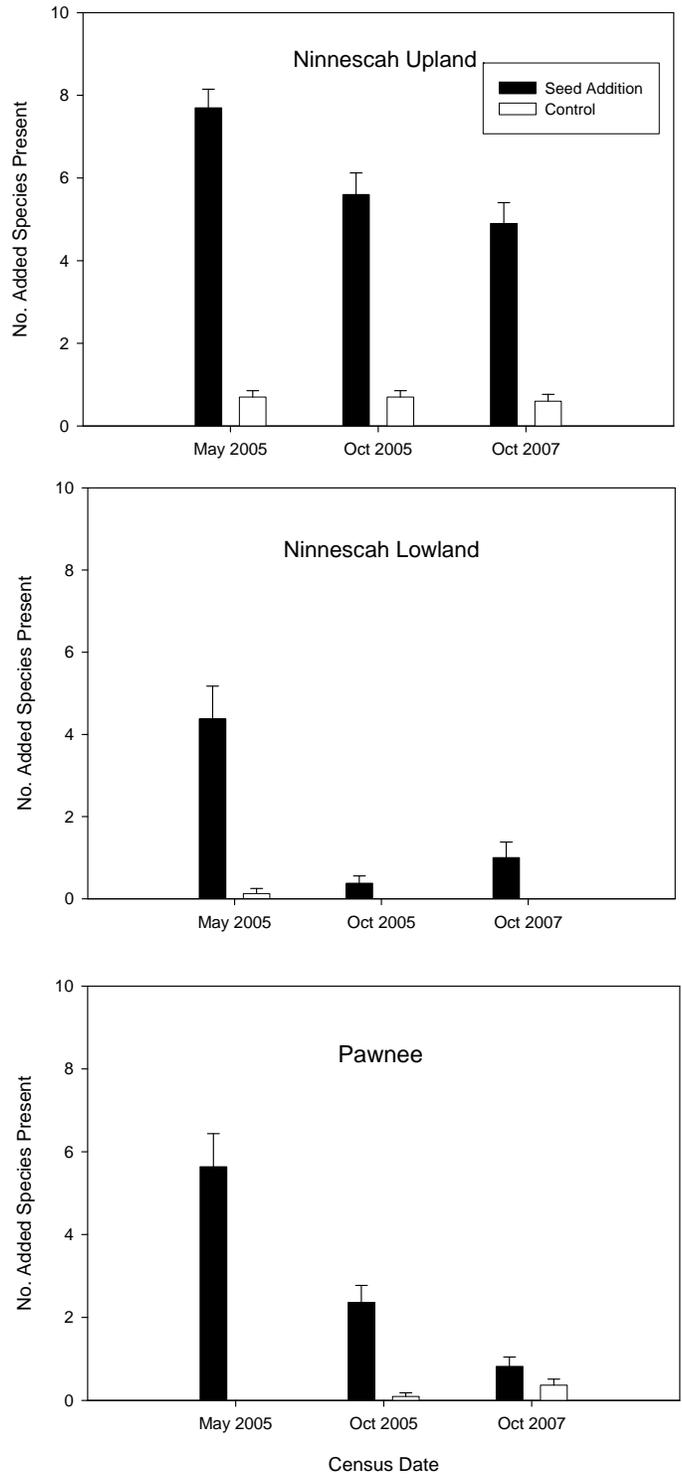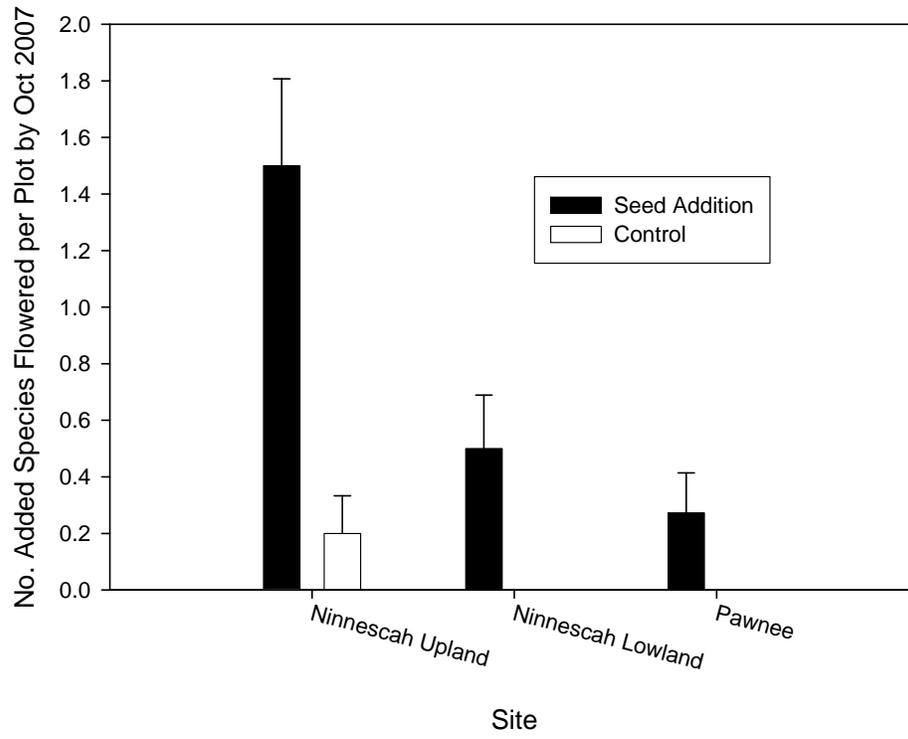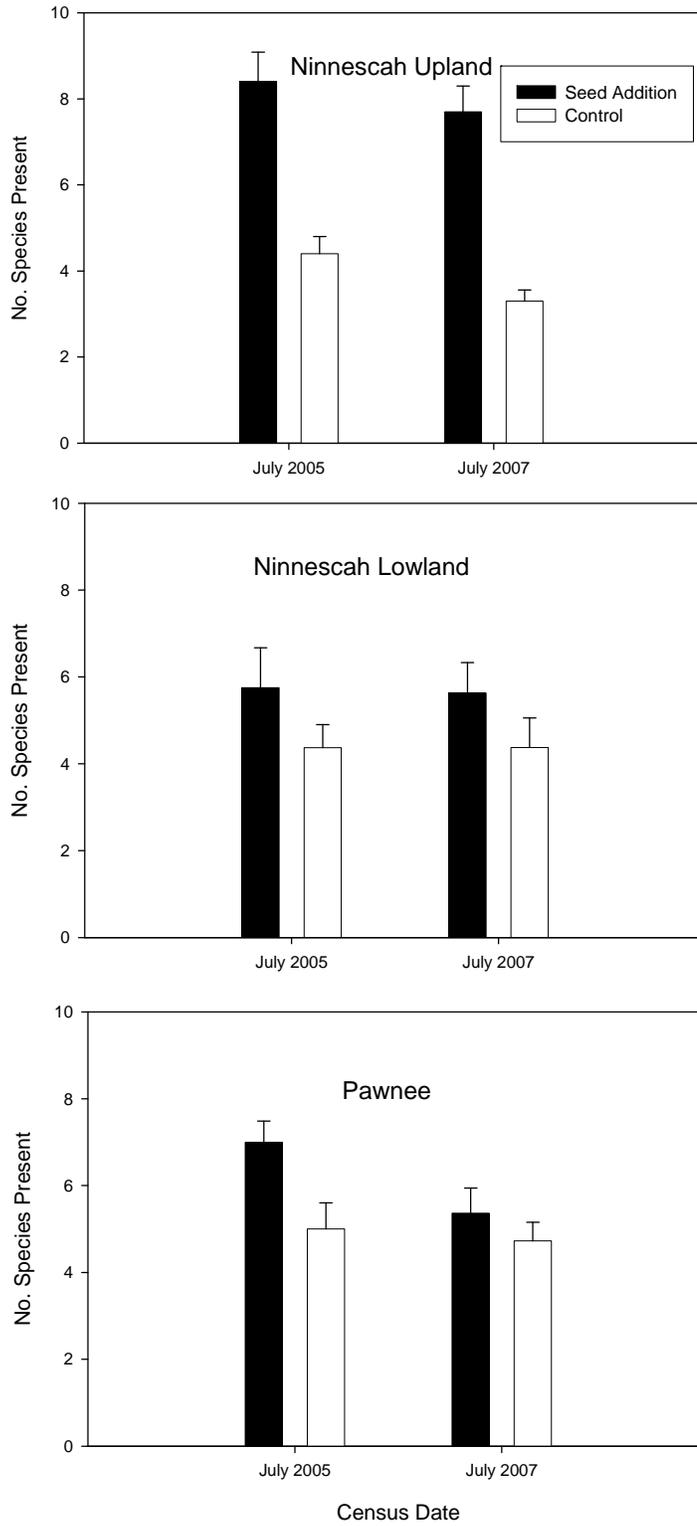
Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.