



Seedling recruitment correlates with seed input across seed sizes: implications for coexistence

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Citation: Maron, J. L., K. L. Hajek, P. G. Hahn, and D. E. Pearson. 2019. Seedling recruitment correlates with seed input across seed sizes: implications for coexistence. *Ecology* 100(12): e02848. 10.1002/ecy.2848

Abstract. Understanding controls on recruitment is critical to predicting community assembly, diversity, and coexistence. Theory posits that at mean fecundity, recruitment of highly fecund small-seeded plants should be primarily microsite limited, which is indicated by a saturating recruitment function. In contrast, species that produce fewer large seeds are more likely to be seed-limited, which is characterized by a linear recruitment function. If these patterns hold in nature, seed predation that disproportionately affects larger-seeded species can limit their establishment. We tested these predictions by comparing recruitment functions among 16 co-occurring perennial forb species that vary by over two orders of magnitude in seed size. We also assessed how postdispersal seed predation by mice influenced recruitment. We added seeds at densities from zero to three times natural fecundity of each species to undisturbed plots and examined spatial variation in recruitment by conducting experiments across 10 grassland sites that varied in productivity and resource availability. Consistent across two replicated years, most species had linear recruitment functions across the range of added seed densities, indicative of seed-limited recruitment. Depending on year, the recruitment functions of only 19–37% of target species saturated near their average fecundity, and this was not associated with seed size. Recruitment was strongly inhibited by rodent seed predation for large-seeded species but not for smaller-seeded species. Proportional recruitment was more sensitive to spatial variation in recruitment conditions across sites for some small-seeded species than for large-seeded species. These results contradict the common belief that highly fecund small-seeded species suffer from microsite-limited recruitment. Rather, they imply that, at least episodically, recruitment can be strongly correlated to plant fecundity. However, proportional recruitment of small-seeded species was inhibited at productive sites to a greater extent than large-seeded species. Results also show that in a system where the dominant granivore prefers larger seeds, low-fecundity large-seeded species can suffer from even greater seed-limited recruitment than would occur in the absence of predators.

Key words: coexistence; microsite limited recruitment; recruitment functions; seed addition; seed limitation; seed predation; seed size.

INTRODUCTION

Maintaining viable populations depends on the successful establishment of propagules (i.e., recruitment into a population). For sedentary species, propagule supply rate (Myers and Harms 2009) and competition for local establishment sites (Roughgarden et al. 1985, Hughes 1990, Turnbull et al. 1999, Maron et al. 2012) are fundamental processes determining recruitment. Importantly, these processes can be strongly influenced by environmental variation that can affect availability of local establishment sites, and by consumers that can destroy propagules and reduce seed supply (Reader 1993, Hulme

1998, Foster et al. 2004, Orrock et al. 2006, Maron et al. 2012). Elucidating the relative importance of these factors and how they contribute to interspecific variation in the magnitude of recruitment is critical for understanding community assembly, community structure, and coexistence (Grubb 1977, Eriksson and Ehrlén 1992, Weiher and Keddy 1999, Rees et al. 2001).

For plants, seed size–fecundity trade-offs have long been considered central to understanding controls on recruitment. Theory predicts that more fecund, small-seeded species are likely to occupy most microsites, and thus recruitment of these species is often thought to be microsite limited rather than limited by the absolute number of seeds produced (Harper 1977, Turnbull et al. 2000, Levine and Rees 2002). By extension, this suggests that the abundance of small-seeded species may be substantially influenced by competition from existing

Manuscript received 27 February 2019; revised 11 July 2019; accepted 15 July 2019. Corresponding Editor: Truman Young.

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dominant vegetation, because existing vegetation can occupy valuable microsites. In annual grasslands small-seeded species may also compete with themselves for germination sites, but in perennial grasslands, most microsite preemption comes from long-lived dominant bunchgrasses. In contrast, recruitment of less fecund, large-seeded species is often considered to be primarily seed-limited (Levins and Culver 1971, Shmida and Ellner 1984, Tilman 1994, Turnbull et al. 2000), because fecundity is typically low relative to microsite availability, and larger seed sizes allow such species to overcome establishment hurdles associated with competitive or stressful microsite conditions. However, consumers can influence these dynamics. For example, in systems where mice are the major postdispersal seed predator, size-dependent seed predation can result in large-seeded species being more vulnerable to seed predation than small-seeded species (Brown and Heske 1990, Reader 1993, Larios et al. 2017, Maron et al. 2018). Hence, seed losses to mice could alter the slope of recruitment functions of larger-seeded species, although this is seldom examined (but see Bricker et al. 2010).

Environmental variation can also play an important role in coexistence among species that vary in seed size. For example, if more fecund small-seeded species are able to disperse to unoccupied microsites, they have inherent advantages over less fecund large-seeded species that cannot reach all of these sites because of poorer dispersal and, more importantly, lower seed numbers (Levine and Rees 2002). However, large-seeded species may have advantages over small-seeded species in productive sites where they can better tolerate local hazards such as deep litter and/or dominant competitors (Leishman and Westoby 1994, Leishman et al. 2000, Moles and Westoby 2004, Maron et al. 2018). Alternatively, if less productive sites are particularly abiotically stressful, large-seeded species might have even greater advantages at these sites (Muller-Landau 2010). Either way, these arguments suggest that spatial variation in productivity and/or resource availability could influence patterns of coexistence.

Central to testing these ideas is understanding how species differ in their recruitment functions (i.e., the relationship between propagule supply and offspring recruitment), and determining the relative importance of underlying biotic and abiotic factors in affecting these functions. Only a few studies have compared recruitment functions among more than a handful of co-occurring species within a given community (Turnbull et al. 1999, Aicher et al. 2011, García-Meza and Martorell 2016). Furthermore, replicated seed addition experiments over multiple years and many sites are rare, so the degree to which spatial and temporal variation in recruitment influences the relative recruitment advantages of large- vs. small-seeded species is often unclear. As a result, we have relatively little insight into whether variation in seed size and seed number among co-occurring species translates to predictable variation in the form of their

recruitment functions, or how environmental variation and consumers can independently and interactively influence these processes.

To address these gaps in knowledge, we conducted a large-scale seed addition experiment, replicated across 2 yr. Our experiment involved adding seeds of 16 co-occurring perennial forb species to plots at a range of seed densities. We also estimated the fecundity of each focal species, so we could add seeds to plots that bracketed the natural fecundity of each species so as to mimic the density of dispersed seeds. To determine how spatial variation in factors that might influence microsite availability differentially affected recruitment of large- vs. small-seeded species, we conducted our experiment across 10 grassland sites that we characterized in terms of underlying productivity and other soil variables. Finally, we performed seed additions inside and outside of rodent enclosures in order to determine how the major postdispersal seed predator at our sites (deer mice, *Peromyscus maniculatus*) might differentially affect recruitment functions among species differing in seed size. We hypothesized that (1) large-seeded species are seed limited, which should be characterized by a recruitment function that saturates beyond seed input densities that approximate average fecundity. In contrast, small-seeded species are microsite limited, which implies a recruitment function that saturates near or before seed input densities near average fecundity. (2) Recruitment limitation, as characterized by the proportion of seeds that germinate, is more extreme at more productive sites because of preemption of microsites, with small-seeded species being more sensitive to this site-level variation than large-seeded species. (3) Seed limited recruitment is exacerbated by rodent granivory, especially for large-seeded species. Based on only 1 yr of recruitment data, we previously reported that proportional recruitment was suppressed to a greater extent for large- vs. small-seeded species (Maron et al. 2018). Here we use data from seed addition replicated in an additional year to test how rodent granivory influences seed limitation.

METHODS

Sites

Our research took place in the Blackfoot Valley in western Montana (47°01' N, 113°07' W) within semiarid perennial grasslands dominated by native rough fescue (*Festuca campestris*) and a rich diversity of native perennial forbs. The primary seed consumer in the study area is the deer mouse (*Peromyscus maniculatus*). Montane voles (*Microtus montanus*) and Columbian ground squirrels (*Spermophilus columbianus*) are present, but *M. montanus* occurs at very low densities (Maron et al. 2010) and *S. columbianus* is mostly folivorous. Seed predation by birds is minimal and ants are primarily carnivorous.

We performed our seed addition experiments at 10 sites spread across a >450-km² area in the Blackfoot

Valley. Sites were all in rough fescue grasslands and chosen based on apparent differences in aboveground productivity (mainly as a result of differences in bunchgrass cover), which we hypothesized could influence the availability of microsites for recruitment. Most study sites were a minimum of 0.75 ha in size, although one site was considerably smaller (0.15 ha). The distance between sites ranged from 200 m to over 50 km. At each site we installed a 10 × 10 m (or in two cases 10 × 15 m) rodent enclosure to determine how postdispersal seed predation by mice might influence the recruitment functions of species. Enclosures were established at three sites in 2003, one site in 2006, four sites in 2008, and two sites in 2016. Enclosures were made of welded wire fencing dug 50 cm into the ground, extending 80–100 cm above the ground and topped with sheet metal to prevent mice from climbing over them (see Maron et al. 2012 for details). Enclosures were effective in excluding mice, but we also placed snap traps inside enclosures to ensure any errant mouse that managed to gain entry was killed.

To quantify differences in abiotic conditions among sites, in May 2016 we sampled soil at each site (see Appendix S1 for sampling methods) and sent soil to A&L Western Laboratories (Modesto, California, USA) for analysis of total nitrogen, organic matter, and available phosphorus. In mid-June 2018 we took additional soil samples at each site to quantify differences in NH_4^+ and NO_3^- (see Appendix S1 for methods pertaining to soil sampling and Potassium chloride (KCl) extraction). During late June 2016, we characterized productivity at each site by placing 22 0.5 × 0.5 m quadrats in a stratified random manner across each site. All live plants within quadrats were clipped at the soil surface, sorted to graminoids, forbs, and litter, and placed into labeled paper bags. Harvested vegetation, the live portion of which represented annual growth, was subsequently dried at 60°C for 3 d and then weighed.

Target species

We selected 17 species of co-occurring perennial forbs as focal species (Table 1). These species are relatively common across the Blackfoot Valley, and frequently co-occur with each other. Although not all focal species occurred at all of our study sites, they all occurred at a subset of these sites (Maron et al. 2018). One of the added species, *Lithospermum ruderales* (LIRU; see Table 1 for key to plant abbreviations henceforth in the paper), has seeds that mostly germinate in their second year (Bricker et al. 2010). Because we only analyze recruitment in the year after seed addition here, this species was excluded from analyses. All other target species germinate most prolifically in the year after dispersal, and our previous work and observations suggest that there is not a dense long-lived seedbank at our sites (Maron et al. 2012, unpublished data).

In June and July 2016 and 2018 we quantified average per capita seed production for most target species (see

Appendix S1 for how fecundity was estimated). A few target species only had fecundity estimated once (Table 1). We also quantified mean seed weight for each focal species by averaging weights from five sets of a known number of seeds that were collected at our sites.

Seed addition experiment

We conducted a seed addition experiment to quantify the recruitment function for each focal species. Because our goal was to gain a broad understanding of how recruitment functions varied across a range of conditions that characterize rough fescue grasslands in western Montana, we replicated plots across sites rather than within sites. At each site in 2016 and again in 2017, we established 11–12 1-m² plots with intact undisturbed grassland vegetation, with corners marked with rebar and a unique numbered metal tag. Six plots were spaced evenly (0.5 m between plots) inside rodent enclosures and five (2016) or six (2017) plots were spread across randomly selected locations within each site. In mid-August 2016 and 2017 we added mixes of seeds containing all of our focal species to each experimental plot. Each plot inside and outside of rodent enclosures received one of the following seed treatments: no seeds added, or 0.25×, 0.5×, 1.0×, 1.5×, or 3× the estimated fecundity of each focal species (as estimated in 2016; Table 1). The no-seeds-added treatment was to control for any recruitment from the seedbank or from dispersal from outside the plots. Plots of a given seed addition treatment received seeds of each focal species at the same multiple of its estimated fecundity. If seed addition plots contained any adult focal species, we made sure to remove all flowers from these individuals to ensure that recruitment would reflect the influence of experimental seed addition as opposed to in situ production of seeds.

We assumed that a 1-m² plot containing seeds added at “ambient fecundity” (i.e., 1.0×) approximates seed density for naturally dispersed seed of each target. This assumption is based on observations indicating that seeds of most target species primarily disperse close to parent plants. It also takes into account the fact that our estimates of plant fecundity incorporate the average number of flowering stems (and seeds per inflorescence) per 1 m². We note, however, that even if our assumption of seed density at ambient fecundity is higher than what occurs naturally, our estimates of each species’ recruitment function should still be reasonable because we added seeds at much lower densities in our 0.25× and 0.5× plots than in our 1× plots.

In 2016 we did not establish 3× plots outside of rodent enclosures because of insufficient seed availability. In 2017, we established 3× plots outside the rodent enclosures at five sites. The seed density treatment was randomly assigned to each plot. In total we established 110 plots in 2016 and 120 plots in 2017. Seeds of all species except ARLU were collected from plants in the Blackfoot Valley in the same year as we performed the seed addition. For BASA, GETR, GEVI, and LUSE we

TABLE 1. Target species, their abbreviated code, mean seed weight, and mean and standard error fecundity “fec” (i.e., mean number of seeds produced per individual plant estimated in 2016 and 2018). Species with NA for fecundity SE only had one estimate for fecundity. “*b*” parameter is the estimated slope of the recruitment function (i.e., proportional recruitment) and “*n*” parameter is the number of total microsites available in a plot. If fecundity is greater than *n*, then the number of recruits would saturate at *bn*. NAs in “*b*” column for ERPU and POGL reflect that we were unable to estimate the recruitment function for these species in 2017. Bolded values are those with the “*n*” parameter within one standard error of mean fecundity.

Species	Species code	Seed WT (mg)	Fec mean	Fec SE	2017 –rodents		2018 –rodents		2018 +rodents	
					<i>b</i>	<i>n</i>	<i>b</i>	<i>n</i>	<i>b</i>	<i>n</i>
<i>Antennaria microphylla</i>	ANMI	0.04	1,244.5	NA	0.005	5,886	0.004	793	0.032	82
<i>Penstemon procerus</i>	PEPR	0.05	2,927.2	2,000.8	0.007	2,109	0.014	1,329	0.015	815
<i>Erigeron pumilus</i>	ERPU	0.07	6,100.4	528.4	NA	NA	0.014	6,623	0.017	6,604
<i>Achillea millefolium</i>	ACMI	0.09	1,230.5	46.5	0.039		0.054	19,970	0.047	
<i>Potentilla glandulosa</i>	POGL	0.10	8,710.4	3876.4	NA	NA	0.029	30,060	0.027	10,160
<i>Arnica sororia</i>	ARLU	0.14	4,919.0	NA	0.007	17,080	0.029	9,049	0.014	19,000
<i>Heterotheca villosa</i>	HEVI	0.58	6,470.6	1,293.4	0.040		0.0228		0.020	33,950
<i>Artemisia ludoviciana</i>	ARSO	0.75	80.6	21.4	0.120	43	0.101	203	0.116	70
<i>Geum triflorum</i>	GETR	0.77	204.4	77.6	0.071		0.077	1,167	0.043	1,141
<i>Zigadenus venenosus</i>	ZIVE	1.38	324.1	33.9	0.151		0.117		0.062	1,495
<i>Eriogonum umbellatum</i>	ERUM	2.16	536.3	42.7	0.291		0.255		0.145	11,340
<i>Lomatium triternatum</i>	LOTR	3.65	81.2	43.8	0.175		0.199	1,618	0.170	72
<i>Gaillardia aristata</i>	GAAR	3.92	230.0	NA	0.045	733	0.118	2,124	0.034	735
<i>Balsamorhiza sagittata</i>	BASA	8.33	150.0	NA	0.131		0.096		0.026	900
<i>Geranium viscosissimum</i>	GEVI	8.68	67.8	32.2	0.130		0.266	74	0.061	
<i>Lupinus sericeus</i>	LUSE	20.92	78.0	NA	0.661	52	0.384	408	0.125	286

used mostly wild collected seed but had to purchase some seed to obtain sufficient stocks for our experiment, which we mixed in with wild collected seed. ARLU seeds were added in October because seeds of this species do not mature until then. In 2016 we were unable to collect enough seeds to add ERPU to the 1.5× plots, and ERPU or POGL to the 3× plots.

In 2017, we used a 1-m² quadrat gridded into 100 10 × 10 cm cells to count and identify seedlings of all seeded species in each plot at all sites. We performed three censuses, in early May, late May, and mid-June. For most species, recruitment peaked in the late-May census, although some species had higher recruitment in the last census in mid-June. In 2018 we performed one census of each plot (mid-May to mid-June), which represented peak recruitment in that year. In a subset of plots (*n* = 17) at six sites where recruitment was extremely high for a subset of species in 2018, we estimated total recruitment using a stratified random sampling design. For ACMI, ARLU, ERPU, ERUM, HEVI, and POGL only we counted seedlings in 32 randomly assigned 10 × 10 cm cells (rather than all 100). The sampling cells were stratified such that each quarter of the quadrat had eight sampling cells. We then extrapolated these results to the entire 1 m² area. In both 2017 and 2018 there was little evidence of seedling herbivory.

Natural recruitment

In order to assess natural recruitment of our target species, we counted seedlings under 15 reproductive adults growing naturally at four of our sites. Some

species (ARLU, BASA, and ZIVI) occurred sparsely, resulting in fewer than 15 individuals being sampled (Table 1). At four study sites, we established 5–10 transects using a systematic random sampling design. We then sampled at five points with a random starting point along each transect. At each sampling point, if a reproductive adult of the target species was growing within a 5-m radius of the point, we centered a 1.8-m² circular plot around the individual. Based on field observations, we know that the majority of a plant’s seeds disperse into an area smaller than the size of this plot, regardless of seed size. However, if some seeds disperse further than our observations indicate, this would likely produce no relationship between estimated fecundity and recruitment for these species. We then counted all conspecific seedlings within the plot. For patchy species, we located a patch then sampled all individuals within the patch. For sparse species, we searched throughout the study area to locate all individuals at the site.

Analytical methods

To determine the shape of the recruitment functions (Hypothesis 1), we fit a Skellam recruitment function to the data for each species (Duncan et al. 2009), which describes the relationship between the number of seeds added *s* and the number of plants that recruited the following year *r*:

$$r = bn(1 - e^{-s/n}) \quad (1)$$

where *n* is the total number of microsites available in a plot and *b* is the proportion seeds that recruit into

seedlings given no microsite limitation. When a large number of seeds are added relative to available safe sites ($s > n$), the number of seedling recruits saturates at bn (i.e., the number of safe sites). If few seeds are added relative to the number of safe sites available ($s < n$), then recruitment is strongly seed limited and the number of recruits increases proportionally to the number of seeds added. In this case Eq. 1 approaches a linear function with a slope of b :

$$r = bs \quad (2)$$

We fit models separately for each year and experimental treatment combination in part because analyzing years and treatments together in one model necessitates examining interaction terms, which can lead to biased estimation (Greene 2010). For data collected in 2017 we used maximum recruitment of each species across the three census periods. Models were fit using the *nls* function in the *nlme* package in R (Pinheiro et al. 2017, R Development Core Team 2018). Visual assessment of the model fit and examination of the residuals ensured that model fits and assumptions of normality and heteroskedasticity were reasonably met. In cases where there was no evidence of saturation and the Skellam models did not converge or the assumptions were severely violated we fit the linear model (Eq. 2). In all cases, this provided a better fit to the data.

To test whether the proportion of seeds that emerged inside of rodent exclosures differed based on seed size, we ran separate regressions (of seed input vs. seedling recruitment) for 2017 and 2018 data, with seed size \log_{10} transformed. The response variable, the proportion of seeds that emerged, was estimated from the recruitment function models (i.e., the b parameter).

To examine how sites varied in conditions that might influence recruitment, we characterized differences in productivity and soil nutrient status among sites using principal-components analysis (PCA) using the *princomp* function in R (R Development Core Team 2018). The analysis was performed on the correlation matrix of standardized variables that included soil organic matter, soil phosphorus, total aboveground plant biomass, and plant available soil nitrogen. In this analysis, soil variables related to productivity and soil nitrogen loaded negatively on PC1 axis, whereas those related to soil phosphorus fell along PC2 axis.

To test how recruitment varied across sites, and whether species differed in their responses to environmental variation (Hypothesis 2), we evaluated proportional recruitment as a function of the site-level principal-component values PC1 and PC2, with all species and interactions between species and PC1 or PC2 included in this model. Proportional recruitment for each species was calculated by dividing the number of seedlings by the total number of seeds added to each plot. This value calculated from the data is similar to the b parameter from the recruitment function models. We only analyzed data from plots inside rodent exclosures,

so that recruitment would not be affected by seed predation. We averaged the proportional recruitment across all seed addition plots within the 0.25 \times and 1.5 \times range at a site to get a mean value for each species at each site in the 2 yr. Averaging proportional recruitment across the 0.25 \times –1.5 \times assumes no microsite limitation. However, the results of this analysis were similar if we included the 3 \times , which suggests the results were not affected by the two species with saturating recruitment functions in 2017 (see below). The response variable (mean proportional recruitment) was \ln transformed to improve normality, with a small number added (+0.001, but the analyses produced similar results if we added 0.01) so that zeros could be transformed (Ives 2015). Transformed mean proportional recruitment was standardized to a mean of zero and a standard deviation of 1 by species so that species with differences in emergence rates could be directly compared across the PC1 and PC2 gradients. Predictor variables included species, PC1, PC2, and interactions between each PC variable and species. Random effects included site and species nested within site to account for the multiple species within sites when estimating the PC1 and PC2 main effects.

Models were fit using the *lmer* function in the *lme4* package in R (Bates et al. 2013). Denominator degrees of freedom were estimated using the Satterthwaite approximation using the *anova* function in the *lmerTest* package (Kuznetsova et al. 2016). Relationships (i.e., slopes) between proportional recruitment and PC axis scores were evaluated using the *emtrends* function in the *emmeans* package (Lenth et al. 2018) when we detected a significant species \times PC axis interaction. To determine how the relationships between proportional recruitment and PC axis scores varied with seed size of the species, we correlated the estimated slopes with seed weight for each species.

To determine how rodent exclusion influenced proportional recruitment, and how this varied among species and years (Hypothesis 3), we used a linear mixed-effects model, with proportional recruitment (\ln transformed) averaged across the 0.25 \times –3 \times seed addition plots at each site as the response variable. Predictor variables were rodent-exclusion treatment, species, and year, and all interactions. Random effects included site and each factor nested within site to account for the multiple measures within each treatment and site. Denominator degrees of freedom were calculated using the Satterthwaite approximation. We used the *emmeans* function in the *emmeans* package to evaluate contrasts on significant interaction terms (Lenth et al. 2018). To determine how the rodent effect varied with seed size of the different species, we correlated the estimated rodent effect with seed weight for each species. For this analysis and the one above that included all species, we considered accounting for a potential phylogenetic signal in seed size among the focal species. However, after examining a pruned phylogeny containing our focal species, it was clear that closely related species did not have similar seed sizes. Because there was clearly no phylogenetic signal in

seed size, we did not include phylogenetically controlled contrasts in our analyses.

RESULTS

In both years there was high emergence of seedlings of target species in our seed addition plots. However, the “0” seeds-added plots had very few seedlings (Fig. 1).

Hypothesis 1: Large-seeded species are seed limited, which should be characterized by a recruitment function that saturates beyond seed input densities that approximate average fecundity. In contrast, small-seeded species are microsite limited, which implies a recruitment function that saturates near or before seed input densities near average fecundity.

In 2017, the saturating parameter (n) was only estimable for 6 of the 14 species for which we had full data, and only three of these species had values of n within

realistic (i.e., near mean) levels of fecundity (Table 1). The remaining eight species had linear recruitment functions (Fig. 1). Thus, 13 out of 16 target species resided on the linear portion of their recruitment functions. In the –rodent plots in 2018, saturating parameters (n) were estimable for 12 of 16 species, but only 4 species had values of n within realistic ranges of fecundity values (Table 1). In the +rodent plots in 2018, saturating parameters (n) were estimable for 14 of 16 species, with 6 species having values within realistic ranges of mean fecundity (Table 1). Thus, most species exhibited seed-limited recruitment and the relative degree of microsite limitation (estimated by n /mean fecundity) was not significantly correlated with seed size in 2017 ($F_{1,3} = 0.02$, $P = 0.88$), or in 2018 (–rodent plots; $F_{1,10} = 0.86$, $P = 0.37$; +rodent plots; $F_{1,12} = 1.8$, $P = 0.20$).

Proportional recruitment as estimated by the b parameter from the recruitment function models was strongly positively correlated with seed size in 2017 ($F_{1,12} = 9.7$, $P = 0.008$) and 2018 (–rodent plots; $F_{1,14} = 31.0$, $P < 0.0001$; +rodent plots; $F_{1,14} = 5.9$, $P = 0.029$).

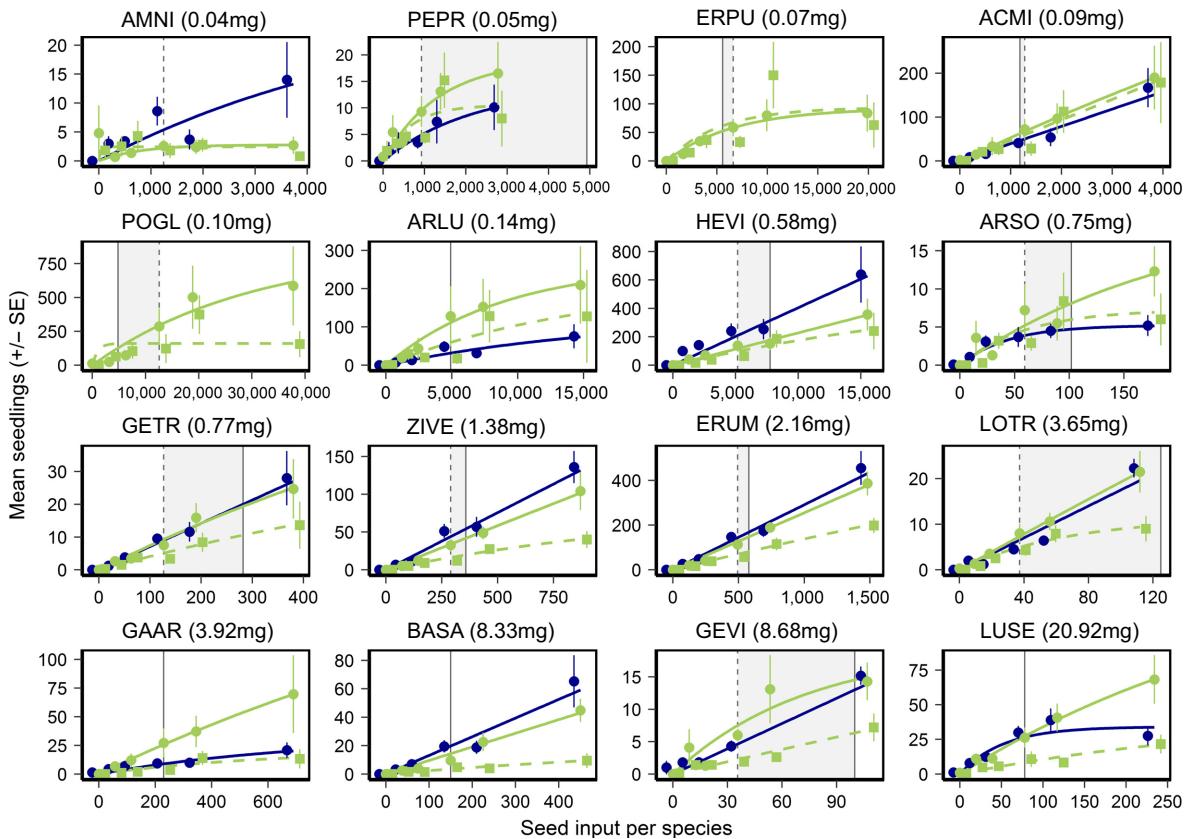


FIG. 1. Recruitment functions for 16 co-occurring forb species (see Table 1 for key to species abbreviations). Recruitment data are from plots where seeds of each target species was added at 0×, 0.25×, 0.5×, 1.0×, 1.5×, or 3× their estimated fecundity (as estimated in 2016). Blue lines, plots initiated in 2016; green lines, plots initiated in 2017. Dashed lines are recruitment functions for species outside of rodent enclosures (2017 only), solid lines are recruitment functions inside rodent enclosures. Vertical lines represent estimated mean fecundity of each species (dashed, estimated in 2016; solid, estimated in 2018); shading depicts range between fecundity estimates in different years. Panels are arranged with smallest-seeded species in upper left corner, largest-seeded species in lower right corner.

Hypothesis 2: Recruitment limitation, as characterized by the proportion of seeds that germinate, is more extreme at more productive sites because of preemption of microsites, with small-seeded species being more sensitive to this site-level variation than large-seeded species.

Sites varied substantially in fertility and productivity (see Appendix S2: Fig. S1). The first PCA axis explained 67.6% of the variation among sites, revealing a strong gradient in total aboveground biomass, total available nitrogen, and soil organic matter. Higher PC1 values indicate less productive sites. The second axis explained 23.6% of the variation among sites and revealed some variation in soil phosphorus among sites (see Appendix S2: Fig. S1).

For all target species combined, the proportion of seeds that recruited as seedlings was not significantly related to site productivity, as summarized by PC1 ($F_{1,7} = 3.5$, $P = 0.10$) or soil P, as summarized by PC2 ($F_{1,7} = 0.04$, $P = 0.86$). However, there was a significant species \times PC1 interaction ($F_{15,105} = 2.3$, $P = 0.007$), with 5 of the 16 species increasing in proportional recruitment at sites with higher PC1 values (i.e., increasing recruitment with decreasing productivity; ACMI, ANMI, ARSO, ERPU, and HEVI; Appendix S2: Table S1). Of the species that had significant correlations between site PCA and the proportion of seeds that established as seedlings, most were small-seeded (Fig. 2A), whereas larger-seeded species did not respond significantly to the productivity gradient (Fig. 2A). The correlation between seed size and response to the environmental gradient was marginally significantly negative ($r = -0.45$, $P = 0.077$, Fig. 2A). The species \times PC2 interaction was not significant ($F_{15,105} = 1.4$, $P = 0.16$).

Hypothesis 3: Seed limited recruitment is exacerbated by rodent granivory, especially for large-seeded species.

Proportional recruitment was greater in rodent exclusion compared to control plots (main effect of rodents: $F_{1,9} = 16.7$, $P = 0.002$), but varied by species (main effect of species: $F_{15,270} = 68.4$, $P < 0.0001$) but not significantly among years (main effect of year: $F_{1,288} = 0.76$, $P = 0.38$). Proportional recruitment of some species differed between the 2 yr (year \times species interaction: $F_{15,288} = 4.1$, $P < 0.0001$); three species had greater recruitment in 2018 compared to 2017 and four species had greater recruitment in 2017; Appendix S3: Table S1) and the effects of rodent exclusion differed among species (species \times rodent exclusion interaction, $F_{15,288} = 1.9$, $P = 0.022$). Recruitment for the 9 largest of the 16 species was significantly reduced in the presence of rodents compared to inside rodent exclusion plots (post hoc comparisons; species with reduced recruitment because of rodents are ARSO, BASA, ERUM, GAAR, GETR, GEVI, LOTR, LUSE, and ZIVE; Appendix S3: Table S1). The year \times rodent exclusion and three-way

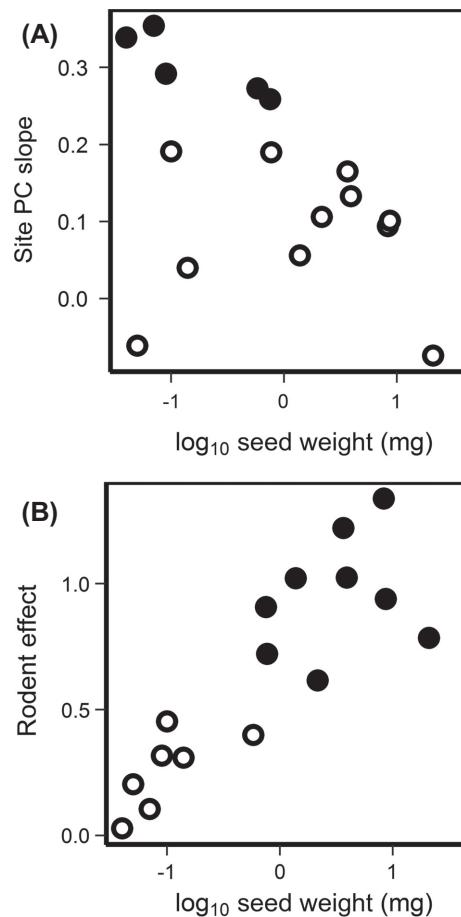


FIG. 2. Relationship between \log_{10} seed weight of target species and (A) the slope of the relationship between site PCA value and proportional recruitment (averaged across $0.25 \times -1.5 \times$ fecundity plots). Each dot represents a focal species. Higher values on the y-axis indicate that a greater proportion of seeds establish as seedlings at less vs. more productive sites. (B) The mean difference in the proportion of seeds that establish as seedlings in vs. out of rodent exclosures (averaged across $0.25 \times -1.5 \times$ fecundity plots). Greater values on the y-axis indicate a greater difference in proportional recruitment in vs. out of exclosures. In both (A) and (B), filled points are those species with statistically significant site PCA or rodent effects.

interactions were not significant ($P > 0.38$). The correlation between seed size and response to rodent exclusion was strongly positive ($r = 0.85$, $P < 0.001$, Fig. 2B).

Patterns of natural recruitment

The average fecundity of target species was significantly and positively related to the average number of seedlings censused around individuals of those species across four sites ($F_{1,14} = 44.0$, $R^2 = 0.76$, $P < 0.0001$; Fig. 3).

DISCUSSION

A paradigm in the literature is that propagule size and number fundamentally influence patterns of plant

recruitment (Harper 1977, Crawley 1990, Eriksson and Ehrlén 1992, Turnbull et al. 2000) and coexistence (Grubb 1986, Rees et al. 2001). More fecund small-seeded species are often assumed to be mostly microsite limited, a phenomenon characterized by a saturating recruitment function. In other words, at mean fecundity, recruitment occurs at or beyond the asymptote of the species' recruitment curve. In contrast, less fecund large-seeded species are thought to be primarily seed limited, which is characterized by a linear recruitment function. Yet counter to these ideas (and in opposition to our Hypothesis 1), we found that most target species had linear recruitment functions estimated over a seed input range from 0 to $3\times$ fecundity (Fig. 1). For species with estimated saturating recruitment functions, saturation occurred at much higher values than the mean fecundity of that species (compare mean fecundity to parameter " n " values; Table 1). Only 3–6 species (depending on treatment and year) exhibited saturating recruitment (i.e., $n > \text{fecundity}$) near their mean fecundity (Table 1). Moreover, we did not find that small-seeded species had recruitment saturation occurring closer to their mean fecundity and large-seeded species had recruitment occurring far away from saturation, as predicted by theory. In fact, there was no correlation between seed size and whether species had linear or saturating recruitment at mean fecundity. These results are similar to those found by García-Meza and Martorell (2016). Thus, in the perennial grasslands we studied it appears that seed limitation is more common than microsite limitation, a result also found in some other systems (Eriksson and Ehrlén 1992, Turnbull et al. 2000, Clark et al. 2007, Poulsen et al. 2007).

The question of how plant species coexist that differ by orders of magnitude in seed size and fecundity is fundamental to plant ecology (Rees et al. 2001, Muller-Landau 2010). It is commonly assumed that microsite availability limits recruitment of highly fecund

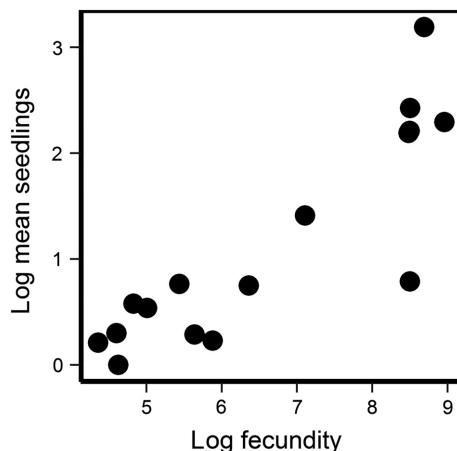


FIG. 3. Relationship between mean natural log fecundity of each target species and mean natural log recruitment around randomly chosen focal plants (see Methods for details). Each point represents a different focal species.

small-seeded species, allowing for more equitable recruitment of small- and large-seeded species. However, we found that recruitment saturated at the mean fecundity of a relatively small subset of our target species, and these species were not exclusively small-seeded. One plausible explanation for this surprising result is that unusually good weather conditions alleviated key stressors on seedling establishment. Rainfall in both spring (April–June) 2017 and 2018 was $>40\%$ higher than the 30-yr average (Menne et al. 2012). Furthermore, there were not prolonged periods with warm temperatures and drying conditions, which can often kill young seedlings in the weeks after emergence. Thus, moderate temperatures coupled with high and consistent precipitation may have alleviated microsite limitation in this normally moisture-limited grassland system. If so, it suggests that episodic recruitment events play an important role in structuring perennial grassland communities by mitigating constraints that often limit the recruitment of small-seeded species. For species that did have saturating recruitment near their mean fecundity, we cannot rule out the possibility that competition for microsites with other seeded species, especially at high seeding rates, might have caused this pattern.

What do the mostly linear recruitment functions we documented imply for coexistence? Owing to higher seed inputs (to mimic their higher fecundity), and limited evidence for microsite limitation, across species there was a strongly linear relationship between seed input at average fecundity and the number of seedlings that established in both 2017 (Maron et al. 2018) and across both years (ANOVA; $F_{1,56} = 53$, $P < 0.0001$). These results were fairly consistent across the 2 yr over which we replicated our experiment (ANOVA; year effect: $F_{1,56} = 0.32$, $P = 0.57$), and suggest that, at least in these wet springs, recruitment mirrors a lottery process (Sale 1977, Chesson and Warner 1981), where the magnitude of recruitment across species is correlated with the number of propagules in the propagule pool. The fact that we found similar patterns of natural recruitment (Fig. 3) bolsters this interpretation. An important caveat in interpreting these results, however, is that we do not know the extent to which compensatory density-dependent mortality operates on older life stages. If strong, compensatory density dependence would cause recruitment functions of small-seeded species that recruited at high density to become flatter through time. Interestingly, however, in censuses of experimental plots we initiated in 2016 in summer 2018, we found surprisingly little evidence of density dependence. Recruitment functions of many focal species were still estimated to be mostly linear (unpublished data). Thus, it does not appear that density-dependent juvenile plant mortality strongly compensates for greater recruitment of more fecund species, but again good precipitation conditions over this interval may have influenced these results.

At the community level, because the magnitude of seedling recruitment was positively correlated with

species' fecundity, it begs the question of what would happen if we added seeds to experimental plots at equal densities. To answer this, we extrapolated the linear recruitment functions of large-seeded species in order to determine how recruitment would be affected if all target species were added to plots at the same seed densities. This revealed that the abundance of seedlings of large-seeded species would be much greater than the abundance of seedlings of small-seeded species (see Appendix S4: Table S1). In fact, at equal seed number input, there was a significant positive relationship between seed weight and seedling abundance ($R^2 = 0.49$, $P = 0.05$). This mirrors results reported by Turnbull et al. (1999), who found that at higher (but equal) rates of seed input among species, large-seeded species predominate. The fact that a lesser proportion of seeds of small- vs. large-seeded species established could be due to these species having lower viability or greater seed dormancy than larger-seeded species. However, in the perennial grasslands studied here, there does not appear to be a large long-lasting seed bank for most species. Recruitment in plots where no seeds were added was nil or very low through time for most species, consistent with other seed addition experiments we have performed (Maron et al. 2012). Nevertheless, lower proportional recruitment due either to viability or dormancy differences would limit the abundance of highly fecund species in any given year, relative to large-seeded less fecund species that have higher proportional germination.

Consistent with our second hypothesis, we found differences among species in the degree to which recruitment was suppressed by site-level productivity. Five species, mostly small-seeded ones, tended to have proportionally lower recruitment at high vs. lower productivity sites (as shown by the significant PC1 \times species interaction). In contrast, this was not the case for larger-seeded species (Fig. 2A, Appendix S2: Table S1). One explanation for this is that smaller-seeded species tend to be more sensitive to competition from the dominant bunchgrasses, whereas larger-seeded species are proportionally less affected. This correlational pattern has been confirmed in experiments where we have removed existing bunchgrass from plots and found that this has a greater positive effect on the recruitment of small- than large-seeded species (Maron et al. 2018). Our results support other empirical work showing that small-seeded species may have greater difficulty than large-seeded species recruiting into sites because of competition from the dominant vegetation and their litter (Leishman and Westoby 1994, Leishman et al. 2000, Moles and Westoby 2004). It also begins to confirm the theory that shows that differential responses to environmental conditions by species based on their seed size can importantly help maintain seed size and species diversity within communities (Muller-Landau 2010).

Our data also affirmed our third hypothesis, that seed predation would have greater suppressive effects on the

recruitment of large- vs. small-seeded species (Fig. 2B). By influencing the recruitment function of large-seeded species, postdispersal seed predation by mice resulted in even greater seed limitation on the establishment of these species. Collectively, these findings build on earlier work from the same system (Maron et al. 2018) and suggest that at the community level, generalist consumers can play an important role in regulating the relative abundance of large-seeded species (Brown and Heske 1990, Larios et al. 2017).

In our perennial bunchgrass system, where dominant bunchgrasses can impede recruitment (Maron et al. 2018), we expected that small-seeded species would generally experience microsite rather than seed-limited recruitment. Surprisingly, however, our experiments suggest that at least in some years, highly fecund small-seeded species can recruit abundantly, and gain numerical advantages because of their higher fecundity. This advantage is tempered, however, by the fact that small-seeded species also have (not surprisingly) lower *proportional* recruitment. As well, some small-seeded species appear more sensitive to variation in recruitment conditions across sites, whereas this is less the case for large-seeded species. However, large-seeded species are disadvantaged because of size-selective postdispersal seed predation. Thus, collectively, our results show that numerous biotic mechanisms (i.e., seed predation by mice and competition from dominant bunchgrasses) simultaneously contribute to variation in the strength of recruitment, and potentially coexistence, for species that vary in fecundity and seed size. A key question moving forward is how patterns that play out at the recruitment stage manifest over longer time periods. By following the fate of plants in our plots through time, we hope to explore the longer-term implications of the patterns reported here, and gain additional insight into how species of different seed sizes and fecundity coexist.

ACKNOWLEDGMENTS

We gratefully acknowledge the field assistance we received from J. Battisti, L. Beegle, T. Brown, R. Butler, R. Hegstad, M. McIntosh, M. Samuli, and C. Skidmore. Thanks to Montana Fish Wildlife and Parks, the U.S. Fish and Wildlife Service, Mary Shaffer Perelman, The Petersons, and the E Bar L Ranch for allowing us to work on their property. Thanks to S. Egge-man, K. Ertl, and J. Tollefson who graciously helped with project logistics. We thank Truman Young and an anonymous reviewer for helpful comments on the manuscript. Research was supported by National Science Foundation grant DEB-1553518 to JLM and DEP.

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DATA AVAILABILITY

Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.0d0k824>