

# The relative importance of the species pool, productivity and disturbance in regulating grassland plant species richness: a field experiment

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## Summary

1. Ecologists have generally focused on how species interactions and available niches control species richness. However, the number of species in the regional species pool may also control richness. Moreover, the relative influence of the species pool and species interactions on plant richness may change along productivity and disturbance gradients.

2. We test the hypothesis that many species from the propagule pool will colonize into habitats of moderate productivity and moderate disturbance, and the number of species in the pool will, therefore, primarily control plant species richness in such habitats. The hypothesis also states that few species from the propagule pool will colonize into habitats of high productivity and minimal disturbance because competitive species interactions primarily control plant richness in such habitats.

3. To test this hypothesis, we experimentally varied resource availability via fertilization and irrigation, the size of the available propagule pool via sowing the seeds of 49 species and disturbance via vegetation clipping.

4. A larger propagule pool increased species richness 80% in the absence of fertilization and the presence of clipping but had no significant effect on richness in the presence of fertilization and the absence of clipping (significant fertilization  $\times$  clipping  $\times$  seed addition interaction). Irrigation increased species richness primarily in the absence of fertilization and the presence or clipping (significant fertilization  $\times$  clipping  $\times$  irrigation interaction).

5. *Synthesis.* These results support the assertion that the control of plant species richness shifts from the number of species in the propagule pool to the intensity of species interactions as productivity due to soil fertility increases and disturbance decreases. However, increases in productivity due to irrigation caused an increase in species richness, probably because of reduced seedling desiccation. Other studies have found similar outcomes along natural productivity gradients where productivity is confounded with other variables, but this study is the first to find such an outcome along an experimental productivity gradient. These results suggest habitats with moderate soil fertility and disturbance may be more open to colonization from the propagule pool than other habitats, a finding that has implications for plant restoration and invasive species management.

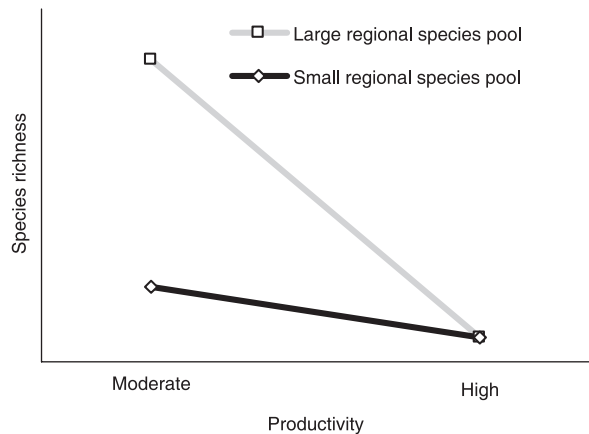
**Key-words:** hump-shaped relationship, local versus regional processes, Midwest, nitrogen, plant biomass, productivity-biodiversity, seed dispersal, successional grassland, temporal variability, water availability

## Introduction

Both species interactions and species pools are likely to affect species richness, but their relative importance may change along gradients of productivity and disturbance (Pärtel *et al.*

2000). We define species interactions as interactions occurring between individuals in close proximity to each other, and we define the species pool, as all species that may potentially disperse to and survive in a given location. Consistent with previous models of plant community organization (Grime 1977; Huston 1979), Huston (1999) and Foster *et al.* (2004) hypothesized that limitations on plant richness shift from the size of the species pool to the intensity of species interactions as one moves from moderate to high productivity (Shifting

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**Fig. 1.** The relative importance of the regional species pool in determining species richness under conditions of moderate and high productivity, as predicted by the Shifting Limitation Hypothesis and Huston's (1999) hypothesis.

Limitation Hypothesis; Fig. 1), and they hypothesized that moderate disturbance would counteract competitive exclusion within habitats of high potential productivity, leaving such habitats open to colonization from the regional species pool. This hypothesis assumes that high productivity will decrease species richness and colonization, which is generally true in the presence of fertilization (DiTommaso & Aarssen 1989). However, fertilization is not the only factor that affects productivity. Water availability can also limit productivity under dry conditions and increases in water availability can increase plant species richness (Stevens *et al.* 2006), possibly because of reduced seedling desiccation. Therefore, studies that examine the role of productivity on plant species richness and colonization should test the effects of both fertilization and increased water availability.

More generally, resources such as water availability vary over time, and species richness should be most limited by a lack of resources during times such as droughts (Foster & Dickson 2004) but should be most limited by the size of the species pool during times when resources for colonization and growth are adequate. Therefore, temporal windows of opportunity may exist for colonization from the species pool (Davis *et al.* 2000), and the relative importance of the species pool in controlling species richness may change over time.

Several recent experiments have simulated larger species pools via seed sowing. These experiments have tested the relative importance of the propagule pool and species interactions in controlling species richness along productivity gradients. Stevens *et al.* (2004) found that increasing the size of the propagule pool did not significantly affect total plant species richness at any fertilization level, which is unexpected since recruitment of new species decreased under fertilized conditions in their study. In contrast, several studies found that larger propagule pools led to a greater increase in richness at low-moderate than at high standing crop biomass in the absence of disturbance (Foster 2001; Foster *et al.* 2004; Houseman & Gross 2006). In addition, the Foster (2001) and Foster *et al.* (2004) studies showed that moderate disturbance

led to increased colonization especially at high biomass levels. However, the experiments of Foster (2001), Foster *et al.* (2004), and Houseman & Gross (2006) were conducted along natural complex topographic gradients where productivity, resource availability (nutrients and water) and disturbance all spatially co-varied and were confounded with topographic position and soil texture (Perring 1959; Hogenbirk & Reader 1989). Therefore, the effects of productivity and disturbance could not be completely separated from the effects of other variables.

Here, we present results from a multi-species sowing experiment conducted within the same grassland as Foster (2001) and Foster *et al.* (2004). Our experiment was designed to experimentally isolate the effects of factors that control productivity (nutrient and water availability) and disturbance (vegetation clipping) from the effects of other factors that co-vary along naturally occurring topographic productivity gradients, and to examine how nutrient availability, water availability and clipping affect richness and colonization from the propagule pool.

## Methods

### STUDY SITE

The study was conducted at the Nelson Environmental Studies Area, which is part of the University of Kansas Field Station and Ecological Reserves (KSR) located in northeastern Kansas (95°19'W, 39°05'N). The experiment was established on a moderately unproductive ridge-top within a 20-ha, secondary grassland dominated by *Bromus inermis* and *Poa pratensis* (introduced perennial C<sub>3</sub> grasses). Although the site has a long history of use as cropland, it was maintained as a seeded hayfield for at least 20 years prior to abandonment in 1984. Soils are Pawnee clay loam and Grundy silty clay loam (montmorillonitic, mesic Aquic Argiudolls). The region has a humid continental climate with a mean annual temperature of 12.9 °C and mean annual precipitation of 93.0 cm. The distribution of precipitation is unimodal, peaking in June (Kettle & Whittemore 1991).

### EXPERIMENTAL DESIGN

In June 2001 we established a grid of 48, 2 × 2 m plots with 2 m walkways between each plot. We randomly applied a 2 × 2 × 2 factorial set of treatments, including fertilization (no fertilizer; 16 g N m<sup>-2</sup> year<sup>-1</sup>), irrigation (no irrigation; 2.54 cm of water m<sup>-2</sup> added if no precipitation had occurred for four consecutive days during the growing season) and seed sowing (no seeds sown; seeds of 49 species sown at 300 seeds m<sup>-2</sup> year<sup>-1</sup> per species). We also incorporated a split-plot design by randomly assigning a clipping treatment to either the north or south 1 × 2 m subplot within each plot. We clipped vegetation to approximately 10 cm in height each June and lightly raked to remove clipped vegetation, which inadvertently removed some litter but did not disturb the soil. The experiment was, therefore, essentially a four-factor design with the clipping factor represented at the subplot level. Each treatment was replicated six times.

We altered the habitat conditions before sowing seeds to make sure the sown species experienced the full effects of the treatments. Therefore, we irrigated, added fertilizer and clipped vegetation in 2001, but we did not sow seeds until 2002. We are unaware of any other studies that have manipulated habitat conditions before seed addition, which makes our results less susceptible to transient, first-year dynamics than other studies.

We used 29-3-4 N-P-K Scotts Turf Builder lawn fertilizer, and from 2001 to 2004 we applied the appropriate amount of fertilizer to add 8 g N m<sup>-2</sup> in April–May and 8 g N m<sup>-2</sup> in mid-summer. A total of 16 g N m<sup>-2</sup> year<sup>-1</sup> was used because this amount of nitrogen (N) is on the high end of what nearby landowners typically use to fertilize cool-season hay fields (B.D. Wood, personal communication).

Irrigation occurred May–September in 2001–03 but ceased at the end of the 2003 growing season to examine whether changes in species richness and percent cover caused by a long-term pulse of moisture availability would persist. The purpose of irrigating every fourth day without rain was to reduce drought stress during the growing season and to increase plant productivity when it was limited by water availability instead of nutrients. During the most severe drought periods in July–September of 2002 and 2003 (NDMC *et al.* 2007), we irrigated 15 and 14 times, respectively. Natural precipitation in July–September 2002 and 2003 was 11.2 and 27.6 cm, respectively, and our addition of 38.1 and 35.6 cm of water probably had large effects on water availability.

Seeds were sown March 2002 and May 2003. The viability of the seeds was found to generally be high in greenhouse germination trials (unpublished data). A rate of 300 seeds m<sup>-2</sup> year<sup>-1</sup> per species is much higher than is typical for prairie restoration (Diboll 1997) and is higher than natural seed rain rates for some species but lower than natural rates for other species (Rabinowitz & Rapp 1980). Our goal when sowing species was to make sure that multiple individuals of every species could germinate in every plot, and our sowing rate should have been sufficient for this goal. All sown species could be found within 2 km of the study site, except *Penstemon albidus*, which typically occurs in the western half of Kansas. Sixteen of the sown species were present within the broader 20-ha field site at the start of the study. Seeds were obtained from area seed suppliers (Stock Seed; Murdock, Nebraska and Delange Seed; Girard, Kansas) and were hand broadcast into plots. We shook the vegetation and litter in every plot to dislodge seeds and allow them to come in contact with the soil. Sown species represent a range of life histories, functional groups, historical origins and habitat affinities (Table 1). The sown species should all thrive in fertilized plots in the absence of competition from other species (Campbell & Grime 1992). It is possible that few species in temperate regions are adapted to competition at high productivity relative to tropical regions (Pärtel *et al.* 2007), which would support our prediction that the intensity of species interactions in our study prevents most colonization from the propagule pool at high productivity. Still, sown species such as *Andropogon gerardii*, *Panicum virgatum*, *Sorghastrum nutans* and *Vicia villosa* are often found under high productivity conditions (Lu *et al.* 2000; Baer *et al.* 2003) and would be expected to successfully colonize into fertilized plots, even though they rarely were observed to do so.

We attempted to neutralize any small changes in soil pH due to fertilization (Murphy 2004) by adding 10.2 g m<sup>-2</sup> of lime with 60% effective calcium carbonate to the soil surface of fertilized plots in May 2004 (Whitney & Lamond 1993).

#### DATA COLLECTION

To determine the effectiveness of irrigation, soil moisture levels were measured during 2003, the last year that irrigation was conducted. On 12 August 2003, volumetric soil moisture was measured using a TRIME-FM, time domain reflectometry system (Mesa Systems Co.; Medfield, Massachusetts) 4 days after an irrigation event and 2 days after 1.5 cm of rain. The addition of 1.5 cm of rain to every plot probably reduced observed treatment differences because the extremely low soil moisture in non-irrigated plots was somewhat ameliorated.

We attempted to measure soil moisture before the rain, but it was impossible because soils in this region become impenetrable when extremely dry (NDMC *et al.* 2007) and the rain was, therefore, necessary to allow insertion of the soil probe in non-irrigated plots. Each measurement integrated soil moisture over the top 11 cm of soil.

The plant species richness and percent cover data reported here were collected during vegetation surveys on 8–12 June and 8–11 August 2004. We also report total live percent cover from 13–18 June and 27–30 September 2002 as an indicator of productivity during a drought year. For each subplot, all vegetation within a 141 × 71 cm (1 m<sup>2</sup>) quadrat was visually identified to species, and each species was assigned a value equal to the percentage of the ground within the quadrat that it covered. Bare ground and litter were also assigned cover values. The percent cover of all species could sum to more than 100%, since the canopy of different species often overlapped. All taxonomy follows the PLANTS database (USDA & NRCS 2007), and the authority for each species name can be found in this database.

Biomass and litter were collected from each subplot by clipping a 7.62 × 80 cm strip of vegetation to ground level. Strips were clipped 21–30 July 2004, and were sorted to live species or litter within 3 days of clipping. Sorted biomass and litter were dried for at least 96 h at 74 °C before being weighed. Biomass generally underestimates net primary productivity, but sites with higher biomass are likely to also have higher productivity (Scurlock *et al.* 2002). We refer to our unfertilized treatment as ‘moderate productivity’ rather than ‘low productivity’ because aboveground biomass in our control treatment (mean 411 g m<sup>-2</sup>; range 288–566 g m<sup>-2</sup>) was moderate compared to the range of aboveground biomass at grassland Long-term Ecological Research sites in the USA (Gross *et al.* 2000).

Light levels were measured 26–31 July 2004 and 25–26 July 2002, using an AccuPAR Model PAR-80 ceptometer (Decagon Devices, Inc.; Pullman, Washington). We avoided biomass collection strips when measuring light. For each subplot, we collected three samples in full sunlight and collected four ground-level samples spaced throughout the subplot. Ground-level samples were below vegetation but above the litter layer as much as possible. We then calculated the percentage of light interception for each subplot [1 – (sunlight at ground level/full sunlight)]. Light data were taken between the hours of 10 AM to 2 PM at times when no clouds obscured the sun. The instantaneous light measurements were taken along an 80-cm probe, and the measurements, therefore, averaged across sunflecks and areas shaded by vegetation.

Soil N availability was measured with cation and anion membrane resin strips (Ionics Inc; Watertown, Massachusetts). One anion (for nitrate + nitrite) and one cation (for ammonium) membrane strip (5 × 2.5 cm) was placed into the top 5 cm of the soil in each subplot on 1 July 2004. All membrane strips were removed 8 days later, washed with deionized water, placed into individual Ziploc bags, and refrigerated for 1 day. The N membrane strips were then extracted for 1 h with 17.5 mL of 1 M KCl. The KCl extract was stored in 20-mL plastic scintillation vials, frozen for 6 months and completely thawed prior to analysis. Ammonium and nitrate + nitrite levels were measured colourimetrically on a Lachat Autoanalyzer (Loveland, Colorado) equipped with ammonium and nitrate + nitrite channels. Ammonium and nitrate + nitrite values were summed to obtain estimates of total N availability over the burial period.

#### DATA ANALYSIS

We combined spring and summer species richness and percent cover data for all statistical analyses. We combined the data by calculating

**Table 1.** Characteristics of sown species and their occurrences and percent cover in non-sown and sown subplots in 2004

Species	Life form*	Origin†	Habitat‡	Number of subplots occupied	
				Non-sown	(sown)
<i>Achillea millefolium</i>	PF	N	G, O, P	0	(0)
<i>Agastache nepetoides</i>	PF	N	F	0	(1)
<i>Amorpha canescens</i>	PL	N	P	0	(19)
<i>Andropogon gerardii</i>	C <sub>4</sub> -PG	N	G, P	0	(10)
<i>Asclepias tuberosa</i>	PF	N	G, P	0	(20)
<i>Astragalus canadensis</i>	PL	N	F	0	(4)
<i>Bouteloua curtipendula</i>	C <sub>4</sub> -PG	N	P	0	(4)
<i>Chamaecrista fasciculata</i>	AL	N	W, O, P	0	(32)
<i>Dactylis glomerata</i>	C <sub>3</sub> -PG	I	F, G, O	0	(0)
<i>Dalea candida</i>	PL	N	P	0	(2)
<i>Dalea purpurea</i>	PL	N	P	0	(1)
<i>Desmanthus illinoensis</i>	PL	N	O, P	0	(24)
<i>Echinacea pallida</i>	PF	N	P	0	(7)
<i>Elymus canadensis</i>	C <sub>3</sub> -PG	N	F, G, P	0	(0)
<i>Eragrostis trichodes</i>	C <sub>3</sub> -PG	N	F, P	0	(0)
<i>Eryngium yuccifolium</i>	PF	N	P	0	(0)
<i>Festuca ovina</i>	C <sub>3</sub> -PG	I	O	0	(0)
<i>Gentiana alba</i>	PF	N	P	0	(0)
<i>Helianthus maximiliani</i>	PF	N	P	0	(3)
<i>Heliopsis helianthoides</i>	PF	N	F, G	0	(14)
<i>Hesperis matronalis</i>	PF	I	O	0	(0)
<i>Lespedeza capitata</i>	PL	N	P	0	(22)
<i>Leucanthemum vulgare</i>	PF	I	G	0	(2)
<i>Liatris pycnostachya</i>	PF	N	G, P	0	(0)
<i>Medicago sativa</i>	PL	I	G, O	0	(0)
<i>Melilotus officinalis</i>	BL	I	G, O	0	(2)
<i>Monarda fistulosa</i>	PF	N	F	0	(26)
<i>Oenothera macrocarpa</i>	PF	N	P	0	(3)
<i>Oligoneuron rigidum</i>	PF	N	G, P	0	(1)
<i>Panicum virgatum</i>	C <sub>4</sub> -PG	N	G, P	0	(8)
<i>Penstemon albidus</i>	PF	N	P	0	(1)
<i>Phleum pratense</i>	C <sub>3</sub> -PG	I	G, O	0	(1)
<i>Ratibida columnifera</i>	PF	N	P	0	(11)
<i>Ratibida pinnata</i>	PF	N	G, P	0	(3)
<i>Rudbeckia hirta</i>	BF	N	G, O, P	0	(13)
<i>Salvia azurea</i>	PF	N	P	1	(8)
<i>Schedonorus phoenix</i>	C <sub>3</sub> -PG	I	G	0	(0)
<i>Schizachyrium scoparium</i>	C <sub>4</sub> -PG	N	G, P	0	(5)
<i>Silphium perfoliatum</i>	PF	N	A	0	(0)
<i>Sorghastrum nutans</i>	C <sub>4</sub> -PG	N	P	0	(4)
<i>Sporobolus cryptandrus</i>	C <sub>4</sub> -PG	N	G	0	(0)
<i>Symphotrichum novae-angliae</i>	PF	N	P	0	(0)
<i>Teucrium canadense</i>	PF	N	G, O	0	(0)
<i>Trifolium pratense</i>	BL	I	G, O	0	(7)
<i>Trifolium repens</i>	PL	I	O	0	(2)
<i>Verbena stricta</i>	PF	N	O	0	(14)
<i>Vicia villosa</i>	AL	I	O	0	(12)
Species not analyzed as sown					
<i>Eupatorium altissimum</i>	PF	N	G, O, P	7	(5)
<i>Poa pratensis</i>	C <sub>3</sub> -PG	I	G, O, P	48	(48)

Number of subplots occupied refers to the number of non-sown or sown subplots ( $n = 48$  for each category) where a given species was recorded in 2004. Average percent cover refers to the average cover for each species when examining data from the spring and summer in subplots where the species was found. Habitat classifications follow Kettle & Whittemore (1991). Taxonomy, life form, and origin follow the PLANTS database (USDA & NRCS 2007).

\*A = annual, B = biennial, P = perennial, F = non-legume forb, G = grass, L = legume.

†I = introduced, N = native.

‡F = forest, G = cool-season grassland, O = successional and disturbed areas, P = prairie, W = wetland.

richness and cover values for each subplot in both the spring and summer and then averaging these values for each subplot. In contrast, for the number of species occurrences per subplot shown in Table 1, we counted one species occurrence whether a particular species occupied a subplot in the spring, summer or both. Percent cover values in Table 1 were calculated by taking all subplots where a given species was present and then averaging percent cover values over the spring and summer for these subplots (for example, if a species was not found in the spring and was found at 1% cover in only one subplot in the summer, its average percent cover would be 0.5%).

When performing analyses on 2004 richness and percent cover, we chose to count *Poa pratensis* and *Eupatorium altissimum* as non-sown species, even though they were sown as seed, since they were present before the experiment began, occurred most often in non-sown subplots, and their percent cover was not significantly affected by sowing. We examined the richness and cover of non-sown species to determine whether those species that must have established from seed during the course of the experiment (annual + biennial) were affected by the experimental treatments in the same way as sown species and differently from species less dependent on colonization (perennial). We, therefore, classified species as annual, biennial, or perennial based on the PLANTS database (USDA & NRCS 2007).

All data analyses were completed in SAS for Windows 8.02 using type III sum of squares and an  $\alpha = 0.05$  level of significance (see Appendix S1 in Supplementary Material). We used twelve response variables in our general linear model analyses: soil moisture, total available N, aboveground biomass, litter, 2004 and 2002 light interception, 2002 total live percent cover, 2004 sown species richness and percent cover, 2004 perennial non-sown species richness and percent cover and 2004 total species richness. Due to violation of normality assumptions, we performed nonparametric Mann-Whitney *U* tests on annual + biennial richness and cover data, except when examining the effects of clipping (paired *t*-tests of non-clipped versus clipped subplots). We transformed other data to reduce heteroscedasticity. We log<sub>10</sub> transformed biomass, litter, and N data and square root transformed 2004 sown species richness and percent cover data and perennial non-sown species richness and percent cover data. We used a Box-Cox transformation (Sokal & Rohlf 1995) for 2004 light interception data ( $\lambda = 4.47$ ) and for the total species richness data ( $\lambda = 0.75$ ). Soil moisture, 2002 light interception and 2002 total live percent cover data remained untransformed.

**Results**

RESOURCE AVAILABILITY AND INDICATORS OF PRODUCTIVITY

Fertilization increased aboveground biomass, soil N availability and litter (Table 2). Fertilization also increased 2004 canopy light interception more in clipped subplots (74–88%) than in non-clipped subplots (88–94%; clipping × fertilization interaction  $F_{1,40} = 6.33, P = 0.016$ ). Irrigation increased soil moisture and litter and decreased soil N availability in the non-clipped subplots only (0.38–0.11  $\mu\text{g cm}^{-2} \text{day}^{-1}$ ; clipping × irrigation interaction  $F_{1,40} = 8.09, P = 0.007$ ). Clipping decreased aboveground biomass, litter and soil moisture, and sowing increased aboveground biomass, litter and 2004 canopy light interception.

We also measured two indicators of productivity in the drought year of 2002 (Table 2). Irrigation affected 2002

**Table 2.** The changes in mean values of different response variables due to treatments

	2004 aboveground biomass	2004 litter	2004 light interception	2004 soil N availability	2003 soil moisture	2002 percent cover	2002 light interception
C	469–299 g m <sup>-2</sup> ***	347–179 g m <sup>-2</sup> ***	C × F interaction	C × I interaction	19.7–18.4%*	C × I interaction	C × F × I and C × S × I interaction
F	333–435 g m <sup>-2</sup> ***	207–319 g m <sup>-2</sup> ***	C × F interaction	0.07–0.29 $\mu\text{g cm}^{-2} \text{day}^{-1}$ ***	NS	84.0–92.1%***	C × F × I interaction
S	351–417 g m <sup>-2</sup> ***	250–275 g m <sup>-2</sup> *	84.1–87.5%**	NS	NS	NS	C × S × I interaction
I	NS	242–283 g m <sup>-2</sup> ***	NS	C × I interaction	18.2–19.9%*	C × I interaction	C × F × I and C × S × I interaction

The changes in means due to interactions are described in the results section.

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

NS = not significant, C = clipping, F = fertilization, S = sowing, I = irrigation.

total live percent cover more than any other treatment, with irrigation increasing cover from 85% to 99% in non-clipped subplots and from 73% to 95% in clipped subplots (clipping  $\times$  irrigation interaction  $F_{1,40} = 9.52$ ,  $P = 0.004$ ). Fertilization also increased total percent cover. Clipping affected 2002 light interception more than any other treatment, and irrigation increased light interception more than either fertilization or seed addition. Irrigation in non-clipped subplots increased light interception from 66% to 78% in the absence of fertilization, but only from 79% to 82% in the presence of fertilization, and irrigation in clipped subplots increased light interception from 26% to 38% in both the absence and presence of fertilization (clipping  $\times$  fertilization  $\times$  irrigation interaction  $F_{1,40} = 4.88$ ,  $P = 0.033$ ). Irrigation in non-clipped subplots increased light interception from 67% to 80% in the absence of seed sowing but only from 78% to 80% in the presence of sowing, and irrigation in clipped subplots increased light interception from 26% to 38% in both the absence and presence of sowing (clipping  $\times$  sowing  $\times$  irrigation interaction  $F_{1,40} = 4.40$ ,  $P = 0.042$ ).

#### RESPONSES OF SOWN SPECIES

In 2004, 32 of the 47 species analyzed as sown were recorded in at least one of the 48 sown subplots (Table 1). To test our hypothesis and examine the effects of treatments on colonization from the sown propagule pool, we analyzed 2004 sown species richness in sown plots only. Fertilization decreased sown richness, clipping increased sown richness and irrigation increased sown richness only in non-fertilized plots (fertilization  $\times$  irrigation interaction; Table 3; Fig. 2a).

We also examined the effects of fertilization, irrigation and clipping on the aggregate cover of sown species in sown plots only. Fertilization decreased sown cover and irrigation increased sown cover, but only in non-fertilized plots (fertilization  $\times$  irrigation interaction; Table 3; Fig. 2a). Clipping increased sown cover the most in non-fertilized subplots that were irrigated (clipping  $\times$  fertilization  $\times$  irrigation interaction).

#### RESPONSES OF NON-SOWN SPECIES

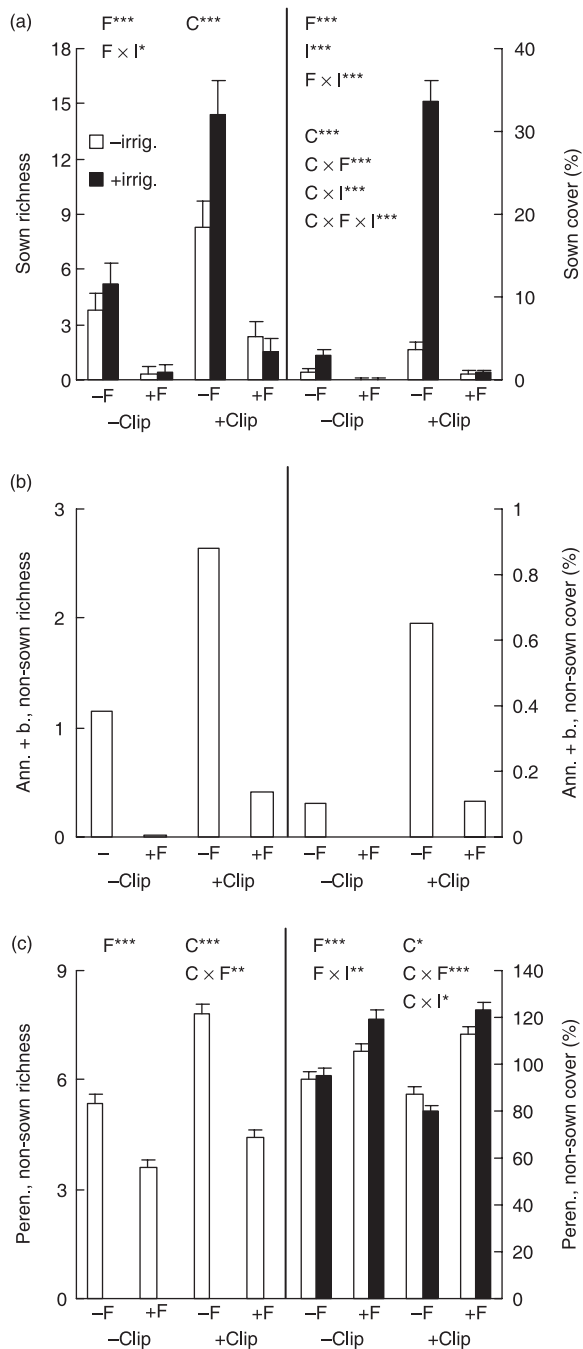
We examined whether non-sown species that must have colonized from seed during the course of the experiment (annual + biennial) would respond to treatments similarly as sown species and differently from species less dependent on colonization from seed (perennial). The effects of treatments on 2004 annual + biennial species richness and cover were very similar to the effects of treatments on sown richness. Annual + biennial richness was decreased by fertilization in both non-clipped ( $U = 90$ ,  $P < 0.001$ ) and clipped subplots ( $U = 32$ ,  $P < 0.001$ ), and was increased by clipping ( $t_{47} = 5.74$ ,  $P < 0.001$ ; Fig. 2b). Only one out of 24 non-clipped and fertilized subplots contained an annual + biennial non-sown species, whereas nine out of 24 clipped and fertilized subplots contained one or more annual + biennial species. Annual + biennial percent cover was decreased by fertilization in both non-clipped ( $U = 88$ ,  $P < 0.001$ ) and clipped subplots ( $U = 65$ ,  $P < 0.001$ ) and was increased by clipping ( $t_{47} = 4.30$ ,  $P < 0.001$ ; Fig. 2b). No other treatment significantly affected annual + biennial non-sown richness or cover.

**Table 3.** Effects of experimental treatments on habitat variables and plant community structure

	Richness			Cover	
	Sown	Perennial non-sown	Total	Sown	Perennial non-sown
<b>Split-plot</b>					
C	38.2+***	99.1+***	206+***	120+***	5.26-
C $\times$ F	2.83	15.6***	50.7***	56.1***	32.3***
C $\times$ S		0.741	32.8***		2.34
C $\times$ I	0.076	0.013	1.14	29.1***	5.38*
C $\times$ F $\times$ S		0.867	7.73**		3.70
C $\times$ F $\times$ I	2.14	1.32	8.20**	32.4***	1.14
C $\times$ S $\times$ I		1.99	0.715		3.36
C $\times$ F $\times$ S $\times$ I		2.27	2.82		0.910
Mean square error	0.320	0.029	0.448	23.7	0.130
<b>Main plot</b>					
F	115-***	80.7-***	262-***	180-***	96.4+***
S		3.37	42.9+***		0.686
I	2.09	1.85	1.91	56.2+***	2.53
F $\times$ S		1.71	21.7***		0.184
F $\times$ I	5.17*	0.001	1.86	54.3***	7.90**
S $\times$ I		0.687	2.32		2.42
F $\times$ S $\times$ I		0.626	4.04		1.77
Mean square error	0.370	0.089	1.01	29.3	0.411

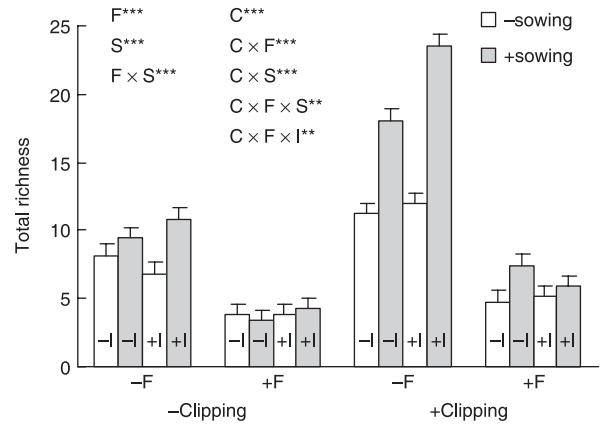
All values (except error values and sown cover and richness values) represent  $F_{1,40}$  values. Sown cover and sown richness values represent  $F_{1,20}$ . \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

C = clipping, F = fertilization, S = sowing, I = irrigation, - indicates a decrease and + an increase in the response variable due to the treatment main effect.



**Fig. 2.** Sown species richness and percent cover (a), annual + biennial non-sown species richness and percent cover (b), and perennial non-sown species richness and percent cover (c). Richness data are to the left of the vertical line and cover data are to the right. All error bars are +1 standard error. (F = fertilization, I = irrigation, C = clipping; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

The effects of treatments on 2004 perennial non-sown species richness and cover were much less dramatic than the effects of treatments on sown species and annual + biennial non-sown species. Fertilization decreased perennial richness and clipping increased perennial richness more in unfertilized plots than in fertilized plots (clipping  $\times$  fertilization interaction; Table 3; Fig. 2c). Fertilization increased perennial



**Fig. 3.** Total species richness. All error bars are +1 standard error. (F = fertilization, S = sowing, I = irrigation, C = clipping; \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

cover more in clipped subplots than in non-clipped subplots (clipping  $\times$  fertilization interaction) and more in irrigated plots than in non-irrigated plots (fertilization  $\times$  irrigation interaction; Table 3; Fig. 2c). Clipping decreased perennial cover only in irrigated plots (clipping  $\times$  irrigation interaction).

#### TOTAL SPECIES RICHNESS

The sum effects of treatments on sown richness, annual + biennial non-sown richness, and perennial non-sown richness led to significant changes in total species richness. Fertilization decreased 2004 total species richness and clipping increased total species richness more in non-fertilized and irrigated plots than in fertilized and non-irrigated plots (clipping  $\times$  fertilization  $\times$  irrigation interaction; Table 3; Fig. 3). Seed sowing generally led to an increase in total richness, but this response was strongly contingent upon fertilization and clipping (clipping  $\times$  fertilization  $\times$  sowing interaction). In the absence of clipping, fertilization prevented seed sowing from significantly increasing total richness (effect of seed sowing in unfertilized subplots,  $F_{1,69.6} = 10.7$ ,  $P = 0.002$ ; effect of seed sowing in fertilized subplots,  $F_{1,69.6} = 0.001$ ,  $P = 0.970$ ). However, in the presence of clipping, seed sowing increased total richness even in fertilized subplots, although sowing increased total richness more in unfertilized than in fertilized subplots (effect of seed sowing in unfertilized subplots,  $F_{1,69.6} = 98.6$ ,  $P < 0.001$ ; effect of seed sowing in fertilized subplots,  $F_{1,69.6} = 5.15$ ,  $P = 0.026$ ).

#### Discussion

Species interactions, and possibly niche availability, appear to control species richness at high fertility levels in the absence of clipping, since few to no species are able to establish from seed. However, a shift occurs at lower fertility in the presence of clipping, and species richness is largely controlled by the number of species in the propagule pool. Fertilization increases an indicator of productivity (aboveground biomass) in our study and clipping increases disturbance. Therefore, our fertilization and clipping results are generally consistent with

the Shifting Limitation Hypothesis and Huston's (1999) hypothesis since species richness is primarily controlled by species interactions at moderate productivity and disturbance levels but is primarily controlled by the size of the propagule pool at high productivity and low disturbance levels. However, fertilization is not the only factor that affects productivity. Water availability can also limit productivity in the Great Plains (Sala *et al.* 1988), and during a drought we found two indicators of productivity (total live percent cover and light interception) were largely controlled by irrigation. Interestingly, irrigation and fertilization have opposite effects on species richness even though both increased productivity at some point in our experiment. Other studies also note different effects of fertilization and irrigation on species richness (Carson & Pickett 1990; Goldberg & Miller 1990; Stevens *et al.* 2006), possibly because irrigation reduces seedling desiccation.

Overall, our results suggest that empirical and theoretical studies of species richness should not focus entirely on either species interactions and niches nor species pools and dispersal. Both species interactions and species pools are important along productivity and disturbance gradients, and both must be considered when studying plant species richness patterns.

#### POTENTIAL MECHANISMS

Our results suggest that the effects of high productivity and disturbance on species richness may be better understood by taking into account colonization from the propagule pool. In cases where high productivity decreases species richness, it has often been assumed that decreased richness is due to local extinction among already established species (Rosenzweig & Abramsky 1993). However, high productivity could decrease plant species richness largely by decreasing colonization from the propagule pool. Sown species and non-sown annual and biennial species must have established from seed during the course of our experiment in order to have been present in 2004. Essentially none of these species established from seed under fertilized conditions in the absence of clipping, whereas many of these species established from seed under non-fertilized conditions in the presence of clipping. We interpret a lack of establishment at high fertility in the absence of clipping as a lack of successful colonization. Species richness may, therefore, decline along fertility and disturbance gradients simply because more species are colonizing from the regional pool in habitats of moderate fertility and disturbance than habitats of high fertility and low disturbance. These declines in species richness along fertility and disturbance gradients could potentially occur irrespective of extinction rates among already established plants, even though extinction rates are also likely to change.

Other studies have found that fertilization decreases colonization rates, but these studies did not control the number of species in the propagule pool and, therefore, could not directly test our hypothesis (Fig. 1). Tilman (1993) showed that colonization decreased at high soil fertility, but extinction also increased. Kitajima & Tilman (1996) found that forb seed germination was significantly reduced by higher soil fertility, suggesting

that lower colonization rates at high fertility may be at least partly due to lower germination.

Although high productivity due to fertilization can apparently affect colonization rates as well as extinction rates, we are not sure of the underlying mechanisms whereby either extinction or colonization are affected by fertilization. Rosenzweig & Abramsky (1993) and Rajaniemi (2003) state in their reviews that no mechanism fully explains why species richness declines at high productivity. These same mechanisms can also be related to colonization, and none of the mechanisms fully explains why colonization declines at high productivity. Although we are not sure of the mechanisms by which fertilization decreases colonization rates, this study and others show that the effects of fertilization on colonization from the propagule pool must be considered along with the effects of fertilization on extinction of established plants. More generally, all of our treatments affect sown species and non-sown annual and biennial species much more strongly than non-sown perennial species, suggesting that many habitat conditions may affect colonization from the propagule pool more than extinction of established plants.

#### TEMPORAL CHANGES

Although we designed this study to determine the role of the propagule pool in controlling species richness at different levels of productivity and disturbance, the role of the propagule pool will also probably change as resources fluctuate over time (Davis *et al.* 2000). Probably more than any other resource in the Great Plains, precipitation shows the most extreme variation over time (Seastedt & Knapp 1993; Knapp & Seastedt 1998), and periods of drought often correspond with high temperatures and thus high rates of evapotranspiration. Seedlings frequently are killed during periods of drought and water stress (Fenner 1987), and severe to extreme droughts occurred while we irrigated and sowed seeds during the growing seasons of 2002 and 2003 (NDMC *et al.* 2007). Irrigation increased sown species richness and percent cover in unfertilized plots (see also Foster & Dickson 2004). Therefore, it seems that seedling establishment and growth may be constrained during times of low moisture availability, suggesting that pulses of adequate moisture availability can create windows of opportunity for establishment from the propagule pool (Davis *et al.* 2000). Pulses of moisture availability in combination with a large propagule pool may have long-lasting effects. In our study we still found strong effects of irrigation one year after the cessation of irrigation, and in other studies at the same field station we have noted that effects of propagule pool establishment due to past irrigation can persist for at least 6 years (unpublished data).

Davis *et al.* (2000) suggest that habitats will become more open to colonization from the species pool as resource supply increases beyond resource uptake. However, colonization from the propagule pool and richness both declined in our study as fertilization increased nutrient supply. As nutrient availability increased, the availability of other resources such as light decreased while litter increased and bare ground

decreased to almost zero percent (unpublished data). Plants compete for light (Wilson & Tilman 1991) and for space free of vegetation (Burke & Grime 1996) and litter (Foster & Gross 1997), and changes in these factors may have decreased species richness and colonization even as nutrient availability increased. Clipping and other disturbances may lessen the effects of fertilization on light levels, bare ground and litter and may reduce resource uptake relative to supply.

Moderate disturbances (Connell 1978) may also decrease the strong competitive interactions that may be present at high productivity (Huston 1979; Wilson & Keddy 1986; Campbell & Grime 1992; but see Wilson & Tilman 1991). Two previous studies completed in the same Kansas grassland as our study (Foster 2001; Foster *et al.* 2004) found that disturbance via winter raking in combination with spring clipping increased species richness and colonization more at high biomass than at moderate biomass. Other studies have also found the greatest abundance of colonizers in the presence of high levels of fertility and disturbance (Hobbs & Atkins 1988; Burke & Grime 1996). We found the opposite in our study, with clipping disturbance causing the largest increase in species richness and colonization in non-fertilized subplots. *Bromus inermis* and *Poa pratensis* were responsible for all of the increase in non-sown perennial cover due to fertilization, and these dominant species probably excluded other species early in the growing season (see also MacDougall & Wilson 2007). Disturbance occurred in our study after *B. inermis* and *P. pratensis* had completed most of their growth, whereas disturbance occurred earlier in the growing season for Foster (2001) and Foster *et al.* (2004). Therefore, our disturbances probably occurred too late to reduce many of the effects of fertilization (see also Howe 1994).

Although most ecological literature has focused on negative relationships between plant species richness and high productivity, meta-analyses have shown that positive or other relationships can occur (Gross *et al.* 2000; Mittelbach *et al.* 2001), even though negative relationships are the most common. Different relationships may occur partly because different factors, such as soil fertility and water availability, control the relationship between productivity and richness at different locations in space and time. The effects of irrigation on productivity are strongest under dry conditions, so irrigation may increase species richness more because of lower seedling mortality than it decreases species richness because of higher productivity. In contrast, where productivity is controlled by soil fertility, there is almost always a negative relationship between productivity and richness (DiTommaso & Aarssen 1989).

## Conclusions

This study is the first to examine the factors underlying productivity and disturbance gradients and find that species richness is primarily controlled by species interactions under fertilized conditions in the absence of disturbance but is primarily controlled by the number of species in the propagule pool under non-fertilized conditions in the presence of distur-

bance. The results of our study are similar to others (Foster 2001; Foster *et al.* 2004; Houseman & Gross 2006), even though these studies were conducted along topographic gradients and could not fully separate the effects of productivity from other co-varying factors. Although many of the plants from the propagule pool were present at low percent cover in our study, sown species have often persisted and grown into plants with high percent cover in other studies at the same field station (unpublished data). This study, along with previous studies, suggests both species interactions and the propagule pool must be considered when examining species richness and colonization across resource and disturbance gradients. This study also suggests that habitats with moderate productivity and disturbance will be open to colonization from the propagule pool in years with adequate precipitation. A habitat open to colonization presents both opportunities and challenges to land managers. Habitats that are open to colonization from the propagule pool may be ideal habitats to restore using seed addition, but habitats that are open to colonization may also be susceptible to invasive plant establishment.

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## Supplementary Material

The following supplementary material is available for this article:

### Appendix S1 SAS statistical analysis code and data.

This material is available as part of the online article from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2745.2008.01420.x>

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