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EFFECTS OF SEED ADDITIONS AND GRAZING HISTORY ON DIVERSITY AND PRODUCTIVITY OF SUBHUMID GRASSLANDS

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Abstract. Recent studies indicate that plant species richness and primary productivity of grasslands may be limited by seed availability. However, it is not known how widespread this limitation is, whether it is affected by disturbance, or which mechanisms underlie any disturbance effect. We tested for seed limitation and explored the role of litter accumulation in explaining effects of recent grazing history on seedling establishment, species diversity (richness and evenness), and plant productivity in a subhumid grassland ecosystem in Texas, USA. We added seeds and removed litter in a factorial treatment arrangement (seeds added, litter removed, seeds added and litter removed, and control [no seeds added nor litter removed]) within each of five fields: currently grazed, no grazing for 1–2, 2–3, or 6–7 yr, and an unplowed prairie remnant. Seeds from each of 20 native species were added to replicated 1-m^2 plots in each field during each of two growing seasons.

Adding seeds increased seedling emergence in all fields, but this increase was much greater when litter was removed, especially in ungrazed fields. Counter to our expectations, seedling emergence increased with time since grazing when litter was removed and was greatest in fields not grazed for 1–3 yr when litter was present. As expected, light availability at the soil surface decreased with time since grazing. However, soil water availability to 15 cm depth increased with time since grazing, suggesting that limits on seedling emergence changed from water to light with time since grazing.

Aboveground productivity during the second year of the study was significantly reduced following seed additions in the four fields along the grazing gradient, primarily because of a decrease in the productivity of C_4 grasses. Species diversity decreased with time since grazing. Seed additions slightly increased species richness but decreased species evenness in fields located along the grazing gradient, resulting in no net effect on diversity. In the more species-rich prairie remnant, seed additions had no effect on aboveground productivity or species richness or evenness. These results suggest that seedling emergence does not relate linearly to time since grazing and that seed availability does not limit productivity and diversity in these subhumid grasslands.

Key words: biodiversity; grassland; light vs. water limitation; prairie; primary productivity; seed addition; species diversity; Texas, USA.

Introduction

For successful plant recruitment to occur from seed, seeds must be produced and stored in the soil, they must germinate, emerge from the soil and litter layer, and seedlings must survive long enough to reproduce sexually or vegetatively. Several recent studies have shown that seed additions can increase species richness in subhumid and humid grasslands, which suggests that richness can be limited by seed availability (Tilman 1997, Zobel et al. 2000). This view is further supported by the positive relationships that usually exist between the richness of the species pool and the number of species that establish at a site (Smith and Knapp 1999, Stohlgren et al. 1999, Levine 2000, Lord and Lee 2001). However, richness of the species pool does not fully explain variation in seedling recruitment. Variation in recruitment also depends on characteristics of the community and on disturbance regimes (Levine 2000, Lord and Lee 2001), both of which may affect seedling establishment indirectly by changing resource availability (Levine and D'Antonio 1999, Davis et al. 2000). For example, fertilization often increases the number of plants that invade a community (e.g., Huenneke et al. 1990, Burke and Grime 1996). Changes in species richness in turn can affect primary productivity. Experimentally increasing species richness often increases short-term primary productivity (Naeem et al. 1994, Tilman et al. 1996, Hector et al. 1999, but see Hooper and Vitousek [1997] and Hooper [1998]). Tilman (1997) found that adding seeds from a number of species increased both species richness and percentage of cover of a Minnesota community. This suggests that both species richness and aboveground productivity might be limited by seed availability in at least some grasslands.

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Large mammal grazing is usually viewed as an intrinsic disturbance (where disturbance is defined as something that damages plant tissue) in subhumid grasslands that can affect seedling emergence and establishment, species diversity, and productivity via several mechanisms. In these highly productive grasslands (net primary productivity [NPP] range: 850–1350 $g \cdot m^{-2} \cdot yr^{-1}$; Risser et al. 1981), release from grazing by large mammals can alter seedling recruitment and productivity by altering the amount of litter accumulation. Litter has been found to inhibit grassland dominants (Carson and Peterson 1990) and to either reduce (Fowler 1988, Hoffman 1996) or increase (Hoffman 1996) seedling recruitment, depending on productivity and water availability of the site (Nash Suding and Goldberg 1999). Bosy and Reader (1995) suggested that lowered light levels and other aspects of microclimate under litter inhibit seedling germination and establishment and lower productivity (Knapp and Seastedt 1986). Litter also can have physical, chemical, and mechanical effects on seed germination and seedling stem elongation (Facelli and Pickett 1991, Bosy and Reader 1995) and can provide cover for seed and seedling predators (Hulme 1996, Edwards and Crawley 1999). Because grazing reduces litter accumulation, grazed areas in subhumid grasslands should have greater light availability at the soil surface and fewer seed predators; these conditions may promote seedling emergence.

Our objectives were to test the following hypotheses: (1) addition of seeds increases both species diversity and primary productivity of subhumid grasslands by increasing species establishment, and (2) this effect is maximal in grazed grasslands and decreases with time since grazing because of litter accumulation and associated changes in water and light availability. To test these hypotheses, seeds were added to plots and litter was removed in a factorial design within fields that differed in recent grazing history. Seedling emergence and light and water availability were then monitored for 2 yr. Net aboveground primary productivity and diversity were measured at the end of the experiment to test whether emerged seedlings had a significant effect on these important variables.

METHODS

Experimental design

The study was conducted during 1999 and 2000 in fields near Temple, Texas (Bell County), USA (31°05′ N, 97°20′ W) at 224 m above sea level. Soils at the site are fine-silty, carbonatic, thermic Udorthentic Haplustolls (type of vertisol). Rainfall, which averages 864 mm per year (mean for 1913–2000), is bimodal, with a large peak in spring and a smaller peak during autumn. Under the classification scheme of Risser et al. (1981), these grasslands are classified as successional subhumid tallgrass prairies. The blackland prairie region of Texas originally supported herds of bison, pronghorn, and probably elk, but grazing by these animals was replaced by cattle grazing after European settlement (Diggs et al. 1999). Seeds were added and litter was removed in five fields: (1) a field that was grazed during the study (hereafter GR), (2) a field that was fenced at the beginning of the experiment (NG1), (3) a field that was fenced 1 yr before the beginning of the study (NG2), (4) a field that was fenced 5 yr before the study (NG6), and (5) a prairie remnant that has never been plowed (based on records from post-European settlement) and that has not been grazed for more than 20 yr (PR). Thus, the experiment was conducted in fields with four levels of time since grazing and, separately, in a prairie remnant. The four fields along the grazing gradient were contiguous and dominated by King Ranch bluestem (*Bothriochloa ischaemum* L.) and Texas wintergrass (*Nassella leucotricha* Trin. & Rupr.). Fields were part of a single large grassland that had been grazed by cattle annually with the same history and management regime for over 60 yr before they were fenced. The prairie remnant is located just south of the other four fields and is typical of native Texas blackland prairie dominated by little bluestem (*Schizachyrium scoparium* Michx.), indian grass (*Sorghastrum nutans* L.), and big bluestem (*Andropogon gerardii* Vitman).

Within each field (size range: 0.4–1.5 ha), five blocks (25 m^2) were established along a north–south direction. In each block, four 1 × 1 m plots were located with a 1-m buffer between plots, and two factorially arranged treatments were randomly applied: (1) seeds added, (2) litter removed, (3) seeds added and litter removed, and (4) control (no seeds added nor litter removed). Thus, there were 20 plots in each field; $20 \times 5 = 100$ total plots.

Litter was removed before the first growing season during January 1999. Because seeds sometimes germinate during autumn and early winter in these grasslands, litter was also removed in October 1999 to obtain a greater range of litter removal dates. Litter was manually removed from the soil surface without disturbing the soil or standing vegetation.

Species added as seed

Twenty common Texas species from five functional groupings (perennial C_4 and C_3 grasses, legumes, and perennial and annual forbs) were added to each seed-addition plot (taxonomy follows Correll and Johnston 1979) following litter removal. Species added as seeds were the perennial C_4 grasses *Schizachyrium scoparium* (little bluestem), *Panicum virgatum* (switchgrass), *Andropogon gerardii* (big bluestem), *Sorghastrum nutans* (indian grass), and *Bouteloua curtipendula* (side-oats grama); the perennial C_3 grass *Elymus canadensis* (Canada wildrye); the legumes *Cassia fasciculata* (partridge pea), *Lupinus texensis* (Texas bluebonnet), and *Petalostemum candidum* (prairie clover); the perennial forbs *Echinacea purpurea* (purple coneflower), *Salvia* *azurea* (blue sage), *Helianthus maximilani* (Maximilian sunflower), and *Liatris lancifolia* (blazing star); and the annual/biennial forbs *Monarda citriodora* (Lemon mint), *Rudbeckia hirta* (black-eyed susan), *Helianthus annuus* (common sunflower), *Verbena bipinnatifida* (vervain), *Gaillardia pulchella* (indian blanket), *Coreopsis tinctoria* (tickseed), and *Corydalis curvisiliqua* (scrambled eggs).

All species are native to the area (i.e., part of the regional species pool), but only a few are found in the prairie remnant (*Schizachyrium scoparium*, *A. gerardii*, *Sorghastrum nutans*, and *Salvia azurea*), and fewer still are present in all fields (*M. citriodora* and *G. pulchella*). Seeds were obtained from local wildflower seed companies. Homogenous seed mixes were created separately for each plot by weighing out each species and then by shaking each mix. Seed germinability was qualitatively verified by germinating seeds from each species in greenhouse flats.

Seeds were evenly distributed on the soil surface to simulate a natural seed rain. Seeds were added on 26 January 1999 and re-added to the same plots during October 1999 (to obtain a wider range of addition times). Seeds were applied at a rate of 4.5 g/m^2 for each species, which results in a constant mass but a variable number of seeds per species, to be comparable to previous studies (e.g., Tilman 1997).

Sampling design

Environmental variables were measured to determine how they varied among treatments and whether they were correlated with seedling emergence and establishment. Soil moisture to 15 cm depth was measured weekly (except for two periods during summer 1999 when equipment failed) in each plot with stainless steel time domain reflectometry (TDR) probes centered in each plot during 1999 (26 February–16 December) and 2000 (10 January–21 September). Propagation time of electromagnetic waves through probes was measured with a MoisturePoint MP-917 instrument (Environmental Sensors, Victoria, British Columbia, Canada). An empirical equation from Topp et al. (1980) was used to calculate volumetric water content of soil from these measurements, and then calculations were corrected to gravimetric measurements using a linear regression equation developed from measurements in the high clay soil where this study was conducted ($r^2 = 0.66$, $n = 32$; Polley et al. 2003). The top 15 cm of soil was sampled because of the presumed importance of shallow soil layers to seedlings (Briske and Wilson 1978). The proportion of light reaching the soil surface was estimated four times each growing season (at noon) with a light bar (1 m long; SunScan Canopy Analysis System, Delta-T Devices, Cambridge, UK) placed 1 m above the vegetation and below the vegetation at the soil surface. The proportion of light at the soil surface was calculated by dividing light levels at the soil surface by light levels above the canopy for each date.

We partitioned soil water loss (in the grazed field only) into evaporation and transpiration components with the use of an herbicide. Twelve additional plots (six pairs) were set up during a 12-d rain-free drying phase (August 1999). One plot of each pair was sprayed with Roundup herbicide (Monsanto, St. Louis, Missouri, USA), which quickly eliminates transpiration, but because the canopy remains intact, does not have any short-term effects on bare soil evaporation. We divided (for each pair) the decline in soil water content to 15 cm depth in the sprayed plots (evaporation only) by the decline in soil water content in control plots that were not sprayed (evaporation and transpiration) to estimate the proportion of soil water lost to evaporation in grazed grassland.

The number of seedlings was counted monthly with a 20 × 50 cm sampling quadrat placed in the center of each plot. Counts began 1 mo after seeds were added and continued until few or no seedlings remained. Because volunteer annual grass seedlings were extremely dense, we also counted grass seedlings during March and April of the second year inside a 15.8 cm diameter ring. All seedling numbers are reported on a per square meter basis.

A point intercept method and biomass sorting by species were both used to estimate relative plant abundances. For the point intercept method, a metal grid (100 × 100 cm) with 25 intercept points located 20 cm apart was placed above the canopy, and the identity of the species (or lack thereof) at each intercept point (first hit only) was determined. Species richness (S) was recorded for each plot by counting species, and a composite index of diversity (Simpson's $D = 1/\Sigma p_i^2$, where p_i is the relative abundance of species i) and an index of species evenness (Simpson's $E = D/S$) were calculated from the relative abundance data (Smith and Wilson 1996, Stirling and Wilsey 2001). Measurements were made twice in 1999 (10 March and 20 May) and again in 2000 (29 March and 2 June).

Aboveground productivity was measured during the final year (2000) using a peak-biomass-by-functional-group approach. In these grasslands, winter frosts kill the aboveground vegetation. Consequently, previous years growth is usually present as standing dead biomass, and productivity estimates were based on living biomass only. Because rainfall is bimodal, we used two harvests to estimate primary productivity. Half of each plot (0.5 m^2) was clipped during July (peak biomass for C_3 grasses and annual forbs), and the remaining half was clipped in October (peak biomass for C_4 grasses and perennial forbs). Plants were sorted by species and then placed into functional groups (annual forbs, perennial forbs, C_3 grasses, C_4 grasses) at each harvest. Biomass was then dried to constant mass and weighed. The larger biomass value for each functional group was taken as an estimate of productivity. Peak biomasses were then summed across functional groups to estimate total productivity for each plot.

Productivity in the grazed field was estimated by adding "off-take" by cattle. Off-take was estimated with five temporary 1-m^2 exclosures, each of which was paired with a 1-m^2 plot that was grazed. Exclosures were relocated monthly during the growing season. Off-take was calculated by subtracting standing crop in grazed plots (outside each exclosure) from standing crop within exclosures (McNaughton et al. 1996). Cattle consumed a mean of 448.8 g/m^2 (range 216–831 g/m^2) during 1999 and 253.4 g/m^2 (range 163–371 g/m^2) during the 2000 growing season. Grazing intensity (consumption/total productivity) was estimated to be 0.84 ± 0.016 (mean ± 1 SE; range 0.83–0.86) for 2000, which means that 84% of plant production in the grazed field was consumed by cattle.

Data analysis

Because the prairie remnant differed in species composition and history from other fields, it was analyzed separately. Data from the two years also were analyzed separately because seeds were added and litter was removed on different dates between years. Response variables from the four fields along the grazing gradient were analyzed with a split-plot ANOVA, with block and field in the main plot (block[field] as the error term) and seed addition, litter removal, and interactions in the subplot. Time effects were analyzed with a repeated measures analysis. All treatments were analyzed as fixed effects. During year 1, most seedlings had disappeared by month 5, so analyses were restricted to the first 4 mo. Linear and quadratic relationships between measured variables and years since grazing (0, 1, 2, or 6 for year 1; 0, 2, 3, or 7 for year 2) were tested with a priori contrasts. Contrast coefficients were calculated with IML of SAS (SAS 1985). Response variables from the prairie remnant were analyzed for seed addition, litter, and interaction effects with a factorial randomized block design ANOVA. Seedling numbers were log transformed, and light levels were arcsine transformed before analysis.

RESULTS

Litter mass removed

Litter mass removed varied among fields (Table 1). At the beginning of the study (January 1999), litter mass did not differ between the grazed field and the first-year fenced plots. Litter mass greatly increased with time since grazing across fields (one way ANOVA, $F_{3,32} = 170$, $P < 0.001$; linear contrast, $F_{1,32} = 443$, $P < 0.001$). Litter mass was intermediate in the prairie remnant.

Litter mass removed during October 1999 was smaller than during year 1, but litter mass again varied among fields (Table 1). In year 2, the relationship between litter mass and time since grazing was quadratic (one-way ANOVA, $F_{3,32} = 20.6$, $P < 0.001$; quadratic contrast, $F_{1,32} = 42$, $P < 0.001$). Litter mass was lowest in the grazed field (as in year 1) and maximal in fields with an intermediate amount of time since grazing.

TABLE 1. Litter mass removed from 10 plots within a grazed field (GR), fields that had no grazing for 1–2 yr (NG1), 2–3 yr (NG2), or 6–7 yr (NG6) during the study, and a prairie remnant (PR; means ± 1 SE).

Field	Litter mass (g/m^2)
January 1999	
GR	60.9 ± 8.4
NG1	45.1 ± 5.6
NG2	156.5 ± 17.7
NG6	806.9 ± 66.5
PR	586.5 ± 23.7
October 1999	
GR	49.9 ± 6.4
NG1	122.1 ± 17.9
NG2	143.6 ± 5.2
NG6	107.3 ± 7.8
PR	84.6 ± 8.6

Seedling emergence

The total number of emerged forb seedlings (all species combined), when averaged across months, was much higher in plots that received seed additions during both years of the experiment and increased across fields with time since grazing ($P < 0.001$). There were few or no seedlings in control plots that did not receive seed additions in fields along the grazing gradient during the first year (Fig. 1). The magnitude of the seed addition effect varied among fields and was smaller without litter removal (Figs. 1 and 2; field × seed × litter interaction, $P < 0.01$). When litter was left intact, seed additions had the greatest effect in fields with an intermediate time since grazing during both years (polynomial contrast, 1999, $P < 0.02$). When litter was removed, the number of forb seedlings increased linearly with time since grazing (linear contrast, $P < 0.01$). In general, largest effects of litter removal on the number of emerged forbs were found in fields with the largest amount of litter (Table 1, Fig. 1), and there was no effect of litter in plots that did not receive seed additions (seed × litter interaction, $P = 0.028$).

There also were few seedlings in the non-seeded control plots of the prairie remnant (Fig. 1). Adding seeds to the remnant increased forb seedling emergence in both years (Figs. 1e, 2e) (seed main effect, $P < 0.001$). Litter removal significantly increased abundance of forb seedlings (litter main effect, $P < 0.033$), and the effects of seed addition were larger when litter was removed during the second year (Fig. 2e, seed × litter interaction, 1999, $P = 0.126$; 2000, $P = 0.03$).

Although many seedlings emerged, not many survived the growing season. During year 1, the number of forb seedlings present declined over time and was close to zero in all fields by month 6 (time effect, $P < 0.001$). During year 2, the greatest number of seedlings was found in month 3 and numbers declined thereafter (time, $P < 0.001$).

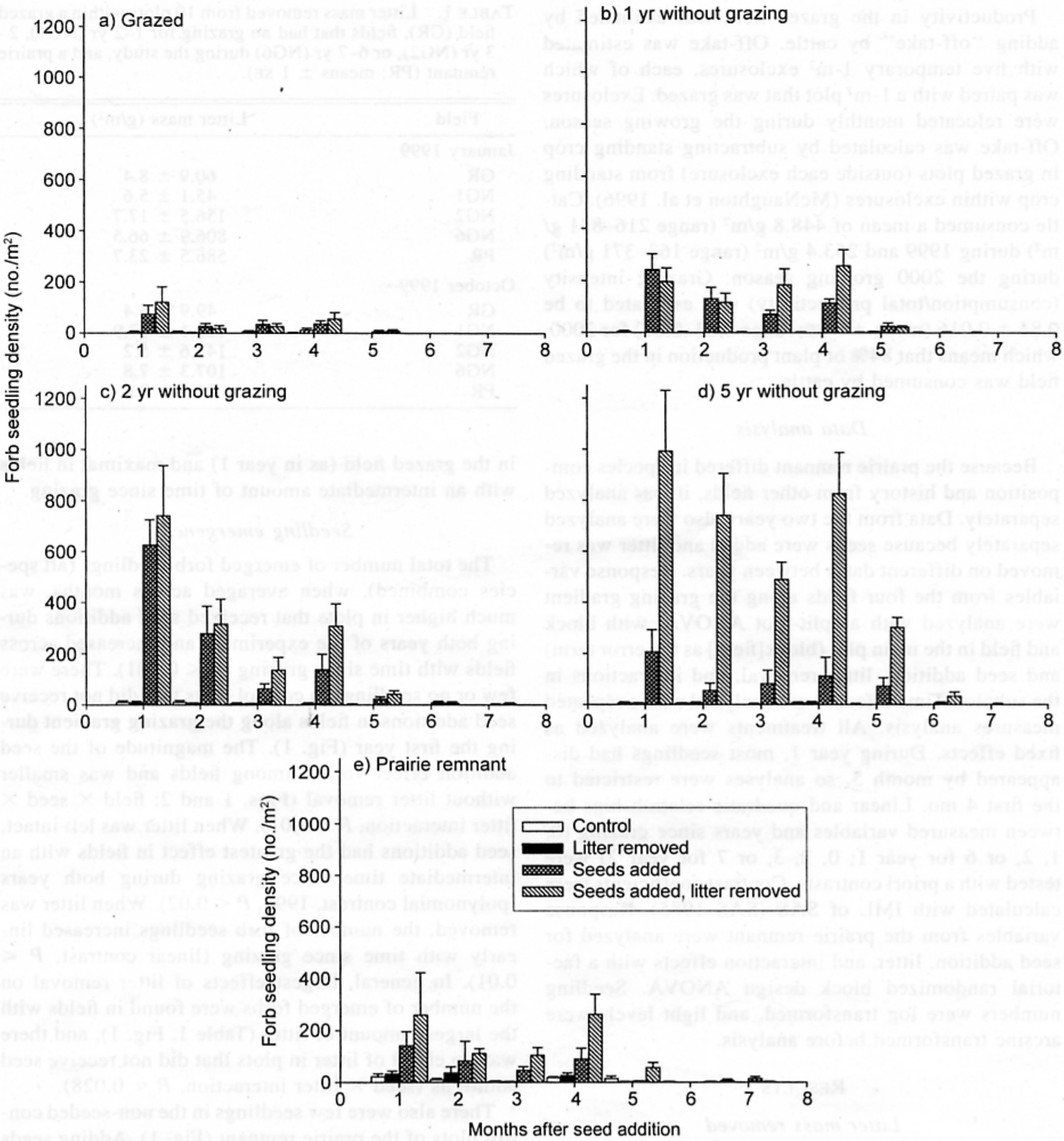

FIG. 1. Forb seedling density in experimental plots in five fields in Texas, USA, with different grazing histories measured at monthly intervals during the first year of the study (1999; means + 1 SE). The first four fields (a–d) were dominated by King Ranch bluestem and Texas wintergrass, and the native prairie remnant was dominated by little bluestem. There are missing histogram bars because some plots had no seedlings.

Most of the added forb species were observed, at least occasionally, as seedlings. The most abundant seedling species were *Monarda citriodora*, *Gaillardia pulchella*, and the two *Helianthus* species (B. J. Wilsey and H. W. Polley, *personal observations*), some of which flowered during the second year of the study. The grass species added as seed did not emerge. Seedlings of these species were not observed in our plots, although seed of all grass and forb species readily germinated in the greenhouse.

During March and April of the second year (2000), a large number of volunteer annual grass seedlings (species that were not experimentally added) emerged in plots. Grass seedling numbers also varied significantly across fields ($P < 0.01$; Fig. 3). Like forb seedlings, grass seedlings were most abundant in fields with

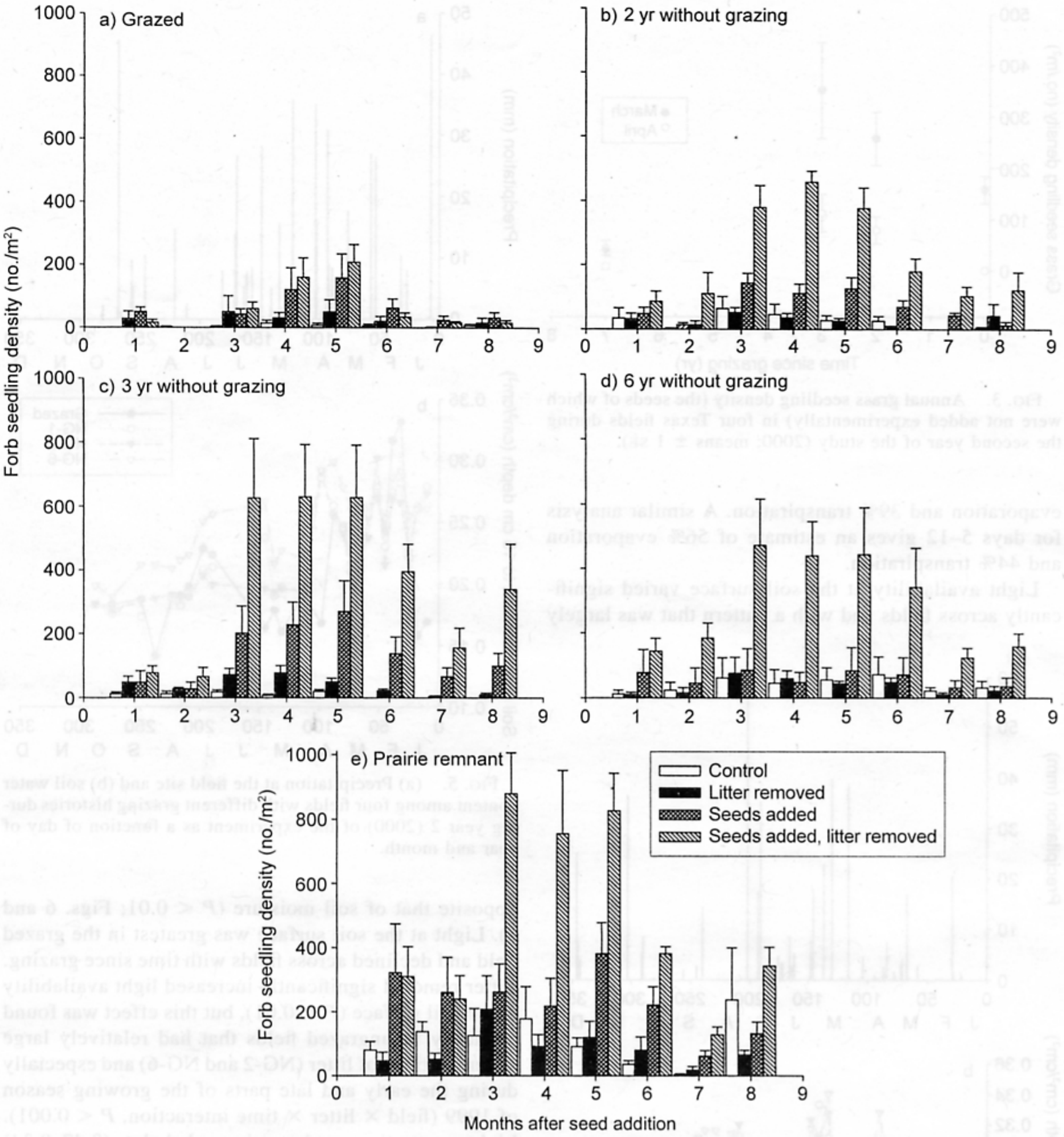

FIG. 2. Forb seedling density in experimental plots in the five fields with different grazing histories measured at monthly intervals during the second year of the study (2000; means + 1 SE). The first four fields (a–d) were dominated by King Ranch bluestem and Texas wintergrass, and the native prairie remnant was dominated by little bluestem. There are missing histogram bars because some plots had no seedlings.

an intermediate amount of time since grazing (quadratic contrast, $P < 0.01$).

Environmental variables

Soil water content to 15 cm depth declined as the growing season progressed during both years (time, $P < 0.001$). In general, soil moisture was lowest in the grazed field and increased across fields with time since grazing (field main effect, $P < 0.001$; linear contrasts, $P < 0.001$; Figs. 4, 5, 6, and 7). However, there was an interaction between field and time ($P < 0.05$), which indicates that grazing effects were not found on every date.

In the sprayed plots, the mean relative decline (per day) in soil water content during the first 5 d after herbicide was sprayed was 0.39 ± 0.19% compared to 0.64 ± 0.21% in plots that were not sprayed. Dividing sprayed by not-sprayed values gives an estimate of 61%

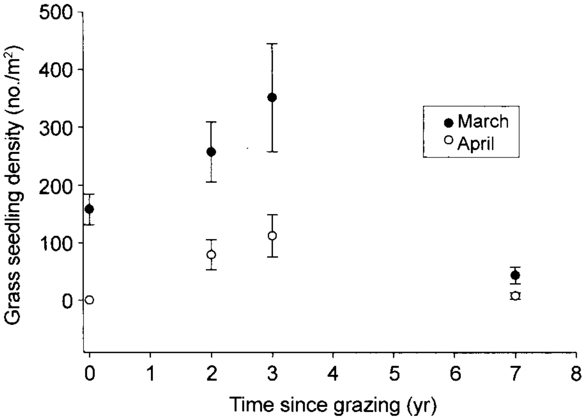

FIG. 3. Annual grass seedling density (the seeds of which were not added experimentally) in four Texas fields during the second year of the study (2000; means ± 1 SE).

evaporation and 39% transpiration. A similar analysis for days 5–12 gives an estimate of 56% evaporation and 44% transpiration.

Light availability at the soil surface varied significantly across fields and with a pattern that was largely opposite that of soil moisture ($P < 0.01$; Figs. 6 and 7). Light at the soil surface was greatest in the grazed field and declined across fields with time since grazing. Litter removal significantly increased light availability at the soil surface ($P < 0.01$), but this effect was found primarily in ungrazed fields that had relatively large accumulations of litter (NG-2 and NG-6) and especially during the early and late parts of the growing season of 1999 (field × litter × time interaction, $P < 0.001$). Light penetration was lower in seeded plots (0.47, 0.34) than in plots that were not seeded (0.54, 0.39) at the earliest sampling date in both 1999 and 2000 (seed addition × time interaction, $P < 0.04$).

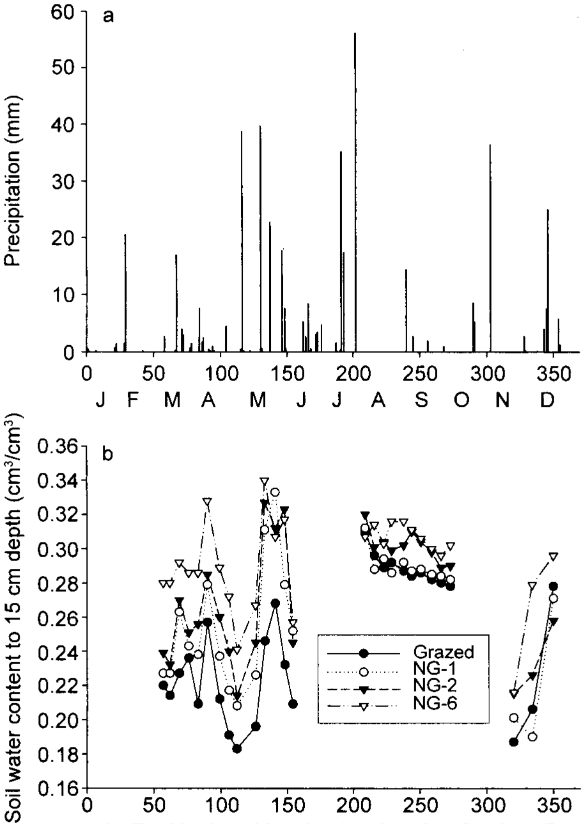

FIG. 4. (a) Precipitation at the field site and (b) soil water content among four fields with different grazing histories during year 1 (1999) of the experiment as a function of day of year and month.

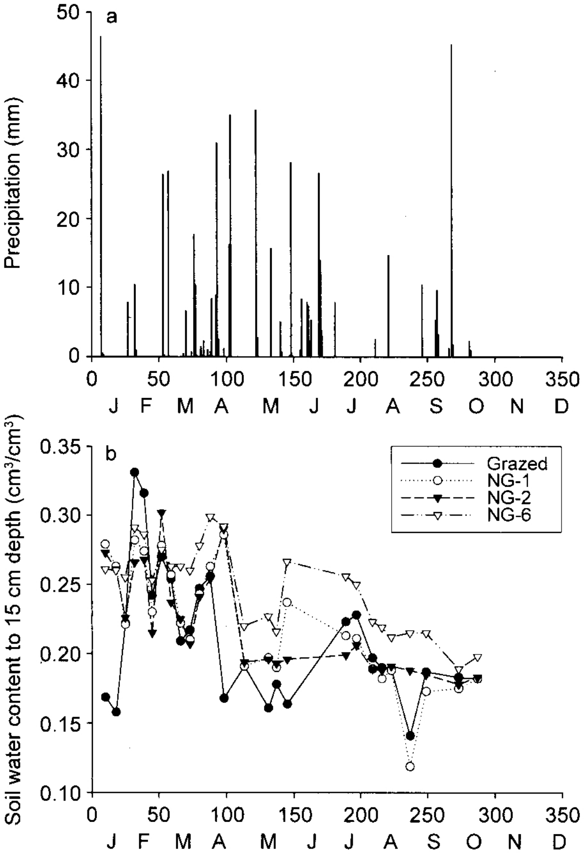

FIG. 5. (a) Precipitation at the field site and (b) soil water content among four fields with different grazing histories during year 2 (2000) of the experiment as a function of day of year and month.

In the prairie remnant, there was no significant effect of seed addition on light availability at the soil surface ($P > 0.537$), nor were there interactions between seed addition and other factors (all $P > 0.13$). Litter removal increased light availability significantly during early 1999 ($P < 0.001$) but not during 2000 ($P = 0.18$; data not shown).

Plant diversity

Both the point intercept method and measures of aboveground productivity yielded largely similar re-

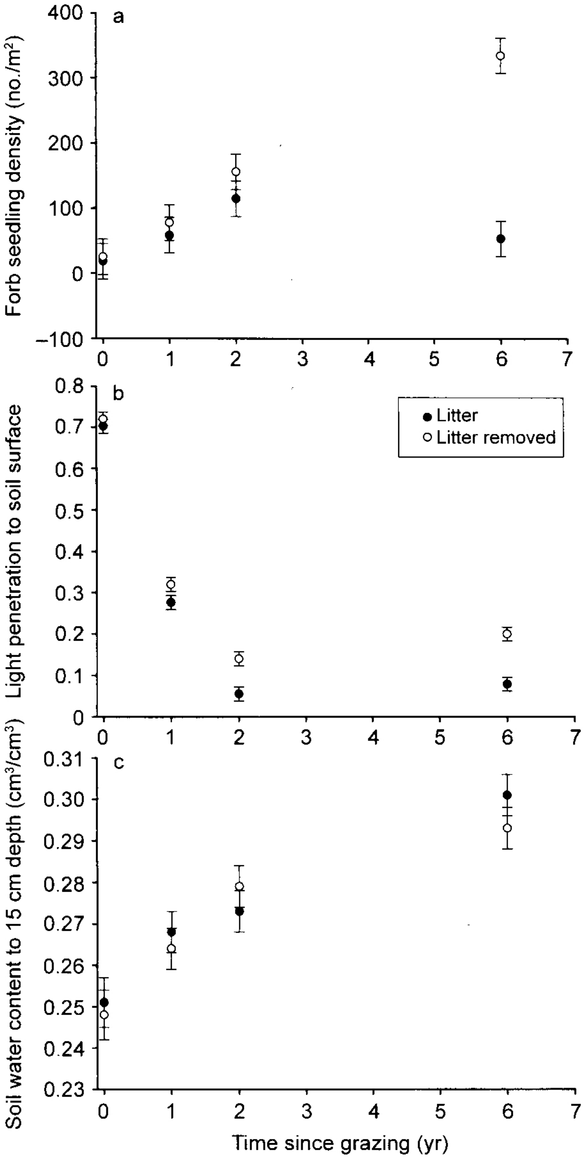

FIG. 6. Forb seedling density (means averaged across dates and seed addition treatments), light penetration to the soil surface (proportion), and soil water content to 15 cm depth in plots with litter intact or litter removed during the first year (1999) of the experiment. Each data point represents means ± 1 SE for a field with a different grazing history.

sults. Both species diversity (D) and species richness (S), as assessed by the point intercept method, declined linearly across fields with time since grazing (Table 2, linear contrasts: S, $P < 0.01$; D, 1999, $P < 0.01$; D, 2000, $P < 0.10$). Evenness did not vary across fields ($P = 0.396$). Adding seeds led to ephemeral increases in plant diversity during 1999 due to increased D ($P = 0.03$) but had no significant effect on S or E. However, by the end of the second year of the experiment (2000), mean D, S, and E all were unchanged by seed additions: 2.50, 6.4, and 0.43 (no additions) and 2.57, 6.6, and 0.43 (seed additions; all $P > 0.12$). Litter removal had no significant effect on any diversity index.

There were also no significant effects of seed addition or litter removal on diversity indices in the prairie remnant (all $P > 0.15$). Species richness, which was much higher in the prairie remnant than in fields along the grazing gradient, averaged 14 in plots with and without seed additions. Evenness averaged 0.36 in both seed-addition and no-seed-addition plots.

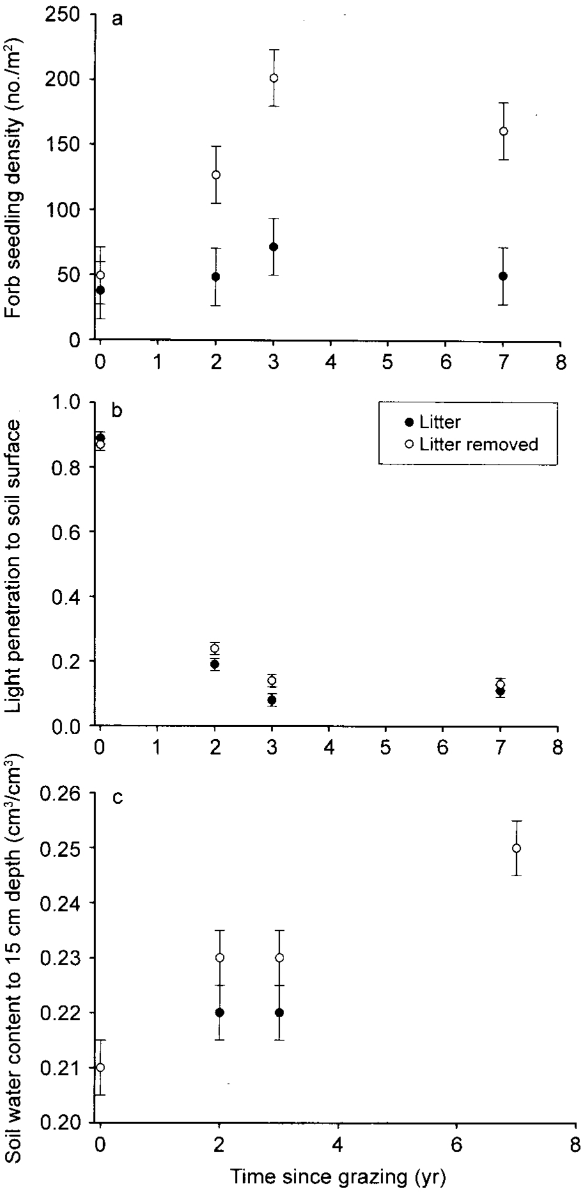

FIG. 7. Forb seedling density (means averaged across dates and seed addition treatments), light penetration to the soil surface (proportion), and soil water content to 15 cm depth in plots with litter intact or litter removed during the second year (2000) of the experiment. Each data point represents means ± 1 SE for a field with a different grazing history.

TABLE 2. Mean species diversity (Simpson's D), richness (S, number of species per 1 m^2), and evenness (E) within a grazed field (GR), fields that had no grazing for 1–2 yr (NG1), 2–3 yr (NG2), or 6–7 yr (NG6), and the prairie remnant (PR).

		D			S			E		
Field	Treatment	Early†	Late‡	Biomass§	Early	Late	Biomass	Early	Late	Biomass
1999										
GR	+AS	2.0	3.7		6.0	10.2		0.33	0.35	
	−AS	2.0	2.7		5.6	9.4		0.39	0.29	
NG1	+AS	2.7	3.1		6.9	9.0		0.39	0.32	
	−AS	2.1	2.8		6.4	11.1		0.33	0.25	
NG2	+AS	1.7	2.8		5.2	8.8		0.32	0.33	
	−AS	1.6	2.5		4.9	8.4		0.34	0.32	
NG6	+AS	1.6	2.2		4.1	7.5		0.47	0.37	
	−AS	1.6	2.4		4.7	6.7		0.37	0.30	
PR	+AS	2.6	4.7		9.6	13.6		0.35	0.35	
	−AS	2.6	4.7		7.4	13.5		0.27	0.35	
2000										
GR	+AS	3.0	3.4	2.2	7.8	8.6	5.5	0.40	0.41	0.44
	−AS	3.0	3.2	2.5	7.7	7.3	5.6	0.39	0.43	0.46
NG1	+AS	2.1	3.2	2.7	6.8	6.7	6.2	0.30	0.49	0.43
	−AS	1.9	2.4	2.1	6.3	6.4	4.4	0.30	0.47	0.51
NG2	+AS	2.3	2.6	2.3	6.8	5.8	5.6	0.36	0.45	0.40
	−AS	2.5	2.6	1.8	6.2	5.8	4.0	0.40	0.44	0.46
NG6	+AS	2.1	1.9	1.5	5.8	5.3	4.5	0.41	0.36	0.32
	−AS	2.4	2.1	1.8	4.7	5.9	4.1	0.56	0.37	0.49
PR	+AS	4.2	4.7	3.3	11.3	13.5	10.3	0.37	0.36	0.36
	−AS	4.1	4.8	3.5	11.3	13.6	10.4	0.37	0.36	0.34

Notes: Experimental plots within each field either received added seeds (+AS) or did not (−AS).

† Point intercept measurements were made on 10 March 1999 and 29 March 2000.

‡ Point intercept measurements were made on 20 May 1999 and 2 June 2000.

§ Based on peak aboveground biomass.

Diversity was also calculated from peak biomass data collected at the end of the final growing season of the study. These data provide a second test for whether early changes in diversity observed with point intercept translated into changes in diversity after 2 yr. Again, diversity and species richness declined across fields with time since grazing (linear contrasts, $P < 0.05$), and evenness did not change significantly ($P > 0.62$). Adding seeds increased S slightly ($P < 0.02$) but decreased E slightly ($P < 0.02$), which resulted in no net change in D ($P = 0.42$). Litter removal had no affect on diversity. There were also no significant changes in S, D, or E in response to seed additions or litter removal in the prairie remnant (all $P > 0.46$).

Aboveground productivity

Primary productivity measured during the second year declined in response to seed addition along the grazing gradient (means: 310.5 $g \cdot m^{-2} \cdot yr^{-1}$ in non-seeded plots, 286.5 $g \cdot m^{-2} \cdot yr^{-1}$ in seeded plots, $P < 0.05$), but did not vary with litter removal or among fields (when off-take to cattle was taken into account). Standing crop biomass, which excludes off-take, was significantly lower in the grazed field and increased with time since grazing among fields (data not shown, $P < 0.001$). In the prairie remnant, neither seed addition nor litter removal affected productivity ($P > 0.20$).

Productivity of individual functional groups varied with seed addition treatments and among fields along the grazing gradient. Productivity of annual forbs was significantly greater ($P < 0.01$) and biomass of C_4 grasses was marginally significantly lower ($P < 0.06$) when seeds were added. Negative effects of seed addition on C_4 productivity tended to be more pronounced in fields that had not been grazed for at least 2 yr (seed × field, $P = 0.06$). In the prairie remnant, annual forb productivity increased when seeds were added ($P = 0.06$), but there were no differences in productivity of the other functional groups, nor between litter treatments.

Productivity of C_4 grasses increased and that of C_3 grasses decreased with time since grazing across fields, especially between NG-2 and NG-6 (linear and quadratic contrasts, both $P < 0.05$). Productivity of perennial forbs increased linearly with time since grazing ($P < 0.05$). Productivity of annuals did not vary consistently with grazing history. C_4 and C_3 grasses made up 66% and 21% of total productivity, respectively. In the prairie remnant, C_4 and C_3 grasses made up 70% and 2% of total productivity, respectively, whereas annual and perennial forbs comprised 17% and 11% (1.1% legumes).

DISCUSSION

We hypothesized that species diversity and primary productivity are seed limited in Texas subhumid grasslands, consistent with trends in other grassland systems (Tilman 1997, Zobel et al. 2000, Lord and Lee 2001). Grazing creates pulses of light and other resources that may enhance seedling recruitment (Olff and Ritchie 1998, Davis et al. 2000). Seedling establishment has been found to increase species richness (Tilman 1997, Zobel et al. 2000, Lord and Lee 2001) and plant cover (Tilman 1997) in some grasslands. We hypothesized, therefore, that positive effects of seed additions on seedling recruitment and species diversity would decline with time since grazing because of a litter-accumulation-mediated decline in light availability at the soil surface.

Our data do not support these hypotheses. In fields along the grazing gradient, adding seeds caused an ephemeral increase in diversity early during the first growing season and a slight increase in species richness (<1 species/m^2) at the end of the second growing season. However, evenness declined with seed additions for no net effect on diversity. Furthermore, seed additions to the prairie remnant caused no changes in any diversity index examined. Because many seedlings successfully emerged from the soil, it is likely that growth and maturation of seedlings were hampered and that low seedling survival prevented any increases in diversity. Seedling emergence was related both to soil water and light availability. The inconsistencies between this study and others in subhumid grasslands suggest that the extent to which seedling recruitment limits diversity depends on which resources are limiting and may be better expressed in areas in which recruitment is limited solely by light and nutrients (Tilman 1997, Zobel et al. 2000, Lord and Lee 2001). For example, reductions in plant canopy size often lower nutrient uptake, which provides more nutrients for recruiting species (reviewed in Davis et al. 2000). Because roots are found throughout the soil profile, this process should occur at all soil depths. Conversely, reductions in plant canopy size in water-limited systems can reduce transpiration but increase soil evaporation rates. This could potentially lead to soils that are drier at the surface, but moister at lower depths. Because of the importance of shallow soil layers to seedlings, we hypothesize that any environmental factor that acts to reduce canopy size in water-limited systems will usually cause lower seed germination and seedling establishment rates, which is opposite from predictions concerning nutrient-limited systems (e.g., Davis et al. 2000).

Aboveground productivity decreased with seed additions in the four fields along the grazing gradient and remained unchanged in the prairie remnant. Adding seeds to field plots along the grazing gradient increased productivity of annual forbs and reduced light at the soil surface early in the growing season, but had no effect on soil moisture. We found a concurrent decrease in the productivity of C_4 grasses. Hooper (1998) found a similar competitive effect of annuals on perennial grass productivity in California. Although it was not measured in our study, the decrease in light with increased forb productivity may have inhibited early tillering of C_4 grasses and contributed to decreased productivity of these dominant species. Regardless of the mechanism involved, the concurrent reduction of C_4 grasses and increase in annual forbs suggests that annual forbs competitively inhibited C_4 grasses. Thus, adding seeds increased the productivity of the annual forb functional group but did not increase overall productivity. Interestingly, this inhibition of C_4 grasses by annual forbs was not found in the more species-rich prairie remnant, perhaps because it contained a greater number of C_4 grass species that could compensate for any inhibition.

Although removing litter or adding seed did not affect plant diversity, removing litter did increase light availability and seedling emergence in plots to which seeds were added. Fowler (1988) also found that seedling emergence was increased by removing litter from a Texas hill country savannah. However, in our study, current grazing reduced seedling emergence and establishment during both years of the experiment. No evidence was found of cattle defoliating the seedlings, and few seedlings were trampled. As expected, the amount of light available at the soil surface was much higher in the currently grazed field (Figs. 6 and 7), but surprisingly (McNaughton 1979, Archer and Detling 1986, Wraith et al. 1987), any positive effect of greater light availability on seedlings apparently was counterbalanced by a decrease in soil water content. Thus, the expected increase in seedling emergence with grazing was not found, because current grazing was associated with drier soil. The companion herbicide spray experiment indicated that evaporation accounted for 56–61% of water loss from upper soil layers during a drying period in the grazed field, which is a greater proportion of evaporation than is usually assumed (Rundel and Jarrell 1989). Water loss to evaporation apparently more than offset any reduction in transpiration caused by grazing. Thus, water availability appears to be at least as important as light availability in this subhumid grassland, as it is in semiarid systems in controlling seedling establishment (Lauenroth et al. 1994, O'Conner 1996, Peters 2000).

The transient-maxima hypothesis of Seastedt and Knapp (1993) predicts that total resource availability and productivity in tallgrass prairie will be highest in situations (or time periods) during which limiting resources are intermediate in relative availabilities. Our data on soil water and light availability support this hypothesis: the fields with an intermediate time since grazing had the highest amounts of light and water available (i.e., intermediate amounts of both resources). Although we failed to find higher productivity in these fields, we did find that these fields had the highest seedling emergence rates. This suggests that the transient-maxima hypothesis may be extended to predict when seedling emergence will be highest.

So why was there a difference between our results in Texas grasslands and earlier results from Minnesota (Tilman 1997) and Eastonian grasslands (Zobel et al. 2000)? Texas grasslands, although they are also classified as subhumid tallgrass systems, may differ from other grasslands studied thus far in being hotter and drier during summers. As mentioned earlier, these grasslands have a bimodal rainfall pattern with a short-duration peak during spring and a smaller peak during autumn. In contrast, Minnesota grasslands have a unimodal rainfall pattern with a maximum during late spring (June); they receive >5 cm/mo from April to September and between 2.5 and 5 cm during March, October, and November. In our Texas grasslands, many seedlings emerged (Figs. 1 and 2) but few survived the very dry conditions of late summer in Texas. In Texas grasslands, seedlings of most species must germinate during the fall or early winter, survive winter frosts, and reach reproductive size before the onset of summer. Because some seedling recruitment obviously occurs into these grasslands, it must occur either among a few species (e.g., *Helianthus*, *Gaillardia*, and *Monarda*) that can survive or avoid typically dry summers or occur in occasional wet summers or warm winters. However, our results may apply more generally, as a variety of abiotic and biotic filters have been shown to reduce seedling recruitment. For example, Wilby and Brown (2001) found that slug herbivory reduced seedling recruitment into British grasslands. Crawley (1990) reviewed several studies in which few or no seedlings emerged following seed additions. Hutchings and Booth (1996) found virtually no emergence in nonmowed old fields. Burke and Grime (1996) found relatively few seedlings in grassland plots that lacked gaps cut into turf and that were not fertilized.

In summary, we found that counter to original expectations, seedling emergence actually increased when grazing was removed. Removing litter from ungrazed grasslands further increased seedling emergence, but because most seedlings did not survive, it did not affect productivity or species diversity. Thus, prevention of litter accumulation is not a primary effect of grazing on diversity in this system. Based on these results, we suggest that controls on plant survival and vegetative reproduction are more important than local seed dispersal rates in determining species richness and productivity in these grasslands.

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