

Early-successional plants regulate grassland productivity and species composition: a removal experiment

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Grime's (1998) "mass-ratio" hypothesis holds that ecosystem processes depend in the short term on functional properties of dominant plants and in the longer term on how resident species influence the recruitment of dominants. The latter of these effects may be especially important among early-successional species in disturbed ecosystems, but experimental tests are few. We removed two groups of early-successional species, an annual forb *Gutierrezia dracunculoides* (DC.) S. F. Blake and annual species (mostly grasses) that complete growth early in the growing season [early-season (ES) species], from a heavily-grazed grassland in central Texas, USA dominated by a C₄ perennial grass. We sought to determine effects of annuals on grassland functioning [productivity, water balance, soil and plant nitrogen (N)] and composition. Removals did not impact N retention in the soil/plant system during the two years of this study, but removing ES annuals increased the amount of water between 30 and 120 cm in the soil profile early in each growing season. Production and N accumulation by vegetation declined following the removal of ES annuals in approximate proportion to the contribution of annuals to aboveground biomass and N, consistent with the mass-ratio hypothesis. By the second year, production and N uptake by initially sub-dominant species increased to fully compensate for the loss of annuals. These results are consistent with the view that ecosystem functions are more strongly linked to species attributes than to diversity per se. Longer-term effects of annuals on grassland composition were evident in a dramatic increase in biomass of perennial forbs after annuals were removed. Because perennial forbs differ from the dominant grass in this grassland in traits that influence ecosystem functioning, ES annuals may affect grassland functioning more by regulating the composition of vegetation than by directly affecting process rates.

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Vegetation in most terrestrial ecosystems has been altered by disturbances and plant invasions. It has become crucial, therefore, that we better understand how the species that are being added to or lost from plant communities affect important ecosystem functions, like productivity, water balance and nutrient cycling, and

influence community recovery from disturbances. Grime (1998) distinguished between immediate and longer-term effects of plant species on ecosystem functioning. According to Grime's "mass ratio" hypothesis, immediate control of ecosystem processes depends primarily on functional properties of dominant species.

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Sub-dominants, by contrast, may regulate ecosystem functioning in the longer term by influencing the recruitment of dominant plants, an impact described by Grime (1998) as a filter effect.

Heavily-grazed perennial grasslands, like other disturbed ecosystems, often support large populations of annual plant species. Because annuals typically succumb to competitive dominants as plant communities recover from disturbances (Wilsey and Polley 2003), these species generally are thought to have little influence on the functioning of recovering grasslands. In regularly disturbed grasslands, however, annual plants may contribute substantially to biomass production, suggesting that these species may have both immediate and longer-term consequences for ecosystem functioning.

Two groups of annual plants, *Gutierrezia dracunculoides* (DC.) S.F. Blake (annual or common broomweed) and the early-season (ES) annuals (mostly grasses) that establish following rainfall in autumn, are particularly abundant in grazed grasslands in central Texas, USA. Spatial and temporal variation in abundances of both groups of plants is large, presumably because of inter-annual variation in precipitation patterns or temperature (Heitschmidt 1979, Haferkamp et al. 1993), differences in soils or local disturbance histories, and the vagaries of seed dispersal.

According to Grime's (1998) mass-ratio hypothesis, immediate effects of these species on functioning should scale with their proportional contribution to biomass production and with the extent to which they differ from co-occurring plants in functionally-important traits (Chapin et al. 1996). Although variation in abundances of both *Gutierrezia* and annual grasses is large, greater than 20% of aboveground biomass in some perennial grasslands may be comprised of these species (Boyd et al. 1983, Haferkamp et al. 1997). Of perhaps greater importance to ecosystem functioning are the large differences in traits between these species and the grassland dominants. Annual grasses like *Bromus tectorum* L. (cheatgrass) and *Hordeum pusillum* Nutt. (little barley) emerge during autumn in central Texas and mature during the spring, shortly after the dominant C₄ perennial grasses begin growth. Consequently, annual grasses use resources like water and nitrogen (N) that might otherwise be available to perennial species or lost from the ecosystem. Although *Gutierrezia* grows while C₄ grasses are active, germinating in early spring and maturing in autumn (Scifres et al. 1971), this forb differs from dominant grasses in patterns of soil water extraction (Yoder et al. 1998).

Annuals also may exert longer-term control on ecosystem functioning by influencing the recruitment of dominant and other perennial species. Because of their growth strategy, ES annuals may be especially effective as a filter on community composition. Annual grasses, for example, form locally dense stands in grass-

lands in central Texas during early spring when soil water content typically is high and recruitment from the seed bank is greatest (Wilsey and Polley 2003). Not surprisingly, annual grasses have been shown to reduce the growth rate or standing crop of dominant grasses and other perennial species in some grasslands (Haferkamp et al. 1997, Humphrey and Schupp 2004). The capacity of both *Gutierrezia* and annual grasses to suppress dominant grasses has been linked to the rapid depletion of soil water by these species (Melgoza et al. 1990, Yoder et al. 1998).

We removed *Gutierrezia* and ES species of annual plants from a heavily-grazed grassland in central Texas, USA that is dominated by a non-native, C₄ perennial grass *Bothriochloa ischaemum* (L.) Keng, var. *songarica* (Rupr.) [King Ranch bluestem], with C₃ perennial forbs as sub-dominants (Polley et al. 2003, Wilsey and Polley 2003). Our objectives were to determine effects of removing annuals on grassland functioning (productivity, water balance, and soil and plant N content) and to assess consequences of removals for grassland composition. Because *Gutierrezia* and ES annuals differ from dominant grasses in growth form or season of activity, we predicted that functional impacts of removing these species would be proportionally larger than expected from their contributions to biomass production. Because ES species dominate vegetation while recruitment from the seed bank typically is greatest, we predicted that removing ES species would increase abundances of perennial forbs that regularly establish from seeds.

Removal treatments also provided a test of mechanisms that regulate plant succession in this grassland. Connell and Slatyer (1977) proposed two models of succession that are particularly relevant to disturbed ecosystems. According to the tolerance model, early-successional species have little influence on later-successional plants. Late-successional species always prevail, as in the more recent "competition-colonization" model (Tilman 1994). By contrast, the inhibition model holds that all species resist invasions. According to this model, initial colonists influence the course of succession. In the grassland that we studied, annuals decline as the dominant C₄ grass and perennial forbs increase after grazers are removed (Wilsey and Polley 2003). Our prediction that abundances of perennial forbs would increase following the removal of annuals is compatible with the inhibition model of succession.

Methods

Study site

This study was conducted during 2000 and 2001 on a site in Bell County, Texas USA (31°05' N, 97°20' W) that has been managed as grassland and grazed by cattle for at least 50 yr (slope = 1 to 2%). Soils at the study site are

classified as fine-silty, carbonatic, thermic Udorthentic Haplustolls. The surface 0.4 m of soil is composed mostly (55%) of clay. Annual precipitation at the site averages 880 mm (92 years record), with peaks in spring and autumn. Annual rainfall was greater than average during both 2000 (903 mm) and 2001 (1029 mm). The year preceding this experiment (1999), however, was among the driest years on record (461 mm). Rainfall from September 1999 through March 2000, the autumn/winter period during which establishment from seed usually is greatest, was 56% of the long-term mean of 490 mm. Rainfall from September 2000 through March 2001 was 106% of the long-term mean.

Experimental design

During December 1999, three blocks (each 14 × 20 m) were located at random in a grazed grassland (9.5 ha) that is dominated by the perennial C₄ grass *Bothriochloa ischaemum* (L.) Keng, var. *songarica* (Rupr.) (Nomenclature follows Diggs et al. 1999). This variety of *B. ischaemum*, introduced to the USA from China in 1917, and the more winter-tolerant variety *ischaemum* have been planted extensively to reclaim marginal farmland and to increase forage production in the southern Plains of the United States (Sims and Dewald 1982). Within each block, we established six experimental plots (each 4 × 4 m). Plots were separated by 2 m walkways and were arranged in a 3 by 2 grid. We randomly assigned three treatments to experimental plots in each block (n = 2 plots per treatment): (1) removal of ES annual species, the mostly grass plants that emerge during autumn and complete growth during spring (RES), (2) removal of *Gutierrezia dracunculoides* (RG), a forb that emerges during early spring and matures during autumn, and (3) control (no plants removed; NR). From January through September of each growing season, each block was fenced to exclude cattle.

Seedlings of *Gutierrezia* were removed by hand from appropriate experimental plots. Early-season annuals were much more numerous and were removed from appropriate plots each year by applying a non-residual herbicide (glyphosate [N-(phosphonomethyl)glycine], formulated as Roundup) with a hand sprayer. The herbicide was applied at the recommended aerial rate to entire plots in early January of each year before *Gutierrezia* germinates and while most perennial species are dormant. To prevent herbicide from contacting the few perennial species that were active during January (the most abundant of which was the C₃ perennial grass *Nassella leucotricha* (Trin. & Rupr.) Barkworth; Texas winter grass), plants with green tissues were covered during spraying.

Aboveground biomass production in each experimental plot was determined from harvests timed to

correspond with periods of peak biomass of early season (May) and late season species (September) in this grassland. At each harvest, we clipped vegetation to 5 cm height in two randomly-located 0.71 × 0.71 m areas (0.5 m²) in each experimental plot. Different 0.5 m² areas were sampled each season and year. At each harvest, plants were sorted by species and weighed after oven drying for 72 h at 60°C. Plant tissue harvested during May and September of each year was assigned to one of five functional groups of species (C₄ perennial grasses, C₃ perennial grasses, perennial forbs, ES annual species, and *Gutierrezia*). Aboveground production each year was estimated by summing values of peak biomass for each group. Nitrogen accumulation of each functional group was calculated by multiplying the value of peak biomass for the group by the N concentration of biomass. Total N accumulation was estimated by summing these values of peak N across functional groups for each experimental plot.

¹⁵N labeling

We used ¹⁵N as a tracer to measure treatment effects on N retention and distribution between soil and plants. Prior to the experiment, one circular plot (0.164 m²) was randomly-located within each experimental plot for ¹⁵N addition. To restrict the movement of added ¹⁵N into surrounding soil, we delimited plots by pressing a 0.4-m length of polyvinylchloride pipe (0.457 m diameter) into soil. Immediately prior to the experiment, we added the equivalent of 1 g of ¹⁵N per m² to each circular plot in the form of 99% ¹⁵N-enriched ammonium nitrate (NH₄NO₃). The NH₄NO₃ for each plot was dissolved in 1 l of distilled water before addition. To limit the movement of ¹⁵N from addition plots, we clipped the ES species that were present in NR and RG treatments after these species senesced in late spring of each year and placed the plants on the soil surface. Experimental plots were grazed following the first growing season in 2000. To prevent ¹⁵N loss to cattle, we clipped plants growing in all ¹⁵N-addition plots to 15 cm height following the final harvest of the 2000 season and covered plots with a steel grid. Cattle were excluded and the grid was removed prior to the second growing season (January 2001). Following termination of the experiment in October 2001, we harvested aboveground biomass (green plus standing dead) to the soil surface and removed 3 soil cores (4.2-cm diameter) to 40-cm depth in each ¹⁵N-addition plot. Crowns of grasses and any litter that was present on the soil surface were harvested. Each core was divided into increments of 0–10 cm, 10–20 cm, and 20–40 cm depth. Roots were separated from each increment by hand.

Plant material was dried at 60°C for 72 h, weighed, and finely ground with a ball-mill grinder. Soil samples

from the three cores per addition plot were composited by depth increment and air dried. For each plot, a mixed sample of soil from each depth increment was ground. The N concentration of plant (shoots, roots, crowns, litter) and soil samples was analyzed by the Soil Testing Laboratory at Texas A&M University (College Station, Texas, USA). The $\%^{15}\text{N}$ of samples was measured by mass spectrometry (Isotope Services, Inc., Los Alamos, New Mexico, USA).

We calculated the N content of each plant component by multiplying the value of biomass for the component by the N concentration of biomass. Quantities of added ^{15}N that were present in soils and plants were estimated by multiplying ^{15}N excess (atom $\%^{15}\text{N}$ of enriched samples minus atom $\%^{15}\text{N}$ of grassland plants at natural abundance; 0.367%) by the N content of each component. We measured bulk density on soil cores to calculate the amount of added ^{15}N that was present per unit of surface area in soil to 40 cm depth.

Soil N and water

During the second growing season, we measured inorganic N in soils from control plots and plots from which ES annuals had been removed. Most species of annuals in this grassland begin growth when perennial species are dormant, implying that annuals capture N that otherwise might accumulate in soil. During February, May, July and September of 2001, we removed three soil cores (4.5-cm diameter \times 15-cm depth) from each control plot and plot from which ES annuals had been removed. Soil samples were immediately returned to the laboratory and refrigerated at 4°C. Roots were removed and soil samples were homogenized by hand. Approximately 15-g samples of soil were shaken in 50 ml of 2 M potassium chloride (KCl) to extract NH_4^+ and NO_3^- . Concentrations of NH_4^+ and NO_3^- in filtered extracts of soil were measured with a Technicon Instruments Auto-analyzer (Technicon Industrial Systems, Tarrytown, New York, USA). Inorganic N concentrations were expressed relative to soil dry weight using gravimetric measurements of soil water content of sub-samples from each core.

Volumetric soil water content to 1.2 m depth was measured in the center of each experimental plot with a neutron probe. Neutron attenuation was measured at approximately weekly intervals at 15 cm depth increments. Because neutron readings taken near the soil surface exhibit instability, we also measured volumetric soil water content from 0 to 15 cm depth using time domain reflectometry (TDR). Single-diode probes with two stainless steel wave guides were installed vertically into soil at two randomly chosen locations in each experimental plot. An empirical equation from Topp et al. (1980) was used to calculate water content of soil

from measurements of the propagation time of electromagnetic waves through the permanently installed probes (MoisturePoint MP-917; Environmental Sensors Inc., Victoria, Canada). Calculations from the TDR technique were corrected to volumetric water content determined gravimetrically using a linear regression developed from measurements in soil near experimental plots ($r^2 = 0.66$, $n = 32$).

Statistical analyses

Aboveground biomass and N accumulation were highly correlated across plots for each species group ($r > 0.88$). To accommodate this correlation between dependent variables, we analyzed treatment effects on biomass and N accumulation with a mixed model multivariate analysis of variance (MANOVA) with block, removal treatment, and year as factors. Removal treatment was analyzed as a fixed effect in the model. Block and year were analyzed as random effects. MANOVA tests for effects of independent variables (year, removal treatment) on a single variate formed from a linear combination of the dependent variables biomass and N accumulation. The experiment was designed as a generalized randomized (complete) block in which each removal treatment was randomly assigned to two experimental plots in each block. Consequently, the MANOVA included a calculation of sampling error to account for differences between the two plots per block of each treatment. Wilk's lambda was used for testing significance. Differences in mean values between removal treatments within each year were assessed in MANOVA with contrasts. Measurements of soil inorganic N concentrations and monthly means of weekly measurements of soil water content were analyzed with a repeated measures technique.

Results

Vegetation

Few plants of *Gutierrezia* established during the dry autumn/winter of 1999/2000, so this forb contributed little to aboveground production during the initial year of the experiment (0.9% to 2.2% in control plots). Biomass of *Gutierrezia* increased substantially during 2001, and contributed an average of 27% of aboveground production in control plots. Early-season annuals comprised an average of between 16% and 44% of aboveground production in control plots in 2000 and between 20% and 31% of production in control plots in 2001.

Most functional groups of species, including C_4 perennial grasses, C_3 perennial grasses, perennial forbs, and ES annuals, were dominated by a few species. At the spring harvest in 2000, for example, 98% of the biomass

of ES annuals belonged to grasses. Grass biomass was about equally divided among 3 genera, *Bromus* (29%), *Hordeum* (31%), and *Limnodea* (29%). Greater than 99% of the C₄ grass component in both years was derived from a single species, *Bothriochloa ischaemum*. The most abundant perennial forb, *Ambrosia confertiflora* DC. (field ragweed), comprised 88% of biomass for this functional group in September 2000 and 97% of biomass for this group in September 2001.

Biomass production and N accumulation

The variate formed by combining the total of above-ground biomass production and N accumulation across plant groups (total production and N accumulation) differed among blocks (MANOVA block effect; $F_{(8,34)} = 4.06$, $P = 0.002$; not shown) and between years (MANOVA year effect; Table 1). Total production (Table 2) and N accumulation (Table 3) were greater across treatments during 2001 than 2000 (633.7 vs 376.4 g biomass m⁻² and 6.78 vs 4.20 g N m⁻²). Total production and N accumulation were not significantly affected by removal of *Gutierrezia* or ES annuals (MANOVA treatment effect; Table 1), although a post-hoc univariate ANOVA indicated that total N accumulation was reduced across years by removing ES annuals (6.25 vs 4.83 g N m⁻² in control and ES annual removal plots, respectively; ANOVA treatment effect; $F_{(2,18)} = 2.65$, $P = 0.10$). Nitrogen accumulation was slightly smaller, across years, in *Gutierrezia* removal than in control plots (6.25 vs 5.40 g N m⁻² in control and broomweed removal plots, respectively), but the difference was not significant. The absence of a statistical response to removals resulted partly from the large variability in total production and N accumulation among blocks. The immediate impact of ES annuals on biomass production and N accumulation

was evident in 2000 in the similarity between the proportional contribution of these species to above-ground production and N accumulation in control plots (0.16, 0.24 and 0.44 for biomass among blocks and 0.18, 0.36, and 0.61 for N among blocks) and the relative decrease in production and N accumulation on removing ES annuals (0.15, 0.15 and 0.44 for biomass, respectively, and 0.05, 0.26 and 0.60 for N, respectively). Compensation by other species mitigated the effect of removals during the second season (2001) when some plots from which ES annuals were removed produced as much or more biomass than did control plots (not shown). There also was no relationship between the relative decrease in N accumulation on removing ES annuals during 2001 (0.13, 0.20 and 0.05 among blocks) and the contribution of annuals to N accumulation in control plots (0.19, 0.36 and 0.40, respectively). Neither was there a consistent relationship between aboveground production in plots from which *Gutierrezia* was removed and the contribution of this species to production in adjacent control plots, even in 2001 when *Gutierrezia* was relatively abundant (not shown).

Grassland composition

Biomass and N accumulation declined relatively little following species removals because production and N accumulation of other species increased to compensate for the loss of annuals. Compensation was greatest among perennial forbs, especially during the second year. During 2001, production of perennial forbs was greater by a factor of almost 2 in plots from which *Gutierrezia* was removed and by a factor of almost 9 in plots from which ES annuals were removed than in control plots (Table 2). That *Gutierrezia* regulated production of perennial forbs also is evident in the

Table 1. Results from a MANOVA test of the influence of year (2000, 2001) and removal treatment (*Gutierrezia dracunculoides* removed, early-season (ES) species of annual plants removed, no plants removed) on a variate formed from a linear combination of aboveground biomass and N accumulation for each of five plant groups and for the total across groups.

Plant group	Source of variation	Wilk's lambda	F value	Numerator df	Denominator df	P value
<i>Gutierrezia</i>	year	0.534	7.40	2	17	0.0049
	treatment	0.417	4.66	4	34	0.0042
	year × treatment	0.575	2.71	4	34	0.0464
C ₄ perennial grasses	year	0.951	0.44	2	17	0.6535
	treatment	0.908	0.42	4	34	0.7938
	year × treatment	0.937	0.28	4	34	0.8893
C ₃ perennial grasses	year	0.988	0.10	2	17	0.9091
	treatment	0.780	1.12	4	34	0.3618
	year × treatment	0.677	1.83	4	34	0.1461
Perennial forbs	year	0.298	20.01	2	17	<0.0001
	treatment	0.292	7.22	4	34	0.0003
	year × treatment	0.317	6.58	4	34	0.0005
ES annuals	year	0.499	8.51	2	17	0.0028
	treatment	0.084	20.75	4	34	<0.0001
	year × treatment	0.609	2.39	4	34	0.0700
Total	year	0.416	11.90	2	17	0.0006
	treatment	0.751	1.31	4	34	0.2867
	year × treatment	0.893	0.49	4	34	0.7416

Table 2. Aboveground biomass production (g m^{-2} ; 1 SE in parentheses) in plots from which the forb *Gutierrezia dracunculoides* was removed (RG), early-season species of annual plants were removed (RES), or no plants were removed (NR) during each of two years (2000, 2001; $n=6$). Values for *Gutierrezia*, perennial forbs, and early-season (ES) annuals do not differ significantly within a year ($F_{(2,17)} < 3.6$, $P > 0.05$) if followed by the same letter, as determined with paired contrasts between removal treatments in a MANOVA in which biomass production and N accumulation were combined into a single variate. Removal treatments did not affect total biomass production or production of either C_3 or C_4 perennial grasses (Table 1).

Plant group	2000			2001		
	RG	RES	NR	RG	RES	NR
<i>Gutierrezia</i>	1.0 (0.4)a	5.4 (4.3)a	6.0 (1.8)a	9.7 (6.2)a	45.8 (42.6)a	177.1 (51.0)b
C_4 perennial grasses	233.9 (45.0)	210.5 (49.0)	234.4 (42.2)	284.0 (93.3)	186.8 (82.5)	197.2 (43.1)
C_3 perennial grasses	42.4 (26.7)	61.2 (37.5)	42.9 (32.6)	41.9 (23.2)	32.6 (20.8)	58.7 (36.7)
Perennial forbs	6.1 (1.8)a	26.7 (9.4)a	10.1 (4.4)a	78.9 (31.9)b	365.5 (148.2)c	41.5 (23.8)a
ES annuals	117.4 (28.7)b	0.7 (0.2)a	130.4 (37.9)c	196.7 (17.3)b	2.5 (1.5)a	182.2 (28.6)b
Total	400.8 (42.3)	304.5 (35.7)	423.8 (56.2)	611.2 (97.5)	633.2 (127.7)	656.7 (63.8)

strong negative relationship between biomass of *Gutierrezia* and production of perennial forbs during 2001 (Fig. 1). Biomass of perennial forbs exceeded 100 g m^{-2} only in plots in which *Gutierrezia* produced $< 50 \text{ g m}^{-2}$. Greater N accumulation by perennial forbs more than compensated for the removal of ES annuals in 2001 (Table 3), but the slight negative (albeit non-significant) effect of removing ES annuals on total N accumulation persisted through 2001 because the pool of N in *Gutierrezia* plants was smaller in plots from which ES annuals were removed than in control plots (Table 3). In contrast, production and N accumulation of the dominant C_4 grasses and of C_3 perennial grasses (Table 2 and 3) were remarkably consistent, and did not differ among removal treatments or between years (Table 1).

^{15}N

An average of 66% of added ^{15}N was retained in plants and soil after two growing seasons (0.66 g m^{-2} , $n=18$), but retention of ^{15}N did not differ among treatments ($P=0.84$). Most of the ^{15}N that was retained was present in soil (mean of 87.8%). The fraction of added ^{15}N that remained in soil was slightly greater in plots from which *Gutierrezia* was removed (91.3%) than in control plots or those from which early-season annuals were removed

(87.0% and 85.1%, respectively). Most of the added ^{15}N that was present in soil was recovered in the upper 10 cm of the profile (mean = 66.2%). Aboveground tissues, including shoots and crowns, contained most of the ^{15}N that was recovered in plants (mean = 81.8%). Neither the distribution of ^{15}N with depth in soil ($P=0.86$) nor the distribution of ^{15}N within plants differed among treatments ($P=0.31$).

Soil N and water

During the second growing season (2001), we measured soil N levels in control plots and plots from which ES annuals were removed. Concentrations of NO_3^- and NH_4^+ varied significantly with sampling date (Fig. 2; $P < 0.0001$). For NO_3^- , the date \times treatment interaction was significant ($P=0.01$). Soil NO_3^- concentrations were greater in plots from which ES annuals had been removed (RES) than in control (NR) plots during early February (DOY 37; $P=0.005$) and early May (DOY 127; $P=0.09$), but the reverse was true on the final sampling date (DOY 255; $P=0.06$). Soil NH_4^+ concentration did not differ between treatments ($P > 0.10$).

Soil water content was measured weekly during each growing season using TDR probes (0–15 cm depth) and the neutron attenuation technique (0–1.2 m depth).

Table 3. Aboveground N accumulation (g N m^{-2} ; 1 SE in parentheses) in plots from which the forb *Gutierrezia dracunculoides* was removed (RG), early-season species of annual plants were removed (RES), or no plants were removed (NR) during each of two years (2000, 2001; $n=6$). Values for *Gutierrezia*, perennial forbs, and early-season (ES) annuals do not differ significantly within a year ($F_{(2,17)} < 3.6$, $P > 0.05$) if followed by the same letter, as determined with paired contrasts between removal treatments in a MANOVA in which biomass production and N accumulation were combined into a single variate. Removal treatments did not affect total N accumulation or N accumulation of either C_3 or C_4 perennial grasses (Table 1).

Plant group	2000			2001		
	RG	RES	NR	RG	RES	NR
<i>Gutierrezia</i>	0.01 (0.005)a	0.07 (0.05)a	0.06 (0.01)a	0.11 (0.06)a	0.62 (0.57)a	2.06 (0.56)b
C_4 perennial grasses	1.94 (0.36)	1.78 (0.32)	1.92 (0.28)	2.62 (0.79)	1.69 (0.50)	1.69 (0.35)
C_3 perennial grasses	0.44 (0.26)	0.96 (0.69)	0.65 (0.49)	0.63 (0.35)	0.53 (0.34)	0.73 (0.47)
Perennial forbs	0.13 (0.03)a	0.40 (0.08)a	0.26 (0.13)a	0.91 (0.27)b	3.57 (1.41)c	0.48 (0.23)a
ES annuals	1.67 (0.37)b	0.01 (0.008)a	2.30 (0.84)c	2.33 (0.26)b	0.03 (0.02)a	2.34 (0.45)b
Total	4.19 (0.50)	3.22 (0.44)	5.19 (0.97)	6.60 (0.86)	6.44 (1.27)	7.30 (0.63)

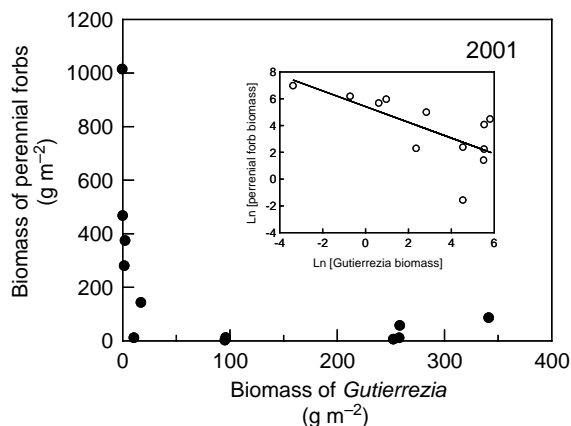


Fig. 1. The relationship during 2001 between aboveground biomass production of *Gutierrezia dracunculoides* and the production of perennial forb species in plots from which *Gutierrezia* plants were not removed ($n=12$). Insert: linear relationship between biomass of perennial forbs and *Gutierrezia* biomass plotted in ln-ln space ($r^2=0.42$, $P=0.01$).

Monthly means of TDR measurements varied with time during each year (not shown; $P<0.02$), but in neither 2000 nor 2001 was soil water content to 15 cm depth affected by treatments ($P>0.19$). Neutron attenuation readings to 30 cm depth also did not vary with treatments (not shown; $P>0.10$). Removing ES annuals increased the amount of water present in deeper soil layers during the early part of each growing season, however (Fig. 3). Treatment effects on monthly means of water content in the 30–60, 60–90 and 90–120 cm increments of the soil profile varied with date during both 2000 and 2001 ($P<0.10$). Water content differed little among treatments during the latter part of each growing season, but water content was greater in plots from which ES annuals were removed during the spring of each year (March through June 2000 at 30–60 cm, May and June 2000 at 60–90 cm, April and May 2000 at 90–120 cm, April and May 2001 at 30–60 cm and 60–90 cm; $P<0.10$).

Discussion

We removed *Gutierrezia* and ES species of annuals (mostly grasses) from a grassland in central Texas, USA to determine immediate effects of eliminating these early-successional plants on grassland functioning and to assess consequences of removals for grassland composition. Removal treatments simulated a non-random extinction event of the type that is widely practiced on intensively-managed grasslands. On grasslands in central Texas, for example, herbicides often are used to remove annual species in an effort to increase the production of warm-season perennial grasses. Immediate effects of removals generally were consistent with predictions

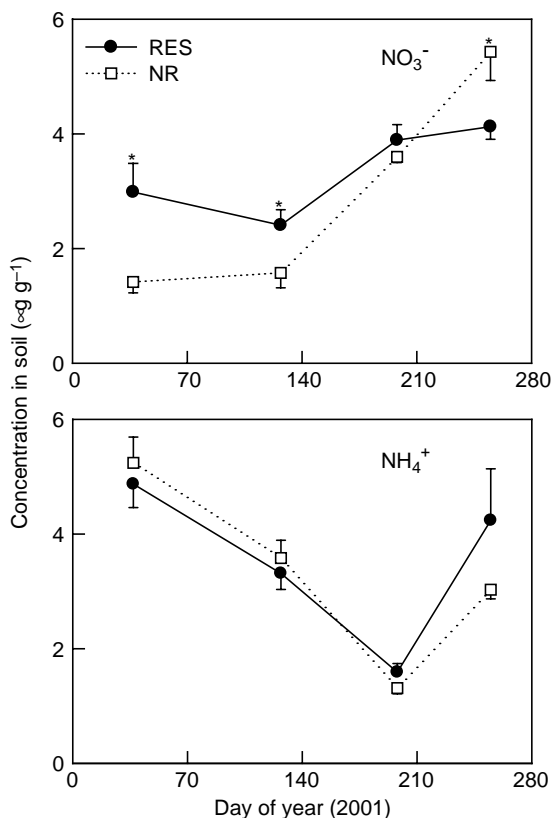


Fig. 2. Soil NO_3^- and NH_4^+ concentrations ($\mu\text{g g}^{-1}$; 0–15 cm depth, $n=18$) during 2001 in plots from which early-season species of annual plants were removed (RES) or no plants were removed (NR). Dates on which soil N differed significantly between treatments are denoted with an asterisk (*). Bars indicate 1 SE.

arising from Grime's mass-ratio hypothesis (Grime 1998). Biomass production and N accumulation of grassland vegetation declined following the removal of ES annuals in approximate proportion to the contribution of annuals to aboveground biomass and N, as predicted by the mass-ratio hypothesis. Because ES annuals contributed disproportionately to N accumulation in this grassland, removals more consistently affected aboveground N than biomass production, again consistent with the mass-ratio hypothesis. Annuals also influenced grassland composition, as was evident in the dramatic increase in biomass of perennial forbs that followed the removal of annuals. Initial impacts of removals on production and N accumulation were largely erased by the second year of the experiment, however, because perennial forbs and other species compensated completely for the biomass removed in annuals. In this heavily-grazed grassland, perennial species in the seed bank or in the pool of established plants rapidly filled the void created by the loss of annual species.

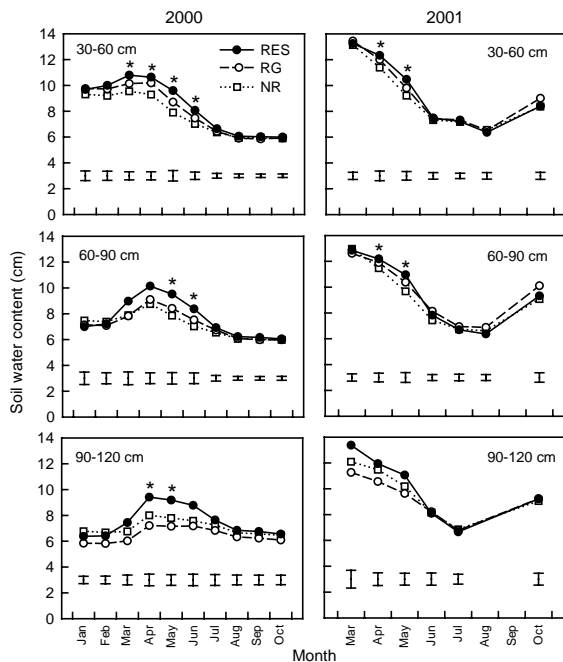


Fig. 3. Water content (cm) of 30–60 cm, 60–90 cm, and 90–120 cm increments of the soil profile in plots from which early-season species of annual plants were removed (RES), the annual forb *Gutierrezia dracunculoides* was removed (RG), and no plants were removed (NR) during each of two years (2000, 2001). Lines were fit to monthly averages of soil water content in each treatment ($n=6$ plots per treatment). Months during which water content was significantly greater in RES plots than in other treatments are denoted with an asterisk (*). Bars indicate the magnitude of the largest SE among the three treatments for each month.

That perennial forbs increased in abundance when annuals were removed is evidence that competition plays a role in structuring this grassland. In this *Bothriochloa*-dominated system, perennial forbs increase in abundance and annual species decline following release from grazing (Wilsey and Polley 2003). Annuals apparently delay this successional shift in species composition while grazers remain. By preempting disturbed sites, early-successional annuals determine which of the potentially dominant functional groups of species is present in greatest abundance. This role of annuals is consistent with the inhibition model of succession (and the successional-niche model of Pacala and Rees 1998) and with evidence that perennial species in this grassland are recruitment limited, rather than seed limited (Wilsey and Polley 2003).

The outcome of management practices and the longer-term functioning of this grassland both depend on how annuals influence plant composition. These grasslands are managed for cattle production and the primary forage for cattle is C_4 perennial grasses. Contrary to the pattern seen in other grasslands, dominant grasses

did not compensate for the loss of subordinate species (Boyd et al. 1983, Haferkamp et al. 1997, Smith and Knapp 2003). Rather, the removal of ES annuals stimulated the growth of perennial forbs. Although not evident in this two-year experiment, an exchange of annual grasses for perennial forbs may in the longer term reduce grass production for cattle and alter ecosystem functioning. Perennial forbs may displace *Bothriochloa*, the dominant C_4 grass in this grassland (Polley et al. 2003), and differ from *Bothriochloa* in traits that likely influence ecosystem functioning. Tissue N concentrations (Polley et al. 2003), rates of leaf gas exchange (Anderson et al. 2001, Maherli et al. 2002), plant water status (Polley et al. 2002), and rates of N mineralization from litter (Gill et al. 2006), for example, all are greater in some perennial forbs than in the dominant grass in this grassland.

Our results also are relevant to the question of whether plant diversity (typically taken as the number of plant species or “functional” groups of species) influences ecosystem functioning. A decrease in diversity may reduce productivity and other ecosystem functions if remaining plants fail to compensate for the functions of species that are lost. The extent to which remaining plants compensate for the functions of lost species depends partly on initial abundances and functional attributes of species that are eliminated. Evidence that compensation among remaining species often is incomplete comes mostly from experiments with plant communities assembled at random without regard to the productivities or relative abundances of species in natural communities (Hector et al. 1999, Tilman et al. 2001). In these synthetically-assembled communities, productivity and other ecosystem functions may decline with diversity loss simply because competitive dominants are excluded from species-poor mixtures (Grime 2002). McNaughton (1983) found that remaining species compensated completely for biomass removed from Serengeti grassland when the species eliminated were initially rare, but compensation was incomplete when dominant and sub-dominant species were removed. Smith and Knapp (2003) observed a similar response to removals in tallgrass prairie. By the second year of our experiment, perennial forbs and other sub-dominant species compensated completely for biomass removed in annuals. Likewise, remaining plants fully compensated for biomass lost when species groups were removed from grasslands in New Zealand (Wardle et al. 1999) and Minnesota, USA (Symstad and Tilman 2001). Annuals had little effect on soil N dynamics in our experiment, and initial impacts of removals on soil water content largely disappeared by the second growing season. Species composition also had little effect on soil N in New Zealand grassland (Wardle et al. 1999). Our experiment was not designed to test diversity effects on ecosystem functioning, but our results are consistent

with the view that ecosystem functions are more strongly linked to species attributes than to diversity per se (Grime 1998, Smith and Knapp 2003).

In summary, the immediate influence of annual species on grassland functioning scaled with the contribution of annuals to biomass production and N accumulation. These early-successional plants also regulated the recruitment of dominant species. Of these effects, the influence of annuals on the composition of vegetation likely is most important for the longer-term functioning and dynamics of these frequently-disturbed grasslands.

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