

Can prescribed fires restore C₄ grasslands invaded by a C₃ woody species and a co-dominant C₃ grass species?

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Abstract. Prescribed fire is used to reduce woody plant invasion and restore herbaceous production and diversity in grasslands and savannas worldwide. Here, we determined whether a concentrated series of repeated-winter, repeated-summer, or alternate-season (winter and summer) fires in a short timeframe (“transition fires”) could catalyze the restoration of C₄ perennial grasses in Southern Great Plains, USA grasslands that had become dominated by a fire-tolerant C₃ woody N₂-fixer (honey mesquite, *Prosopis glandulosa*) and a C₃ perennial bunchgrass (Texas wintergrass, *Nassella leucotricha*). We applied transition fires over a 5-yr span and maintenance fires on a portion of each plot 7 or 8 yr later. We measured herbaceous standing biomass and cover and soil variables (soil organic C, N, δ¹³C, and δ¹⁵N) in unburned, transition-burned, and maintenance-burned treatments. Greater δ¹³C at 10–20 (–17‰) than 0–10 (–20‰) cm depth increment confirmed that vegetation was historically mostly C₄ grassland that shifted toward C₃ dominance. Transition treatments with summer fire were most effective at top-killing mesquite, but no treatments root-killed >3%. Regrowth of top-killed mesquite was similar in all treatments and reached pre-fire height by 9–10 yr post-fire. Herbaceous production and cover responses showed that: (1) Alternate-season transition fires increased C₄ mid-grass, but did not change Texas wintergrass; (2) repeated-summer fires reduced Texas wintergrass, but did not change C₄ mid-grass; and (3) repeated-winter fires did not change C₄ mid-grass or Texas wintergrass compared with the unburned control. All maintenance fires stimulated Texas wintergrass biomass and cover, thus eliminating the reduction of Texas wintergrass caused by repeated-summer transition fires. There were no long-term effects of transition fires on soil C, N, δ¹³C, or δ¹⁵N. Results advance our understanding of the expectations and limitations of prescribed fire in shifting a woodland alternate state toward what was historically a fire supported C₄ grassland/savanna.

Key words: brush management; honey mesquite; *Nassella leucotricha*; *Prosopis glandulosa*; Texas wintergrass; woody plant encroachment.

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INTRODUCTION

Livestock overgrazing, fire suppression, rising atmospheric CO₂ concentration, and alteration of temperature and precipitation patterns associated with climate change appear to be shifting the competitive balance in favor of C₃ vs. C₄

plants in grasslands, savannas, and other dryland ecosystems throughout the world. This often results in an increase in C₃ woody plants (Van Auken 2000, Bond et al. 2005, Briggs et al. 2005, Midgley and Bond 2015, Archer et al. 2017, Stevens et al. 2017, Osborne et al. 2018, Zhang et al. 2019). These often-dramatic vegetation

changes have strong potential to alter biodiversity, rates of ecosystem processes, and the productivity and economic viability of animal production systems (Dalle et al. 2006, Eldridge et al. 2011, Ratajczak et al. 2012, Sala and Maestre 2014). Invasion by N₂-fixing woody legumes may increase soil N storage, accelerate N cycling processes, and alter soil microbial community structure and function (Liao and Boutton 2008, Boutton and Liao 2010, Hollister et al. 2010, Soper et al. 2015, Creamer et al. 2016), resulting in soil legacy effects that modify plant species interactions and hinder efforts to restore native grasslands (Nsikani et al. 2018). Consequently, there is great interest in identifying ecosystem management practices that can (1) reduce existing woody cover in areas that were once grass-dominated, and (2) limit the potential for continued woody encroachment (Roques et al. 2001, Archer and Predick 2014, Ding et al. 2020).

In some mixed C₃/C₄ grasslands, C₃ grasses are gaining dominance without facilitation by woody encroachment. Collatz et al. (1998) predicted this would be due to increasing atmospheric CO₂ levels. Climate change mediated shifts in seasonal rainfall patterns that favor C₃ grasses may also be a factor (Osborne 2008). For example, C₃ grasses outcompete C₄ grasses in some Mediterranean-type biomes in South Africa due to a higher proportion of precipitation in cooler months (Vogel et al. 1978, Sage et al. 1999). Similarly, the multi-year summer drought and high temperatures during the Great Plains USA “Dust Bowl” in the 1930s facilitated the advance of C₃ grasses in predominantly C₄ grassland due to a proportional increase in cool season precipitation (Knapp et al. 2020). Additionally, there are an increasing number of situations in C₄ grasslands or mixed C₃/C₄ grasslands worldwide where woody encroachment has not only reduced C₄ grass abundance directly but has facilitated the expansion of C₃ grasses by providing a subcanopy microenvironment that is more favorable to C₃ grasses. This has led to the displacement of C₄ grasses in Argentina (Rossi and Villagra 2003, Rauber et al. 2014), South Africa (Stuart-Hill and Tainton 1989), Australia (Prober et al. 2007), and the United States (Connell et al. 2020).

The grasslands and savannas of the Southern Great Plains (SGP), USA, have experienced an

increased abundance of the woody legume, honey mesquite (*Prosopis glandulosa*; hereafter, mesquite) (Johnson and Mayeux 1990). Primary causes are reduced fire frequency, heavy grazing pressure on grasses by cattle, and mesquite seed dispersal by cattle via consumption and fecal deposition (Ansley et al. 2017). Increased mesquite encroachment has led to an associated increase in the native C₃ perennial mid-grass, Texas wintergrass (*Nassella leucotricha*) (Simmons et al. 2008), identified as the *Prosopis/Nassella* association (Ansley et al. 2019). Both of these species have reduced the abundance of the once dominant C₄ grasses (Ansley et al. 2013). The increase in Texas wintergrass is related in part to its early spring growth prior to mesquite leaf emergence, while C₄ grasses initiate growth later and must compete with mesquite for light and soil moisture. In addition, N₂ fixation by mesquite may alleviate the consequences of the lower N-use efficiency of C₃ Texas wintergrass, allowing it to compete more effectively with the more N-use efficient C₄ grasses (Yuan et al. 2007, Ghannoum et al. 2011).

Texas wintergrass is inherently less productive than most C₄ mid-grasses that occur in the SGP (Ansley et al. 2013). Early studies noted the value of this species as winter and early spring forage (Whisenant et al. 1984). However, it is unpalatable most of the year and does not provide sufficient structure for nesting cover and thermal protection for many wildlife species, including gallinaceous birds, compared with C₄ mid-grasses (Tomecek et al. 2017). For these reasons, a landscape dominated by the *Prosopis/Nassella* association is undesirable for ranches interested in livestock production or for conservationists interested in grassland biodiversity. Currently, there are no economically viable landscape-scale solutions to transition this association back to original C₄ grassland (Murray et al. 2021).

The anthropogenic re-introduction of fire (i.e., “prescribed fire”) has been applied in many fire-dependent grasslands and savannas to reduce woody plant invasion and restore C₄ grasslands (Roques et al. 2001, Fensham et al. 2005, Keeley and Rundel 2005). Many woody species from flammable biomes, including mesquite, have dormant subterranean buds that are insulated from fire and resprout following above-ground “top-kill” caused by fire (Hoffmann and Solbrig

2003, Bond and Keeley 2005, Clarke et al. 2013, Pausas et al. 2018). The historical fire return interval in most SGP grasslands prior to European settlement in the late 1800s was 1–4 yr (Guyette et al. 2012). These included lightning-caused fires in summer and fires ignited by indigenous Americans in other seasons for hunting or gathering food (Engle and Bidwell 2001, Stewart et al. 2002). Similar to recent findings in Australia (Barker and Price 2018) and South Africa (Gordijn and O’Conner 2021), the SGP landscape was likely a changing mosaic of growing-season and dormant-season burned areas (Anderson 1990, Fuhlendorf and Engle 2004). Such a fire frequency would have prevented the *Prosopis/Nassella* association from attaining its current dominance. A lower density of mesquite would have been more common, because fire kills most mesquite seedlings <2 yr old (Ansley et al. 2015). Because of this, the grass community would have remained largely dominated by C₄ mid-grasses before and after each fire. Texas wintergrass and other C₃ perennial grasses likely remained a minor component of this mixed grass community because mesquite canopy size and density never attained levels that facilitated Texas wintergrass dominance. This assertion is supported by measurements of soil $\delta^{13}\text{C}$ values that indicate this region was once a relatively open C₄ grassland, but with some presence of C₃ species (Dai et al. 2006).

The present *Prosopis/Nassella* association with mature mesquite and Texas wintergrass monocultures beneath mesquite canopies may be particularly resistant to transition back to C₄ grasslands using fire. Like fire management plans in C₄ grasslands in South Africa (Archibald et al. 2005) and tallgrass prairie, USA (Briggs et al. 2005), prescribed fires in the SGP are typically applied during late winter or early spring when C₄ grasses are dormant (Wright and Bailey 1982, Whisenant et al. 1984). However, Texas wintergrass is often green during these months, resulting in reduced fire intensity and little effect on mesquite. Moreover, climate models predict climate change will generate warmer winters with little change in precipitation in the SGP and that may increase the proportion of green tissue in Texas wintergrass patches (Karl et al. 2009, IPCC 2021). Accordingly, there is increased interest in application of summer fires when C₃

grasses are dormant. These fires potentially yield greater mesquite top-kill than winter fires (Drewa 2003, Ansley et al. 2008). However, it remains uncertain if they can affect a transition from C₃ to C₄ grass dominance since mesquite regrowth quickly re-establishes the micro-environmental conditions that favor C₃ subcanopy species such as Texas wintergrass (Briske et al. 2003). In addition, some C₄ grasses may be slow to recover after summer fire (Trollope 1987, Engle et al. 2000, Ansley et al. 2006). Some evidence suggests that historic fire regimes may no longer be sufficient to prevent and/or control woody encroachment due to global change drivers (rising atmospheric CO₂ concentrations, altered rainfall regimes) that favor C₃ woody plant performance and may shorten the window for C₄ grass recovery (Bond and Midgley 2000, 2012, Case and Staver 2017). Thus, a series of intense fires within a short time span (i.e., a *pyric transition regime*) may be necessary to sufficiently top-kill mesquite and facilitate a shift toward C₄ grass species. In addition, a mixture of seasonal fires (i.e., a component of “pyrodiversity”) may be necessary to restore ecosystem plant diversity in mixed C₃/C₄ grassland (Bradstock 2010, Barker and Price 2018, He et al. 2019, Gordijn and O’Conner 2021). Because of the potential for woody regrowth after top-kill, maintenance fires to continually suppress mesquite would also be necessary.

Historically, C₄ grasslands maintained by frequent fire have high soil organic C, due to high root and litter production, but low soil N (Turner et al. 1997, Johnson and Matchett 2001). Most studies show that woody encroachment into grasslands increases soil organic C (Mordelet et al. 1993, Liao et al. 2006, Eldridge et al. 2011, Connell et al. 2020), and encroachment by N₂-fixing woody legumes enriches soil N (McCulley et al. 2004, Boutton and Liao 2010). However, Connell et al. (2021) demonstrated that both total soil C and N increased with increasing size of the non-leguminous woody shrub *Cornus drummondii* in tallgrass prairie, USA; thus, soil N enrichment is not limited to woody legumes. It is unknown how a concentration of fires in different seasons over a few years aimed at woody legume suppression may affect soil C or N, or their isotopes. In a global meta-analysis, Li et al. (2021) demonstrated that wildfires more than

prescribed fires and fires in other seasons more than winter season fires have greater potential for reducing soil C and N. However, some studies show little long-term effect (Larson et al. 2020, Pellegrini et al. 2020).

Here, we compared effects of repeated seasonal fires on herbaceous standing biomass and cover, soil organic C and total N, and their isotopic composition in a mixed C₃/C₄ SGP grassland that had shifted to C₃ dominance. We applied two or three fires within a 3- to 5-yr time span as prescribed “transition” fires to top-kill and possibly root-kill mesquite and stimulate a transition away from mesquite/Texas wintergrass woodland to a C₄ grassland/savanna state. We followed these transition fires with maintenance fires 7–8 yr later. We hypothesized that: (1) Treatments that included summer fire would be more effective than winter-only fires in top-killing or possibly root-killing mesquite and shifting the grass community away from Texas wintergrass and toward C₄ grasses; (2) there would be no long-term negative effects of the transition or maintenance fires on soil organic C or soil N stores; and (3) the maintenance fires would maintain or strengthen any changes in herbaceous community dynamics attributable to the transition fire treatments.

METHODS

Study area

Research occurred on a 200-ha savanna in north central Texas, USA (33.852603, –99.436555; elev. 384 m), near the north-south mid-point in the SGP (Appendix S1: Fig. S1; USDA-NRCS 2006) and northern edge of the subtropical climate zone. Mean annual rainfall (30 yr; 1977–2006) is 698 mm, and mean annual air temperature is 17.1°C. Mean daily air temperatures range from 35.9°C in July to –2.4°C in January (NOAA-NCDC 2019). Soils are 1–2-m deep clay loams on 1–3% slopes and classified as fine, mixed, superactive, thermic Vertic Paleustolls of the Tillman series (USDA-NRCS 2019a). Ecological site description is Clay Loam R078CY096TX (USDA-NRCS 2019b). Pre-treatment overstory at study initiation in 1991 consisted of 2- to 3-m tall mesquite at 30–50% canopy cover. No fire occurred on the site for at least 40 yr prior to the beginning of this study. Mesquite were aerial

sprayed with 2,4,5-T in 1973 that root-killed ~20% and top-killed the remaining plants. Thus, most mesquite were 18-yr-old regrowth mixed with newly recruited plants of various ages. The herbaceous layer consisted of a mixture of C₃ and C₄ perennial grasses, dominated by C₃ Texas wintergrass, and the C₄ perennial short-grass, buffalograss (*Bouteloua dactyloides*). Several C₄ perennial mid-grass species were infrequently scattered on the site. Forbs and annual grasses comprised a small component of the herbaceous layer. Typical growing seasons are from March to May for C₃ grasses and April to September for C₄ grasses. This site had a history of moderate continuous cow–calf cattle grazing for at least 80 yr before we began our study. Between 1960 and 1991, the site experienced moderate continuous cow–calf grazing at a stocking rate of 12 ha/cow (Asner et al. 2003, Ansley et al. 2013). Livestock (cattle) grazing was excluded for the entire duration of the study period.

Fire treatments

The study had three replicate plots (each 3–5 ha) in unburned mesquite woodland (“control”) and in each of six fire treatments. The first three fire treatments, referred to as “Transition” fires, consisted of repeated intense fires (as “fire steps”) within a 3- to 5-yr span (1991–1995) and included repeated-winter (TWF), repeated-summer (TSF), and repeated alternate-season (TAF) fires (Table 1). In 2002, 25% of each transition fire treatment plot was burned as a “Maintenance” fire treatment in either winter or summer, with the remaining portion unburned. As a continuation of the Transition fires, the Maintenance fire treatments included repeated-winter (MWF), repeated-summer (MSF), and alternate-season (MAF) fires (Table 1). All winter fires occurred between late January and early March when mesquite were leafless. All summer fires occurred in late summer (September) with mesquite in full foliage. All fires were headfires burned toward pre-burned blacklines (Wright and Bailey 1982). Air temperature, relative humidity (RH), and wind speed were measured on site within 10 min prior to headfire ignition using a sling psychrometer and hand-held wind gauge at 2 m height. We estimated herbaceous fine fuel amount by harvesting 10 randomly located 0.25-m² quadrats in intercanopy spaces

Table 1. Transition and maintenance fire treatments in the study, and fire steps within each treatment.

Treatment number and long name	Treatment short ID†	Steps in each fire treatment‡	Time burned
1. Control	Control	None	None
2. Repeated-winter transition fires	TWF	w91 + w93 + w95	Late January to early March
3. Repeated-summer transition fires	TSF	s92 + s94	Early September
4. Alternate-season transition fires	TAF	w91 + s92 + w94	Winter—Late January to early March; Summer—early September
5. Repeated-winter maintenance fire	MWF	w91 + w93 + w95 + w02	Late January to early March
6. Repeated-summer maintenance fire	MSF	s92 + s94 + s02	Early September
7. Alternate-season maintenance fire	MAF	w91 + s92 + w94 + s02	Winter—Late January to early March; Summer—early September

Notes: The treatment short ID is used in the figures. The control is unburned mesquite woodland.

† T = Transition, M = maintenance, W = winter, S = summer, A = alternate-season, F = fire.

‡ Steps in each fire treatment include season and year (e.g., w91 = winter fire, 1991; s02 = summer fire, 2002).

between mesquite trees the day of the fire. Fire temperatures were measured at 1-s intervals at six heights (0, 0.1, 0.3, 1, 2, and 3 m) at each of three to six locations per plot using thermocouples and a datalogger (Ansley et al. 2010a). Fire-line intensity was determined in each plot using the flame length equation of Byram (1959): $I = 269.37 \times L^{2.2}$, where I = fireline intensity (kW/m) and L = flame length (m). We estimated flame length in each plot by videotaping or photographing the flame front as it passed 3-m tall metal standards (after Ansley et al. 2015).

Vegetation and soil measurements

Fifty mesquite trees were evaluated along two parallel 10-m wide \times ~ 50-m long belt transects (length varied depending on mesquite density) in each plot at the end of the second growing season following each fire step. Response variables included percent whole plant mortality (root-kill), percent of trees with complete above-ground mortality but with basal sprouting (top-kill), and percent of trees partially top-killed or unaffected. New belt transects and a different set of trees were evaluated in each plot each sample period. Heights of live mesquite trees were measured in each plot every 3–4 yr from 1994 to 2006. Mesquite density was quantified in each plot from low-level aerial images taken in the year 2000, 5 or 6 yr after the transition fire treatments. ArcMap GIS was used to randomly locate three 20 \times 20 m subplots in each plot, and mesquite plants were counted manually in each subplot. Prior to the 2002 fire treatments, mesquite

canopy cover was measured along two parallel 60-m lines in each plot using the line intercept method.

Herbaceous standing biomass (live + standing dead; hereafter “biomass”) and litter were measured in all treatments from 2002 to 2006 so that responses to transition fires at 8–13 yr post-fire and maintenance fires at 1–5 yr post-fire were measured during the same years. Sampling occurred in summer, 2002 before the 2002 summer fires, in summer and fall in 2003–2005, and in fall 2006. Sampling occurred in intercanopy spaces between mesquite at 8 randomly determined points in each replicate plot. All herbaceous material was clipped within a 0.25-m² quadrat at each sample point and separated into 5 functional groups: C₃ annual grasses, C₃ perennial mid-grasses (C₃ mid-grasses), C₄ perennial mid-grasses (C₄ mid-grasses), C₄ perennial short-grasses (C₄ short-grasses), and forbs (Table 2). We separated mid-grasses from short-grasses based on vegetative height and production potential. Most of the mid-grass species were bunchgrasses, but a few were stoloniferous. Most of the short-grass species were stoloniferous. Herbaceous litter was collected and included non-woody material on the ground that had separated from standing dead material. No areas were clipped more than once. Samples were oven-dried at 60°C until dry and weighed. We calculated total perennial grass (TPG) biomass (TPG = C₃ mid-grasses + C₄ mid-grasses + C₄ short-grasses), percent of TPG biomass of each of the three perennial grass groups, and TPG-to-litter biomass ratio.

Table 2. List of grass species alphabetically by scientific name found at the site and their classification into functional groups.

Group	Scientific name and author(s)	Common name
C ₃ annual grass	<i>Bromus japonicus</i> Houtt.	Japanese brome
	<i>Hordeum pusillum</i> Nutt.	Little barley
C ₃ mid-grass	<i>Elymus smithii</i> (Rydb.) Gould†	Western wheatgrass
	<i>Nassella leucotricha</i> (Trin. & Rupr.) R.W. Pohl.	Texas wintergrass
C ₄ mid-grass	<i>Poa arachnifera</i> Torr.	Texas bluegrass
	<i>Aristida purpurea</i> Nutt.	Purple threeawn
	<i>Bothriochloa laguroides</i> (DC.) Herter	Silver bluestem
	<i>Bouteloua curtipendula</i> (Michx.) Torr.	Sideoats grama
	<i>Bouteloua gracilis</i> (Kunth) Lag. ex Griffiths‡	Blue grama
	<i>Digitaria californica</i> [Benth.] Henrard	Arizona cottontop
	<i>Eriochloa sericea</i> (Scheele) Munro ex Vasey	Texas cupgrass
	<i>Hilaria mutica</i> (Buckley) Benth.§	Tobosagrass
	<i>Panicum obtusum</i> Kunth	Vinemesquite
	<i>Sporobolus giganteus</i> Nash	Giant dropseed
	<i>Sporobolus compositus</i> (Poir.) Merr.	Tall dropseed
	<i>Sporobolus cryptandrus</i> (Torr.) A. Gray	Sand dropseed
	<i>Tridens albescens</i> (Vasey) Wootton & Standl.	White tridens
C ₄ short-grass	<i>Bouteloua hirsuta</i> Lag.	Hairy grama
	<i>Bouteloua rigidisetia</i> (Steud.) Hitchc.	Texas grama
	<i>Buchloe dactyloides</i> (Nutt.) Engelm.¶	Buffalograss

Note: All nomenclature by Stubbendieck et al. (2017).

† Recent synonym *Elytrigia smithii* (Rydb.) Nevski.

‡ *Bouteloua gracilis* is a highly productive bunchgrass in this ecosystem and is thus placed in the C₄ mid-grass group.

§ Recent synonym *Pleuraphis mutica* Buckley.

¶ Recent synonym *Bouteloua dactyloides* (Nutt.) J.T. Columbus.

Basal cover of individual grass functional groups in response to the transition fires is reported in Ansley et al. (2010a). For the present study, we report TPG basal cover and percent of TPG cover of each of the three perennial grass groups from 1994 to 2005 in response to transition and maintenance fire treatments. Texas wintergrass comprised 98 and 95% of C₃ mid-grass basal cover when averaged over all treatments in 2003 and 2005, respectively. Therefore, for this paper, C₃ mid-grass and Texas wintergrass are considered synonymous.

Soil samples were collected at 0–10 and 10–20 cm depths at the end of the C₄ grass growing season in 1994, 1996, 2001, and 2006 within patches of Texas wintergrass in intercanopy spaces between mesquite trees. Ten 2.5 cm diameter soil cores were collected in each plot and mixed into two subsamples (five cores per subsample) prior to analysis. Litter was removed from the soil surface before coring. Samples were oven-dried at 50°C for 24 hr and passed through a 2-mm sieve to remove coarse organic debris (Nelson and Sommers 1982). Soils were then homogenized thoroughly and pulverized for elemental and isotopic analyses. Soil organic C (hereafter “soil C”) and total N (hereafter “soil N”) concentrations were determined by combustion/gas chromatography using a Carlo-Erba NA-1500 (Milan, Italy) elemental analyzer. Soils containing CaCO₃ were pretreated with HCl to volatilize inorganic C (Nieuwenhuize et al. 1994, Harris et al. 2001). Soil bulk density was used to convert concentrations (g/kg) to pool sizes (g/m²). C/N ratios were calculated by dividing soil C by soil N. Soils were also analyzed for δ¹³C and δ¹⁵N using a Carlo-Erba EA-1108 (Milan, Italy) elemental analyzer interfaced with a Delta Plus (Thermo-Finnigan, San Jose, CA) isotope ratio mass spectrometer operating in continuous flow mode. Precision was <0.1‰ for δ¹³C and <0.2‰ for δ¹⁵N. The δ¹³C values were expressed relative to the V-PDB standard (Coplen 1995), while δ¹⁵N values were expressed relative to atmospheric N₂ (Mariotti 1983). Appendix S1: Table S1 shows the timeline when herbaceous and soil data were collected relative to number of years post-fire (hereafter: years post-fire refers to the number of C₄ grass growing seasons post-fire).

Statistical analysis

Data from individual trees, herbaceous quadrats, or soil samples were averaged at the replicate plot level prior to analysis ($n = 3$). Repeated-measures analyses (Proc GLM, SAS 9.4, SAS Institute Inc., Cary, NC, USA) determined main effects of transition or maintenance fire treatment and sample date and their interactions on each herbaceous and soil variable. Fire treatment was the whole plot, and year was the split plot. We used the replicate × treatment mean square was the error term to test treatment

effects, and the pooled error to test year effects and treatment \times year interactions. Sample dates that occurred within the time-period of the transition fires (1994), or in the year of the maintenance fires (2002), were not included in the repeated-measures analyses. Subsequently, one-way GLM analyses were performed to assess effects of transition or maintenance fire treatments vs. the control on each sample date for each variable. For herbaceous and soils variables, these per date analyses were divided into control vs. transition fires (TWF, TSF, TAF), or control vs. maintenance fires (MWF, MSF, MAF). Mean separations were performed with LSD at $P \leq 0.05$. Percentage data were arcsine transformed prior to analysis to meet assumptions of normality and homoscedasticity.

RESULTS

Precipitation, fire weather, and fire behavior

Total annual precipitation was below average in 6 of the 11 yr (1996–2006) after the transition fires (Fig. 1). Annual precipitation was 41% below average in 2001, the year prior to the 2002 fires, and was below average in 3 of the 5 yr during the 2002–2006 herbaceous sampling period. Precipitation was 42% and 31% below average during the C₃ grass (March–May) and C₄ grass (April–September) growing seasons, respectively, from 2003 to 2006. The normal pattern of highest precipitation in May and June never occurred from 2001 to 2006.

Averaged across all fire steps within each treatment, pre-burn air temperature, RH, fine fuel, peak fire temperature, and fireline intensity were greater, and wind speed slightly lower, in summer than winter transition fires (Table 3). Peak fire temperature mostly occurred at the 10 cm height. Trends in these variables were similar for the 2002 maintenance fires with the exception that peak fire temperature was greater in the winter than the summer fires. This occurred because fine fuel in the 2002 winter fires had very little green tissue, due to the 2001 drought. In contrast, fine fuel in the summer 2002 fires contained a large amount of green tissue due to above normal June and July precipitation. Summer fires typically had lower peak temperature, but greater duration of high temperature than winter fires (Appendix S1: Fig. S2).

Mesquite responses

Mesquite top-kill was 63% in repeated-winter, and >85% repeated-summer and alternate-season transition fire treatments (Table 4). After the 2002 fires, mesquite top-kill was >80% in all fire treatments. Root-kill remained <3% in all treatments. Regrowth height of top-killed mesquite increased at similar rates in all transition fire treatments from 1994/95 to 2005 and nearly attained levels in the control by 2005 (Fig. 2). The 2002 maintenance fires reduced mesquite height to zero, and regrowth height again increased equally among treatments. Mesquite density in 2001, 7–8 yr after the transition fires, was not different ($P \leq 0.05$) among treatments and averaged 892 ± 119 trees/ha. Mesquite canopy cover was $58.1 \pm 7.0\%$ in the control, $48.3 \pm 4.1\%$ in repeated-winter, $45.8 \pm 8.3\%$ in repeated-summer, and $42.4 \pm 3.7\%$ in alternate-season fire treatments.

Herbaceous biomass

Repeated-measures analyses revealed that at 8–13 yr after the transition fires, the main effect of sample date was significant for most herbaceous biomass variables, but the main effect of fire treatment was significant for only C₄ mid-grass, C₄ mid-grass percent of TPG biomass, and forbs (Table 5). There was a significant treatment-by-date interaction for litter. In contrast, in the 1–4 yr following the 2002 maintenance fires, most of the herbaceous biomass variables had a significant treatment-by-date interaction. Functional group responses when averaged over all sample dates, or over all treatments, are shown in Appendix S1: Tables S2 and S3.

The greatest declines in C₃ mid-grass biomass over the duration of the study were in the unburned control (Fig. 3a). The transition fire treatment that had the greatest effect on reducing C₃ mid-grass biomass compared with the control was repeated-summer fire. Following the 2002 maintenance fires, C₃ mid-grass biomass increased in all fire treatments to levels similar to the control by 1–2 yr post-fire (Fig. 3b). C₄ mid-grass biomass was greater in the alternate-season transition fire treatment than the control on most sample dates from 2002 to 2006 (Fig. 3c). C₄ mid-grass biomass increased more rapidly after winter than summer maintenance fires and was

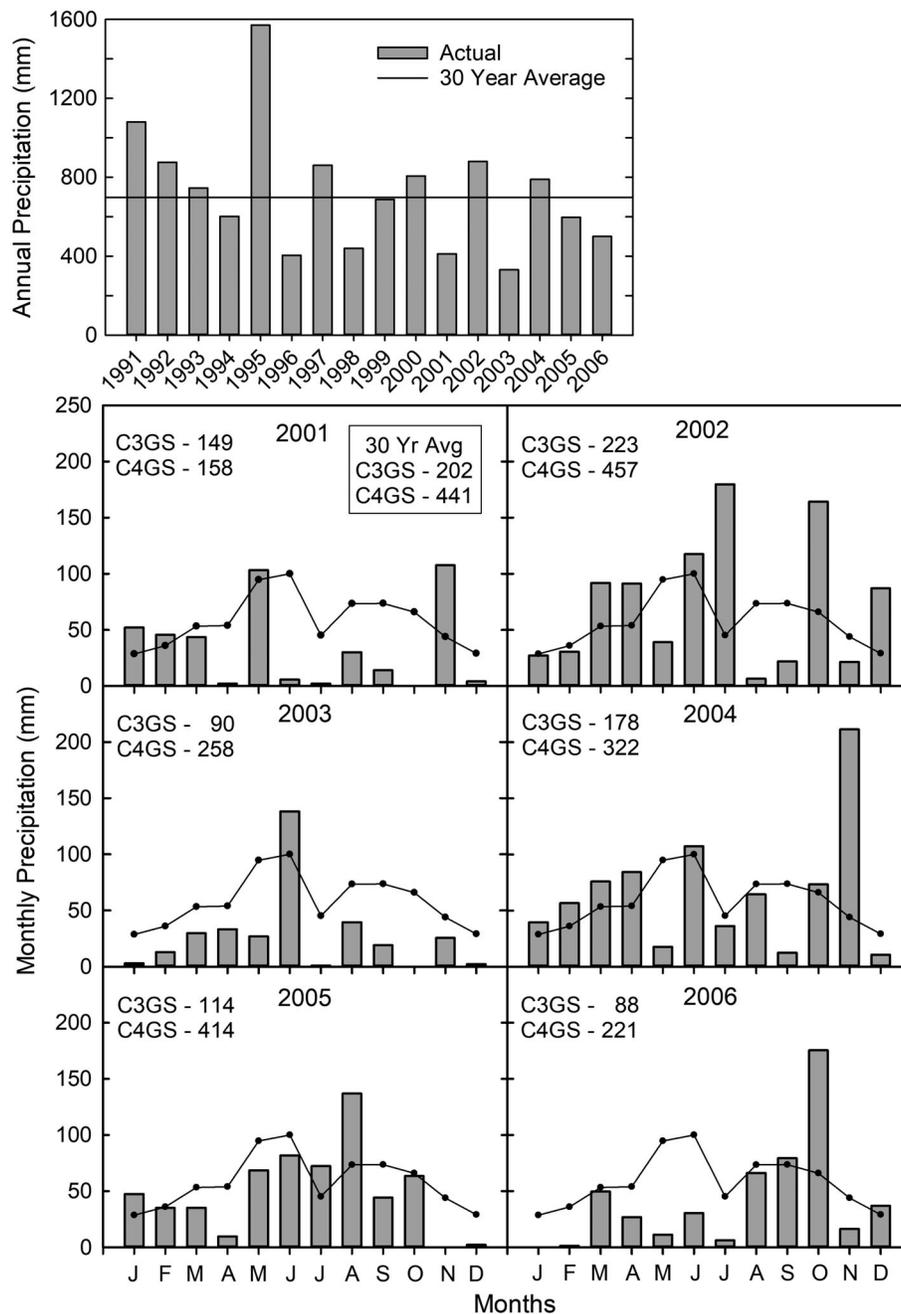


Fig. 1. Annual precipitation, 1991–2006 (top panel), and monthly precipitation in 2001, before the 2002 fires, and during the 2002–2006 herbaceous sampling period (bottom six panels). Totals for C_3 grass growing season (C3GS; March–May) and C_4 grass growing season (C4GS; April–September) are shown for each year (30-yr averages in box within the 2001 panel).

greater in all 3 maintenance fire treatments than the control from fall 2005 to fall 2006 (Fig. 3d). C_4 mid-grass biomass spiked in the control in fall 2003, but rapidly declined after that.

There were no differences in C_4 short-grass biomass between transition fire treatments and the control from 2002 to 2006 (Fig. 4a). C_4 short-grass biomass increased more rapidly after

Table 3. Pre-burn weather and herbaceous fine fuel of each fire step in repeated-winter, repeated-summer, or alternate-season transition (t) and maintenance (m) fire treatments.

Treatments†	Fire step	Air temperature (°C)	Relative humidity (%)	Wind speed (m/s)	Fine fuel (g/m ²)	Peak fire temperature (°C)	Fireline intensity (kW/m)
Rep. Winter	w91 (t)	14.4 ± 2.6	33.0 ± 8.9	4.5 ± 1.5	249 ± 43	590 ± 16	183 ± 30
Rep. Winter	w93 (t)	20.9 ± 0.8	29.3 ± 1.8	5.0 ± 1.3	266 ± 11	627 ± 39	2518 ± 472
Rep. Winter	w95 (t)	25.4 ± 2.1	26.3 ± 2.9	4.2 ± 1.6	263 ± 48	469 ± 40	770 ± 154
Rep. Winter	w02 (m)	21.9 ± 1.9	25.3 ± 6.8	4.8 ± 0.1	217 ± 11	654 ± 10	2766 ± 474
Rep. Summer	s92 (t)	33.5 ± 1.0	27.0 ± 3.8	2.1 ± 0.4	278 ± 54	632 ± 29	3827 ± 369
Rep. Summer	s94 (t)	32.2 ± 1.1	45.3 ± 3.3	3.2 ± 0.4	267 ± 13	649 ± 17	2690 ± 358
Rep. Summer	s02 (m)	33.5 ± 0.9	38.0 ± 1.0	1.6 ± 0.4	347 ± 71	521 ± 43	4548 ± 1356
Alt. Season	w91 (t)	23.8 ± 1.5	25.3 ± 4.3	6.6 ± 1.0	269 ± 19	--nd--	543 ± 356
Alt. Season	s92 (t)	33.1 ± 0.7	41.3 ± 3.0	4.6 ± 0.8	390 ± 18	627 ± 37	7796 ± 960
Alt. Season	w94 (t)	24.2 ± 0.2	32.0 ± 2.1	2.5 ± 0.1	143 ± 18	441 ± 58	2186 ± 1477
Alt. Season	s02 (m)	33.5 ± 0.7	35.7 ± 0.7	1.0 ± 0.1	367 ± 75	581 ± 17	4124 ± 249
Winter (t)	w91–95	21.7 ± 1.2 b	29.2 ± 2.0 b	4.5 ± 0.6 a	238 ± 18 b	526 ± 32 b	1240 ± 368 b
Summer (t)	s92–94	33.0 ± 0.4 a	37.9 ± 2.8 a	3.3 ± 0.4 b	312 ± 22 a	636 ± 15 a	4771 ± 835 a
Winter (m)	w02	21.9 ± 1.9 y	25.3 ± 6.8 y	4.8 ± 0.1 x	217 ± 11 y	654 ± 10 x	2766 ± 474 y
Summer (m)	s02	33.5 ± 0.5 x	36.8 ± 0.7 x	1.3 ± 0.2 y	357 ± 46 x	551 ± 25 y	4386 ± 583 x

Notes: Except for the two bottom rows, all values are means (\pm s.e.) of three plots. The last four rows are the average of all winter or summer fire steps. Means with similar letters are not significantly different ($P \leq 0.05$).

† Rep. = repeated; Alt. = alternate; Winter (t) = mean for all transition winter fire replicates ($n = 12$ or 15). Summer (t) = mean for all transition summer fire replicates ($n = 9$); Winter (m) = mean for all maintenance winter fire replicates ($n = 3$); Summer (m) = mean for all maintenance summer fire replicates ($n = 6$).

Table 4. Mesquite responses to repeated-winter, repeated-summer, or alternate-season transition (t) and maintenance (m) fire treatments.

Fire treatment	Step in each fire treatment†	Root-kill (%)	Complete top-kill (%)	Partial or no top-kill (%)
Repeated winter	w91 (t)	2.2 ± 1.7 a‡	8.2 ± 5.6 c	89.6 ± 5.8 a
Repeated winter	w93 (t)	0.0 ± 0.0 a	57.8 ± 2.2 b	42.2 ± 2.2 b
Repeated winter	w95 (t)	1.4 ± 0.9 a	63.2 ± 3.5 b	35.4 ± 4.2 b
Repeated winter	w02 (m)	0.7 ± 0.3 a; x	86.7 ± 5.9 a; y	12.7 ± 6.2 c; x
Repeated summer	s92 (t)	0.4 ± 0.4 a	95.3 ± 2.9 a	4.4 ± 2.6 ab
Repeated summer	s94 (t)	1.7 ± 1.3 a	89.2 ± 3.9 a	9.0 ± 2.7 a
Repeated summer	s02 (m)	0.7 ± 0.7 a; x	98.0 ± 1.0 a; x	1.3 ± 0.3 b; y
Alternate season	w91 (t)	0.0 ± 0.0 a	30.3 ± 10.1 c	69.7 ± 10.1 a
Alternate season	s92 (t)	0.7 ± 0.7 a	81.6 ± 2.6 b	17.7 ± 3.2 b
Alternate season	w94 (t)	1.4 ± 0.9 a	85.8 ± 6.5 ab	12.8 ± 7.2 b
Alternate season	s02 (m)	1.0 ± 0.6 a; x	97.7 ± 0.3 a; x	1.3 ± 0.3 c; y

Notes: Responses represent the cumulative effect of multiple fires after the first step in each treatment. All values are means (\pm s.e.) of three plots.

† Step followed by (t) is part of transition treatment. Step followed by (m) is maintenance fire.

‡ Means with similar letters (a–b) are not significantly different ($P \leq 0.05$; $n = 3$) among steps within each fire treatment. Means with similar letters (x–y) are not significantly different ($P \leq 0.05$; $n = 3$) between fire treatments after the last step of each fire treatment.

winter than summer 2002 fires and never increased in the alternate-season fire treatment (Fig. 4b). TPG biomass declined in transition fire treatments and the control from 2002 to 2006 with few differences between treatments (Fig. 4c). TPG biomass increased more rapidly after winter than summer maintenance fires and

exceeded the control in several of the fire treatments from fall 2004 to fall 2006 (Fig. 4d). Maintenance fires caused brief increases in C₃ annual grass and forb biomass that lasted no more than 2 yr (Appendix 1: Fig. S3).

Transition and maintenance fire treatments that included summer fire occasionally reduced

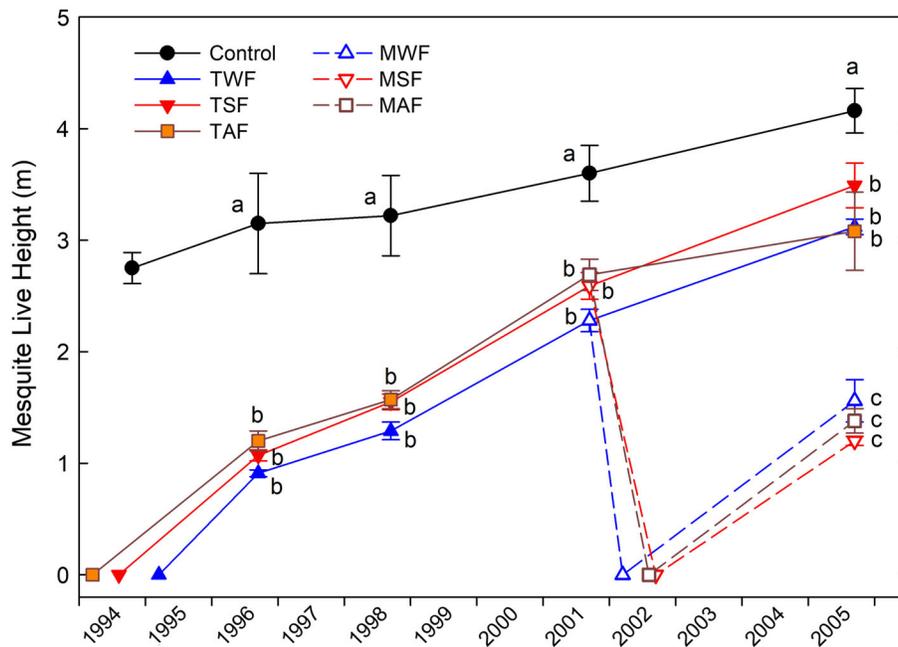


Fig. 2. Mean (\pm SE) mesquite live canopy height following the last fire step in the transition fire treatments in 1994 or 1995 (solid lines) and following the maintenance fires in 2002 (dashed lines), compared to the control. Means with similar letters within a sample date are not significantly different at $P \leq 0.05$. The x -axis ticks are starting and ending points of each year.

C_3 mid-grass percent of TPG biomass compared with the control (Fig. 5a, b). In contrast, all transition and maintenance fire treatments increased C_4 mid-grass percent of TPG biomass compared with the control on most dates from fall 2004 to fall 2006 (Fig. 5c, d).

Litter biomass peaked in summer 2004 in all transition fire treatments and the control and declined with some exceptions from 2004 to 2006 (Fig. 6a). Litter increased more rapidly after winter than summer 2002 maintenance fires (Fig. 6b). As a result, TPG-to-litter ratio was greater in treatments that had a 2002 summer fire than the control from fall 2004 to fall 2005 (Fig. 6d).

Grass basal cover

Repeated-measures analyses revealed a significant main effect of transition fire treatment and sample date on all grass basal cover variables and a significant treatment-by-date interaction with TPG basal cover (Appendix S1: Table S4). Averaged over all sample dates, TPG cover and C_4 mid-grass percent of TPG cover were greatest

in the alternate-season transition fire treatment (Table 6). C_3 mid-grass percent of TPG cover was lowest, and C_4 short-grass percent of TPG cover was greatest in the repeated-summer fire treatment.

TPG basal cover was greatest in the alternate-season transition fire treatment on most dates from 1998 to 2005 (Fig. 7a). Following the 2002 maintenance fires, TPG cover increased at the same rate in all fire treatments and there was no difference between treatments by 2005 (Fig. 7e). C_3 mid-grass percent of TPG cover was lower in the repeated-summer fire treatment than the control from 1998 to 2005 (Fig. 7b). Following the maintenance fires, there were few differences in C_3 mid-grass percent of TPG cover between fire treatments and the control (Fig. 7f).

C_4 mid-grass and C_4 short-grass differed in their percent of TPG cover responses to the transition fire treatments. C_4 mid-grass percent of TPG cover was greatest in the alternate-season fire treatment, and C_4 short-grass percent of TPG cover was greatest in the repeated-summer fire treatment (Fig. 7c, d). Following the maintenance

Table 5. *P*-values of repeated-measures analysis of main effects of treatment and sample date and their interaction ($T \times D$) on herbaceous biomass variables during the eight sample dates from 2002 to 2006 following transition fires in 1991–1995, and seven dates (2003–2006) following the maintenance fires in 2002 (2002 sampling excluded).

Variable	Treatment	Date	T × D
Transition fires			
C ₃ mid-grass biomass	0.1118	<0.0001	0.7694
C ₄ mid-grass biomass	0.0225	<0.0001	0.3877
C ₄ short-grass biomass	0.2023	<0.0001	0.8635
Total Perennial Grass (TPG) biomass	0.0568	<0.0001	0.0933
C ₃ mid-grass percent of TPG biomass	0.0703	0.0037	0.9030
C ₄ mid-grass percent of TPG biomass	0.0141	0.1033	0.6231
C ₃ annual grass biomass	0.5793	<0.0001	0.2568
Forb biomass	0.0216	0.0069	0.2984
Litter biomass	0.3026	<0.0001	0.0256
TPG:litter ratio	0.3196	<0.0001	0.9892
Maintenance fires			
C ₃ mid-grass biomass	0.8729	<0.0001	0.0126
C ₄ mid-grass biomass	0.2671	0.5852	0.0045
C ₄ short-grass biomass	0.1611	0.0006	0.0545
Total perennial grass (TPG) biomass	0.3283	<0.0001	<0.0001
C ₃ mid-grass percent of TPG biomass	0.6669	0.0153	0.0498
C ₄ mid-grass percent of TPG biomass	0.3637	0.0590	0.0125
C ₃ annual grass biomass	0.1469	<0.0001	0.1303
Forb biomass	0.3645	<0.0001	0.0021
Litter biomass	0.0004	<0.0001	<0.0001
TPG:litter ratio	0.0015	<0.0001	<0.0001

Note: Significant values ($P \leq 0.05$) are in bold.

fires, there were no consistent differences in C₄ mid-grass or C₄ short-grass percent of TPG cover between fire treatments and the control (Fig. 7g, h).

Soil C and N responses

Repeated-measures analyses revealed no significant ($P \leq 0.05$) main effects of treatment on any variable at either depth increment, although effect of treatment on soil C and N had *P*-values < 0.10 (Table 7). At 0–10 cm depth increment, there was a significant main effect of sample date on soil C/N ratio and $\delta^{13}\text{C}$, and a significant treatment-by-date interaction with soil C, N, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$. At 10–20 cm depth increment, there was a significant main effect of

sample date on soil N and C/N ratio, and a significant treatment-by-date interaction with $\delta^{13}\text{C}$.

Averaged over all sample dates, soil C was greatest in the alternate-season fire treatment, and soil N was greater in the alternate-season than the repeated-summer fire treatment at 0–10 cm depth (Table 8). Averaged over all treatments, soil C/N ratio increased, and $\delta^{13}\text{C}$ decreased (became more negative) from 1996 to 2006 at 0–10 cm depth. Soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ tended to be lower in the 0–10 cm than the 10–20 cm depth in all treatments and sample periods.

The alternate-season transition fire treatment increased soil C in the 0–10 cm depth increment compared with the control from 1994 to 2001 (Fig. 8a). Soil C was greater in the repeated-summer transition fire treatment than the control in 1996, 2 yr post-fire, but was not different from the control after that, although it showed a trend ($P = 0.085$) of being lower than the control in 2006, 12 yr post-fire. A similar pattern for soil total N occurred among treatments at 0–10 cm (Fig. 8c). Soil C and N in the 10–20 cm layer were lower in the repeated-summer and repeated-winter fire treatments than the control in 1994 only (Fig. 8b, d). Responses in 2006 to the 2002 maintenance fires revealed no differences in soil C or N among treatments. Soil C/N ratio was lower in the control than some transition fire treatments from 1994 to 2001 (Fig. 8e, f).

Soil $\delta^{13}\text{C}$ was lower (more negative) in the repeated-summer transition fire treatment than the control in both depth increments in 1996, 2 yr post-fire (Fig. 9a, b). Soil $\delta^{13}\text{C}$ values increased (less negative) in the repeated-summer fire but decreased in the control and repeated-winter fire treatments from 1996 to 2006, explaining the significant treatment-by-date interaction from Table 8. Conversely, soil $\delta^{15}\text{N}$ decreased in the repeated-summer fire, but increased in the alternate-season and repeated-winter fire treatments from 2001 to 2006 (Fig. 9c, d).

DISCUSSION

Prescribed transition fires

If frequent fire and fire in different seasons maintained C₄ grasslands in the SGP prior to European settlement, can the anthropogenically managed return of fire transform the present

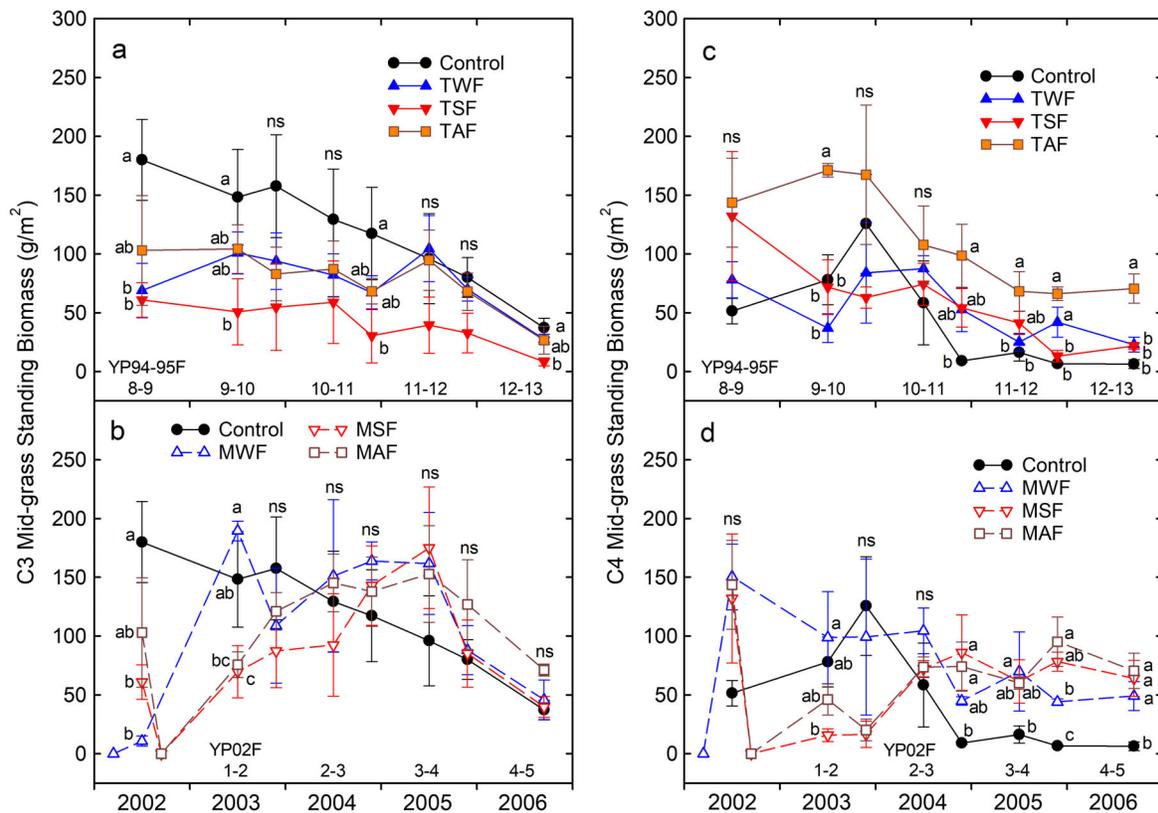


Fig. 3. Mean (\pm SE) C₃ mid-grass (left) and C₄ mid-grass (right) biomass, 2002–2006, in response to repeated transition fire (a, c) and maintenance fire (b, d) treatments. Values for the control are repeated in top and bottom panels for each variable. Means with similar letters within a sample date in each panel are not significantly different at $P \leq 0.05$ (ns = no significant differences). YP94-95F = Years post-1994 or 1995 fires; YP02F = Years post-2002 fires.

dense stands of mature mesquite with Texas wintergrass understories (the *Prosopis/Nassella* association) back toward the relatively open C₄ grasslands that once characterized the area? If so, how frequently must fires occur to maintain mesquite suppression if very few mesquite plants are root-killed, and if woody overstory growth has progressed to a dense stand of mature trees? Additionally, is a mosaic of seasonal fires needed to re-establish certain herbaceous species diminished by woody encroachment (Fuhlendorf et al. 2009, Gordijn and O'Conner 2021)?

The anthropogenic re-introduction of fire regimes in grassland/savanna ecosystems that were once sustained by fire varies greatly among ecosystems (Fynn et al. 2005, Govender et al. 2006, Peterson et al. 2007, Twidwell et al. 2016,

Connell et al. 2020, Larson et al. 2020). A detailed understanding of the constraints of each ecosystem is critical in developing long-term fire management strategies. Certain grassland/savanna ecosystems, such as the SGP described in this study, have shifted into an alternative state of mature woody encroachment and associated degradation of the herbaceous layer that may be difficult to reverse using only fire (Briske et al. 2003). Research in tallgrass prairie, USA, demonstrated that once resprouting shrubs established in the previous tree-less C₄ grassland due to the loss of the historic fire regime, they were difficult to remove through the re-introduction of fire (Briggs et al. 2005).

The main constraints to the application of prescribed fire in the SGP are semi-arid precipitation

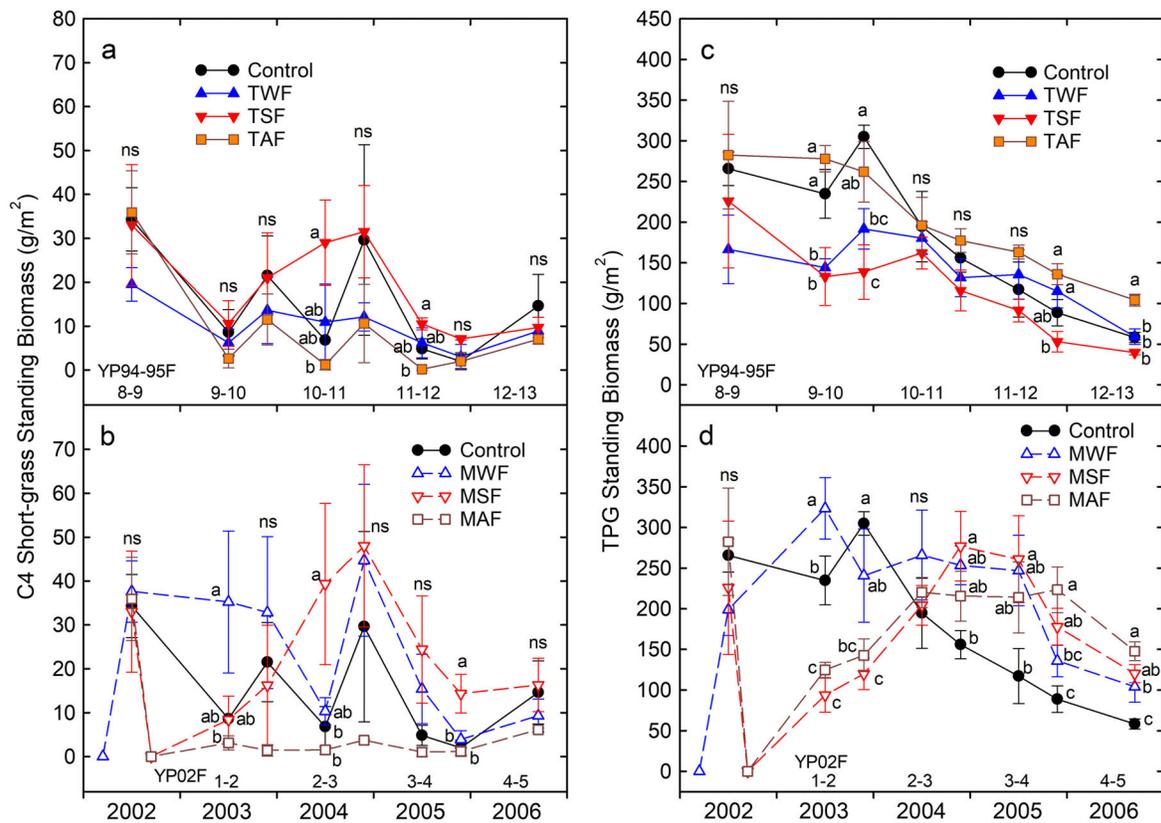


Fig. 4. Mean (\pm SE) C₄ short-grass (left) and total perennial grass (right) biomass, 2002–2006, in response to repeated transition fire (a, c) and maintenance fire (b, d) treatments. Additional descriptions same as in Fig. 3 caption.

that limits grass growth, a high percent woody canopy cover that limits growth of the most productive C₄ mid-grasses (Ansley et al. 2013), and cattle grazing operations that, because of the two previous constraints, are limited regarding opportunities to defer grazing and accumulate grass as fine fuel to carry a fire. Under these constraints, managers have not been able to burn any single area more than once every 10 yr (Ansley et al. 2010b). Such a prescribed burning schedule would not be adequate to transition the *Prosopis/Nassella* association to C₄ grassland. Thus, the rationale for our study was to accelerate the return of fire to the ecosystem via a *pyric transition regime* (a series of intense, repeated headfires, including some in summer, within a few years) that would top-kill most and possibly root-kill some mesquite compared with single fires, and facilitate the transition to C₄ grassland.

This plan assumed the presence of C₄ mid-grass species in the seed and bud bank (Russell et al. 2015, Hiers et al. 2021) and recognized that C₄ grass recovery might be slower after summer fire (Trollope 1987, Engle et al. 2000, Briske et al. 2003, Ansley et al. 2006).

The transition fire treatments were mostly of sufficient intensity to achieve a high level of mesquite top-kill. In Africa, Govender et al. (2006) estimated that a fire intensity of 3000 kW/m top-killed 90% of woody plants 1 m tall, but only 40% of those 2 m tall. Most of our summer fires exceeded 4000 kW/m but all winter fires were <3000 kW/m. Govender et al. (2006) found higher fire intensities with winter than summer fires. Our results were opposite likely because Texas wintergrass is green in winter.

The second or third fire step in each transition fire treatment top-killed resprouting mesquite

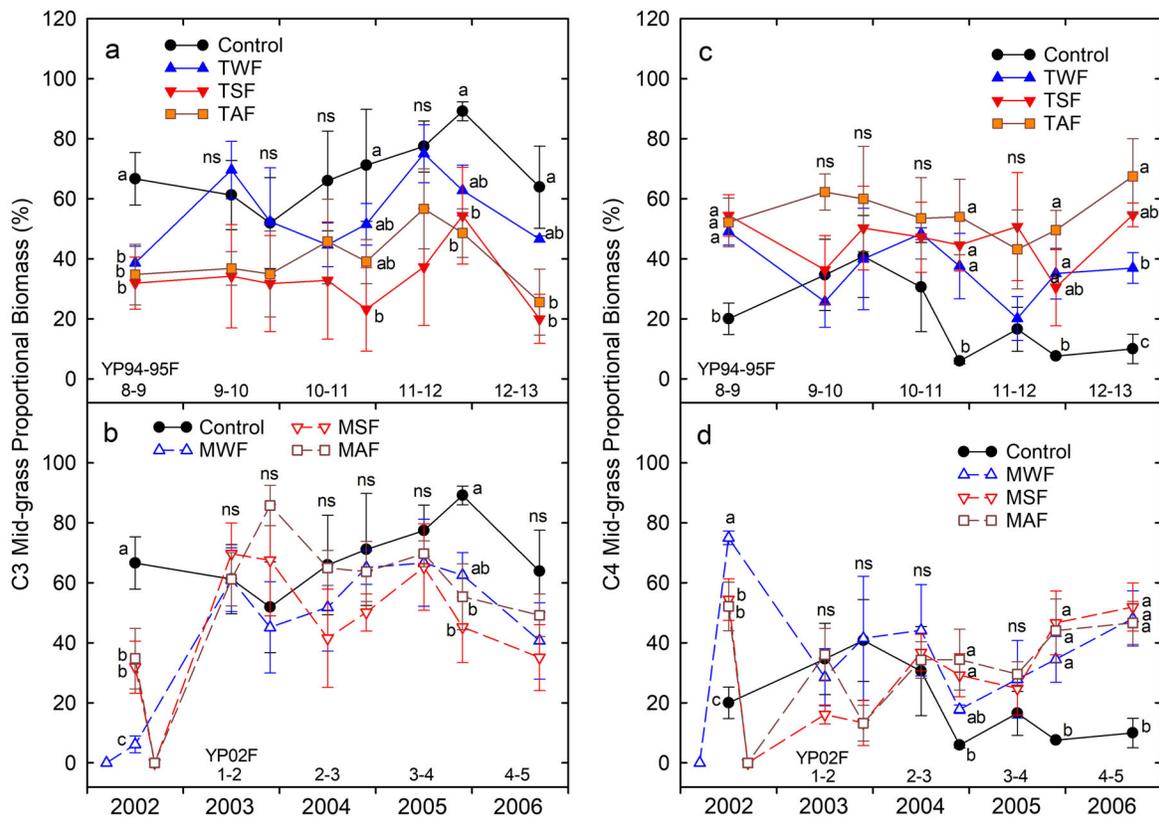


Fig. 5. Mean (\pm SE) of C₃ mid-grass (left) and C₄ mid-grass (right) as a percent of TPG biomass in response to repeated transition fire (a, c) and maintenance fire (b, d) treatments, 2002–2006. Additional descriptions same as in Fig. 3 caption.

that had been top-killed by the previous fire step. Thus, these fires repeatedly “suppressed” the ability of mesquite to compete with grasses. However, even the most severe fires employed in this study were not able to kill dormant subterranean buds and mesquite resprouted after each fire. Most studies have found that even the most extreme fires root-kill no more than 5% of mature mesquite plants (Drewa 2003, Ansley et al. 2008). Twidwell et al. (2016) reported 16% mesquite root-kill from extreme summer fire during an historic drought. However, natural mortality from drought was 12%, so net due to fire was 4%. After we completed the series of transition fires, mesquite post-fire regrowth was similar in all fire treatments and attained canopy heights similar to pre-fire levels by 10 yr post-fire. This study highlights that ecosystem services provided by brush management are often short-lived (Archer and Predick 2014).

Mesquite top-killing fires may carry greater ecological risk today than in pre-European settlement times because of higher mesquite density. Shifting such a higher-density woody species into a regrowth physiognomy has the potential to increase the competitive impact of mesquite on the herbaceous understory compared with low-density regrowth in historic times, or the current arborescent woodland, because regrowth mesquite is more competitive for soil water (Ansley et al. 2018). Our study revealed such consequences as fire-mediated changes in herbaceous community responses declined to levels similar to the unburned mesquite woodland 8–10 yr after the transition fires due to mesquite regrowth. Thus, subsequent “maintenance” fires as a component of a long-term “fire regime” are necessary to maintain mesquite in a so-called fire-trap of low regrowth that can be easily top-killed by

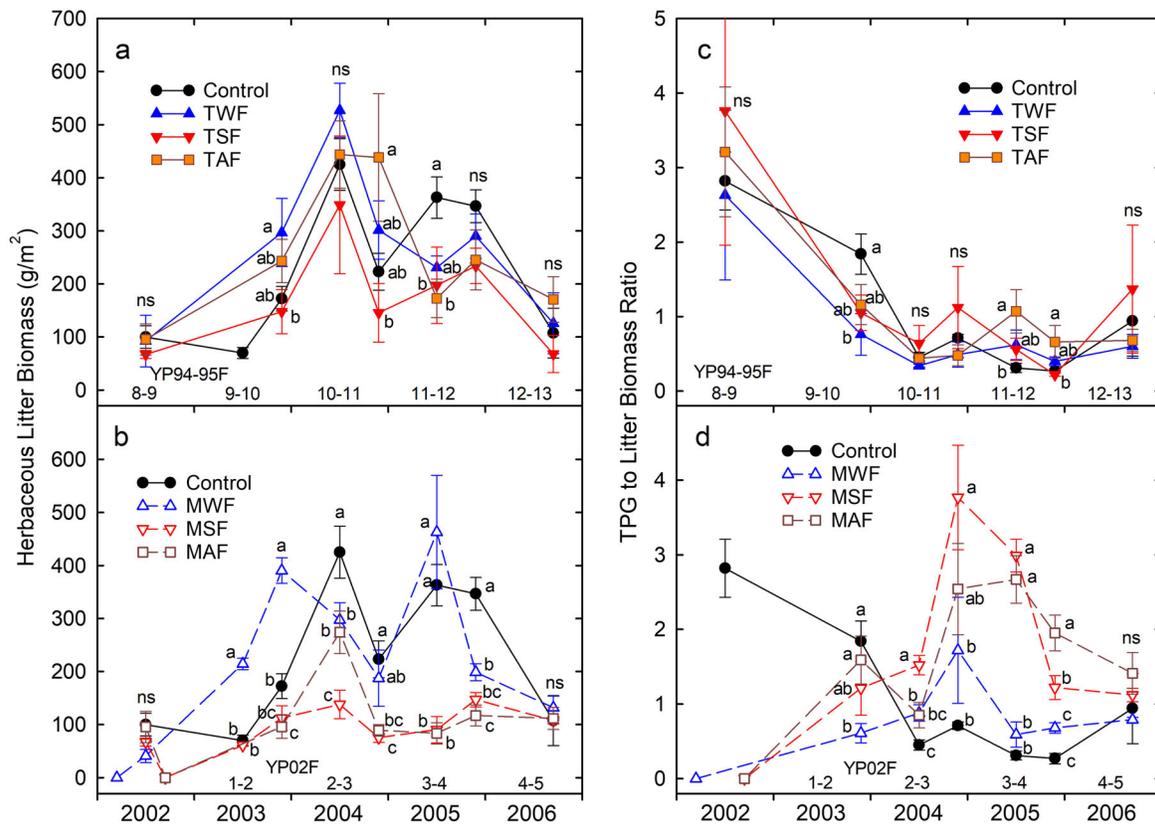


Fig. 6. Mean (\pm SE) herbaceous litter biomass (left) and total perennial grass (TPG) biomass to litter biomass ratio (right), 2002–2006, in response to repeated transition fire (a, c) and maintenance fire (b, d) treatments. Additional descriptions same as in Fig. 3 caption.

Table 6. Means of herbaceous cover variables within each transition fire treatment averaged over four sample dates 1998–2005, and within each sample date averaged over all fire treatments.

Treatment or year	TPG percent cover (%)	Percent of TPG cover		
		C ₃ mid-grass	C ₄ mid-grass	C ₄ short-grass
Control	24.0 b	71.0 a	14.3 b	14.7 b
TWF	26.0 b	57.7 ab	22.0 b	20.3 b
TSF	29.9 b	37.2 c	22.6 b	40.3 a
TAF	43.7 a	43.4 bc	44.7 a	11.9 b
LSD 0.05	12.3	14.6	14.5	10.8
1998	39.1 a	24.9 b	34.8 a	40.3 a
2001	24.5 b	63.6 a	18.0 c	18.4 b
2003	35.8 a	54.9 a	29.3 ab	15.8 b
2005	24.2 b	65.8 a	21.4 bc	12.7 b
LSD 0.05	4.0	11.2	8.0	9.5

Note: Means with similar letters within each group are not significantly different at $P \leq 0.05$.

subsequent fires (Bond and Keeley 2005, Gordijn and O'Conner 2021).

Maintenance fires applied 8–10 yr after the transition fires again completely top-killed nearly all mesquite, but post-fire regrowth rate remained similar to that following the transition fire treatments. These responses reinforce the management reality that repeated intense fires will not reduce density of resprouting woody species. Moreover, due to the rapid regrowth rate, a fire return interval longer than that employed in this study risks woody regrowth becoming too large and fine fuel too limited to support a fire of sufficient intensity to maintain woody suppression.

Our study was conducted in the absence of livestock grazing, although the site had a history of moderate continuous cow–calf cattle grazing for at least 80 yr before we began our study. If a

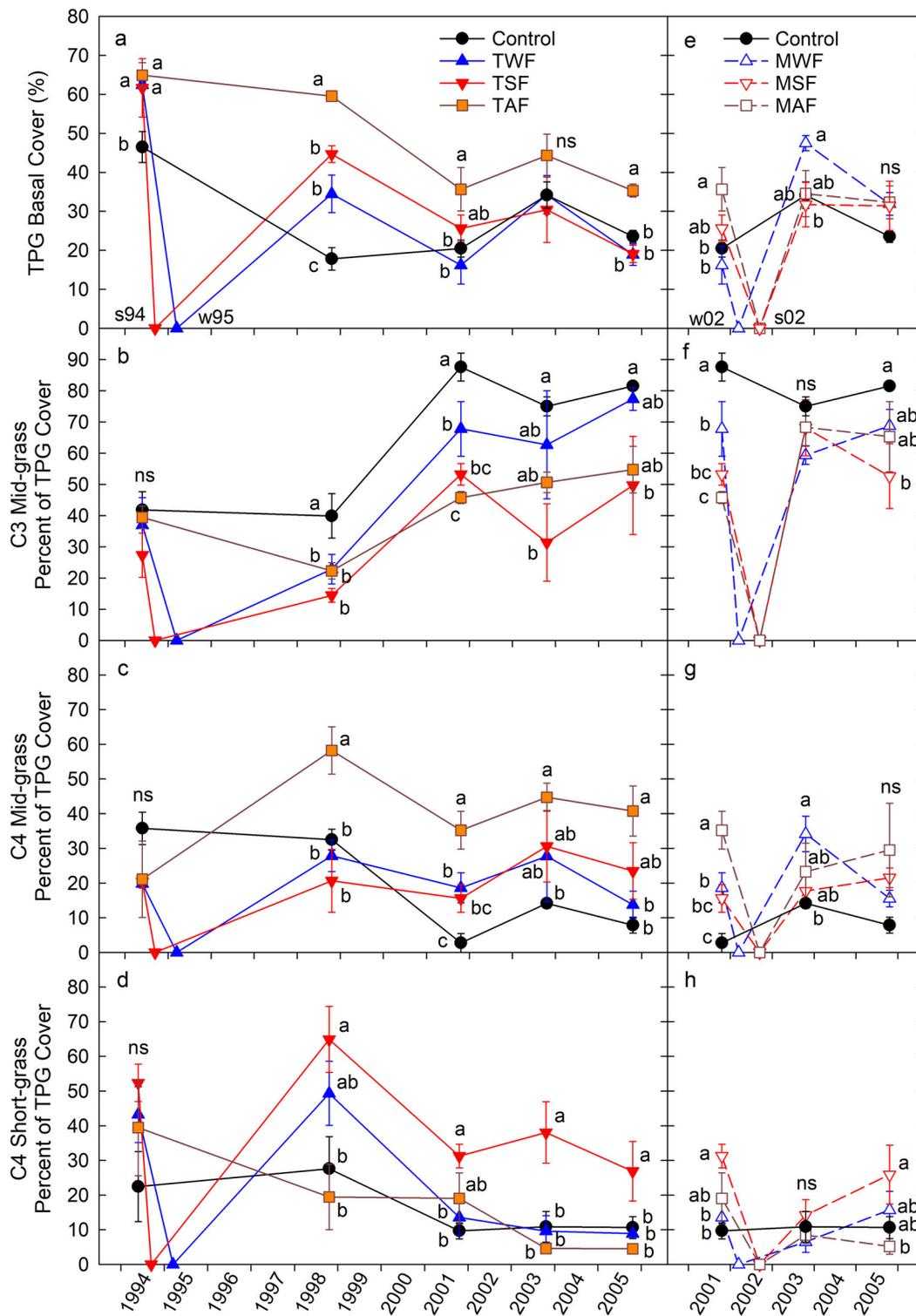


Fig. 7. Mean (\pm SE) of TPG basal cover (a, e), and C₃ mid-grass (b, f), C₄ mid-grass (c, g) and C₄ short-grass (d, h) percent of TPG cover following the 1991–1995 transition fire (panels a–d), and the 2002 maintenance fire

(Fig. 7. *Continued*)

(panels e–h) treatments. Means with similar letters within a sample date in each panel are not significantly different at $P \leq 0.05$ (ns = no significant differences). Means from 2001 and control means are same for left and right panels. Fire steps s94, w95, w02, and s02 are shown.

Table 7. P -values of repeated-measures analysis of main effects of transition fire treatment (T) and sample date (D), and their interaction ($T \times D$) on soil C and N variables at two depth increments during 1996–2006 sample dates.

Variable	Treatment	Date	$T \times D$
0–10 cm depth			
Soil C	0.0598	0.1229	0.0034
Soil N	0.0931	0.5697	0.0033
C/N ratio	0.4206	0.0003	0.0259
$\delta^{13}\text{C}$	0.6703	0.0026	0.0032
$\delta^{15}\text{N}$	0.8074	0.2564	0.0762
10–20 cm depth			
Soil C	0.4439	0.2012	0.9457
Soil N	0.7678	0.0485	0.8302
C/N ratio	0.4219	0.0010	0.1852
$\delta^{13}\text{C}$	0.2824	0.1662	0.0368
$\delta^{15}\text{N}$	0.4552	0.5094	0.0777

Note: Significant values ($P \leq 0.05$) are in bold.

similar level of cattle grazing occurred during the study, the potential for accumulating sufficient fine fuel to support the concentration of intense transition fires we implemented would have been more limited (van Langevelde et al. 2003, Starns et al. 2019). Given these constraints to prescribed fire in this region and situation, an alternative method in conjunction with fire, such as aerial spray of a mesquite-specific herbicide (Ansley and Castellano 2006, Ansley et al. 2010b) or mechanical extraction of the mesquite root crown, may be necessary to root-kill the majority of mesquite and thus prevent basal regrowth. However, these treatments are very expensive and cannot be applied economically over large land areas.

Pyric herbivory (i.e., “patch burning”) is a useful strategy for implementing prescribed fire without removing grazing from a pasture as we did in our study (Fuhlenndorf et al. 2009, Larson et al. 2020). Pyric herbivory has been shown to increase floral and faunal heterogeneity (Fuhlenndorf et al. 2009) and can be more effective than fire alone in limiting woody (Roques et al. 2001,

Table 8. Means of soil variables at 0–10 and 10–20 cm depth increments within each transition fire treatment averaged over three sample dates (1996–2006), and within each sample date averaged over all fire treatments.

Treatment or year	Soil C (g/m ²)	Soil N (g/m ²)	C/N Ratio	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
0–10 cm depth					
Control	1376 b	137.9 ab	10.0 a	–19.7 a	6.5 a
TWF	1392 b	137.3 ab	10.1 a	–20.0 a	6.6 a
TSF	1359 b	131.5 b	10.4 a	–19.8 a	6.3 a
TAF	1508 a	146.4 a	10.3 a	–19.5 a	6.2 a
LSD 0.05	112	11.5	0.6	1.0	1.1
1996	1338 a	136.5 a	9.8 b	–19.2 a	6.5 a
2001	1437 a	136.7 a	10.6 a	–19.9 b	6.2 a
2006	1451 a	141.6 a	10.3 a	–20.2 b	6.5 a
LSD 0.05	119	11.2	0.3	0.5	0.4
10–20 cm depth					
Control	1044 a	109.7 a	9.6 a	–16.9 a	7.4 a
3WF	1013 a	105.3 a	9.7 a	–17.2 a	7.3 a
2SF	964 a	103.4 a	9.4 a	–17.3 a	7.1 a
3ASF	1099 a	110.2 a	10.0 a	–16.8 a	7.2 a
LSD 0.05	192	18.7	0.8	0.6	0.6
1996	1053 a	113.3 a	9.3 b	–17.1 a	7.3 a
2001	1056 a	102.9 b	10.3 a	–16.9 a	7.2 a
2006	981 a	105.2 ab	9.4 b	–17.2 a	7.3 a
LSD 0.05	95	8.5	0.5	0.3	0.3

Note: Means with similar letters within each group and soil depth are not significantly different at $P \leq 0.05$.

Capozzelli et al. 2020) or prickly pear (*Opuntia polyacantha*) (Augustine and Derner 2015) encroachment in some ecosystems. However, no studies have demonstrated that this burning strategy can overcome a dense stand of tall, mature mesquite as found in our study. Pyric herbivory may thus have more utility in facilitating application of maintenance fires once the majority of mesquite are top-killed or root-killed. Alternatively, a rotational grazing strategy with fenced paddocks may provide a better opportunity to completely defer grazing for a period to accumulate enough fine fuel to support fires intense enough to top-kill mature woody plants (Ansley et al. 2010b). Future strategies may include training of livestock such as goats to

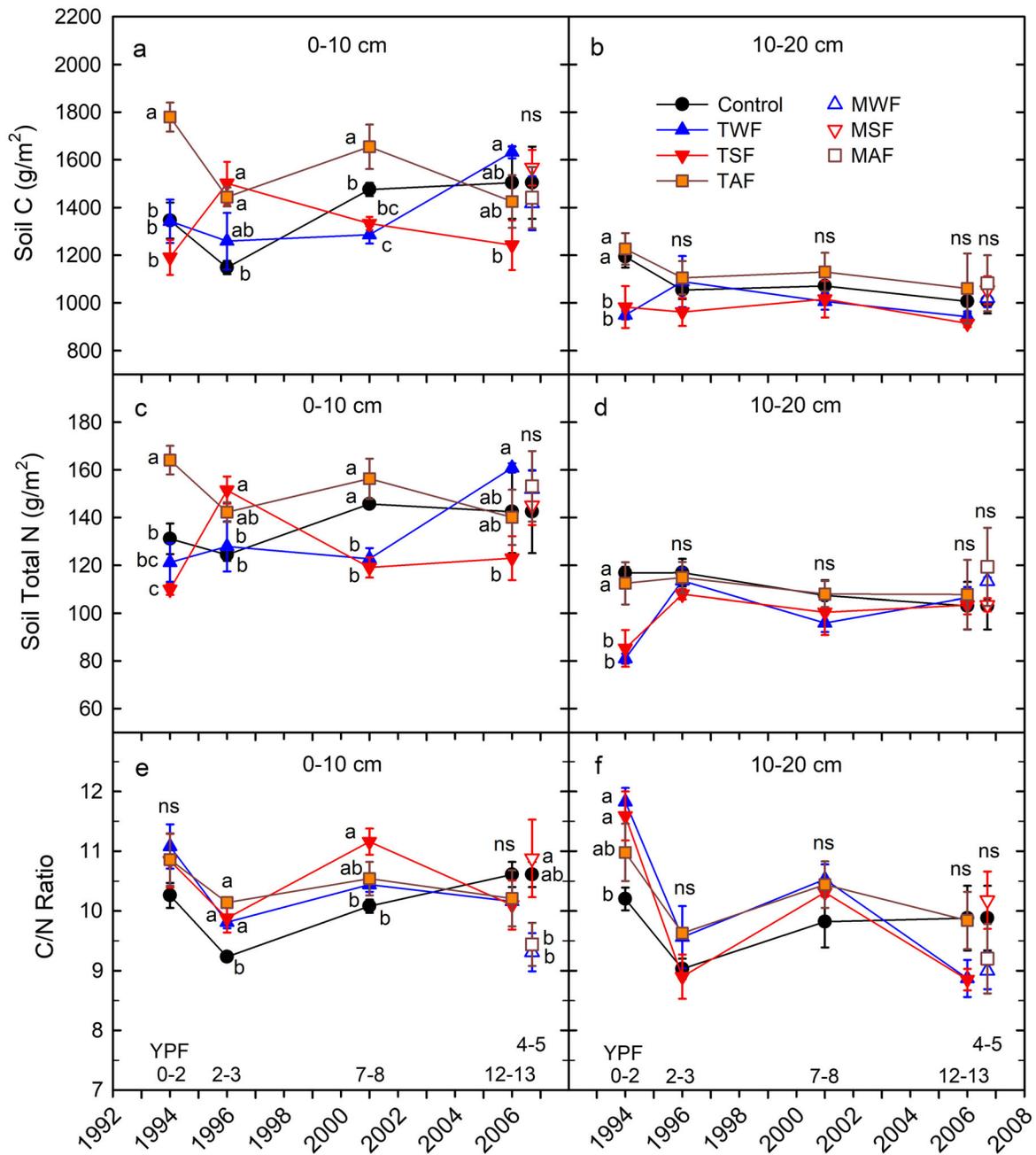


Fig. 8. Mean (\pm SE) soil organic C, total N, and C:N ratio at two depths in response to transition fire and maintenance fire treatments compared with the control. Responses to the 2002 maintenance fires measured in 2006 only are to the right of transition fire treatment means in 2006, but sampling was on the same date. Means with similar letters within each date are not significantly different ($P \leq 0.05$) (ns = no significant differences). YPF = Years post-1994 or 1995 fires (lower row; 0-2, 2-3, etc.), or years post-2002 fires (upper row "4-5" in 2006 only).

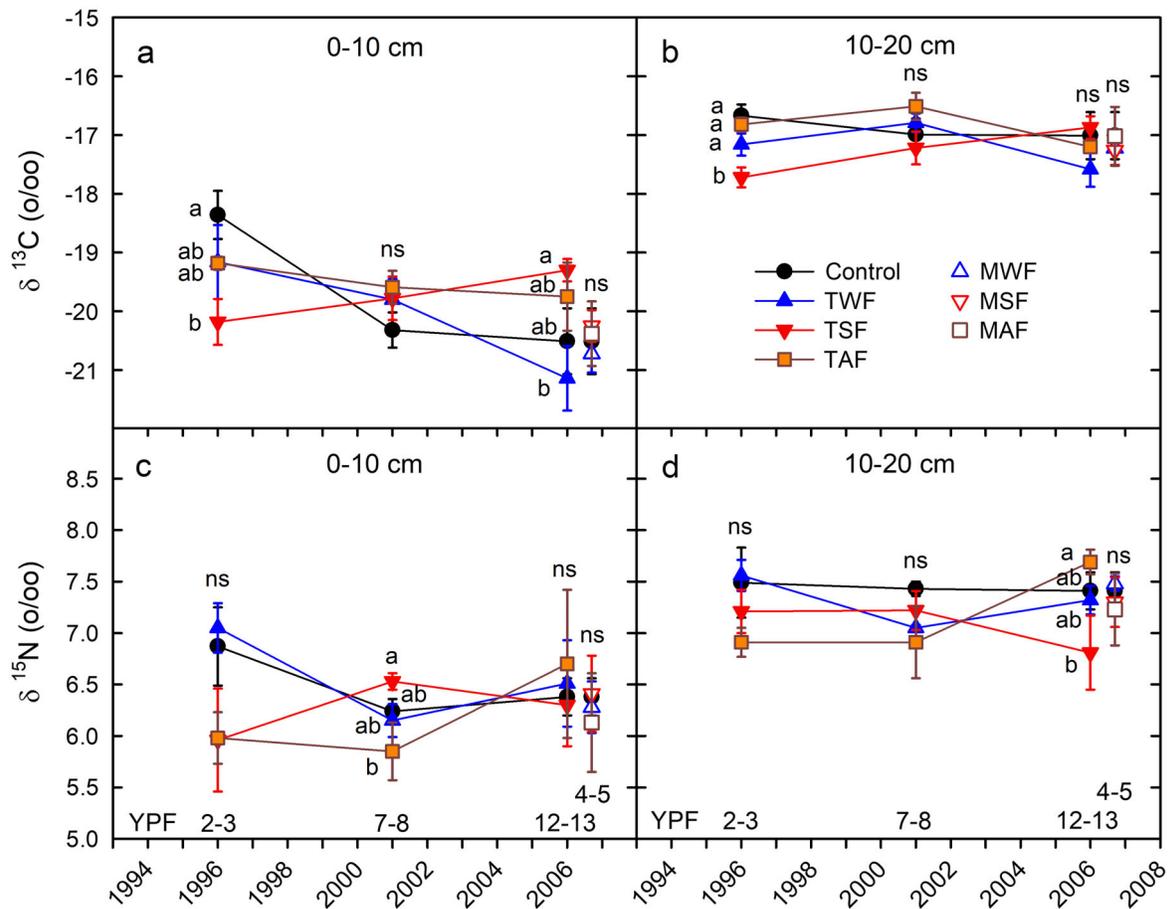


Fig. 9. Mean (\pm standard error) soil $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at two depths in response to transition and maintenance fire treatments compared with the control. Additional descriptions same as in Fig. 8 caption.

browse invasive woody plants (Frost et al. 2008), although little progress has been made related to browsing suppression of mesquite. Unlike endemic browsers that help limit woody encroachment in African savannas (van Langevelde et al. 2003), or cactus encroachment in short-grass prairie in the USA (Augustine and Derner 2015), there are no natural browsers of mesquite in the SGP.

Grass responses to transition fires

Some studies show that frequently applied winter or early spring fires in mixed grass communities favor C_4 over C_3 grasses in (Howe 2000, Peterson et al. 2007, Prober et al. 2007), and summer fires favor C_3 over C_4 grasses (Howe 1995, Engle et al. 1998). Frequent winter or spring fires

benefitted C_4 bunchgrass *Themeda triandra* in South Africa (Uys et al. 2004) and Australia (Morgan and Lunt 1999) compared with unburned, but there was no C_3 grass component. However, several studies have found no long-term changes in C_3 vs. C_4 grasses species composition after summer fires (Engle et al. 2000, Howe 2000, Taylor et al. 2012). Our results did not follow any of these responses, possibly due to the presence of a fire-tolerant, resprouting, N-fixing woody legume that facilitated growth of a co-dominant C_3 perennial grass.

Our findings that the repeated-summer transition fire treatment reduced Texas wintergrass biomass at 8–13 yr post-fire, and percent of TPG cover at 7–11 yr post-fire compared with the control are novel results. No previous studies have

found that fire in any season causes a long-term reduction in Texas wintergrass production or cover (Whisenant et al. 1984, Engle et al. 1998, Ansley and Castellano 2007, Reemts et al. 2019). The reason why the repeated-summer transition fire treatment was more effective than the other transition fire treatments in reducing Texas wintergrass biomass and cover is not clear. It was apparently not due to differences in shading by regrowth mesquite canopies since mesquite regrowth rate was similar among all transition fire treatments. It may relate to the relatively large increase in C₄ short-grass percent of TPG cover in this fire treatment (40.3% vs. 14.7% in the control; Table 6) that displaced areas occupied by Texas wintergrass. The ecological “costs” of this treatment were that it limited herbaceous litter accumulation and did not increase C₄ mid-grass biomass or basal cover.

We are uncertain why C₄ mid-grasses replaced C₄ short-grass biomass in the alternate-season but not the repeated-summer transition fire treatment. It may have been the result of a less intense fire sequence in the alternate-season fire treatment. Many fire studies address C₄ grasses as a single group. We separated C₄ short-grasses from C₄ mid-grasses because their sensitivities to mesquite encroachment and fire are different (Engle et al. 1998, Ansley et al. 2013). The increase in C₄ short-grasses under the more severe repeated-summer fire transition treatment occurred only through lateral advancement (increased ground cover) and not production per unit land area of this mostly stoloniferous group. A less severe alternate-season transition fire treatment enabled C₄ mid-grasses to advance both vertically (via increased production) and laterally (via increased cover by new plant recruitment or lateral expansion of stoloniferous species). The combination of alternating seasons of fire may also have stimulated growing buds in certain C₄ mid-grasses (Russell et al. 2015). The shifting of grass functional group dominance in response to the different fire disturbances reinforces the conclusion of Komatsu et al. (2019) that “species re-ordering or replacement is an important mechanism of community responses to global change drivers.” Their synthesis paper did not include fire disturbances, however. Gordijn and O’Conner (2021) in a South Africa study noted the importance of establishing pyrodiversity in an

anthropogenically managed fire regime to maintain or enhance plant diversity.

Grass responses to maintenance fires

All the 2002 maintenance fire treatments initially (first 3 yr post-fire) increased Texas wintergrass biomass and percent of TPG cover to a greater degree than C₄ grasses. These results mimicked a similar Texas wintergrass production spike the first few years after the transition fire treatments shown in Ansley and Castellano (2007). None of the maintenance fires reduced Texas wintergrass biomass compared with the control, and only the repeated-summer maintenance fire reduced Texas wintergrass percent of TPG cover compared with the control. Thus, while we theorize that maintaining mesquite in a suppressed condition may eventually decrease Texas wintergrass due to the reduction of the mesquite subcanopy microenvironment that favors Texas wintergrass, fires in summer or winter initially stimulated Texas wintergrass growth even after previous fires appeared to reduced Texas wintergrass biomass relative to the control. The reason for this may be related to the mesquite microenvironment re-establishing quickly following top-kill (Ansley et al. 2019) and providing a nitrogen boost to Texas wintergrass in the first few years following fire. A recent study found that growing buds in caespitose Texas wintergrass are positioned lower than those of stoloniferous short-grass curly mesquite (*Hilaria belangeri*) and this may explain the tolerance of this species to even the most intense fires (Hiers et al. 2021). We thus reject our hypothesis that the maintenance fires would restore or strengthen trends in herbaceous community responses that were established by the transition fire treatments.

The delayed response of C₄ mid-grass biomass to the 2002 summer maintenance fires compared with response to the 2002 winter fires reinforced observations in other SGP studies (Engle et al. 2000, Ansley et al. 2006) and South Africa (Everson et al. 1985, Trollope 1987) of a delayed production response of C₄ mid-grasses to summer fire. However, by 3 yr post-fire, C₄ mid-grass biomass was at similar levels in both winter and summer fire treatments. This suggests that in a pyrodiversity fire regime of alternating winter and summer fires that an effective fire return

interval for promoting shifts in herbaceous composition toward C₄ mid-grass dominance in this ecosystem may be at least 3–5 yr. More frequent fires may slow the ability of this functional group to assert dominance as has been predicted for some understory species in tropical *Eucalyptus* savannas in Australia (Werner and Peacock 2019). Post-fire cattle grazing may disproportionately add more pressure on C₄ mid-grasses and likely would have lessened any advances of this grass group that were found in some of our fire treatments (Ansley et al. 2006).

The need for a longer fire return interval for C₄ mid-grasses when summer fire is part of the fire regime contrasts with annual or biennial late winter/early spring burning schedules needed to maintain C₄ grass dominance tallgrass prairie, USA (Connell et al. 2020) but is similar to a fire return interval of ≤ 5 yr that was necessary to maintain the competitiveness of bunchgrass *Themeda triandra* in Australia (Morgan and Lunt 1999). In contrast, a study in South Africa showed that while annual burning favored *Themeda* abundance, taller C₄ grasses such as *Cymbopogon* and *Eragrostis* were more responsive to less frequent burning (Fynn et al. 2005). Dormant-season fires alone (without grazers) at intervals of ≤ 3 yr were projected to eliminate C₄ lawn grasses (i.e., short-grasses) and promote C₄ bunchgrasses in Hluhluwe–Umfolozzi Park, South Africa (Archibald et al. 2005). These studies illustrate the need for more research into effects of seasonally diverse fire regimes (pyrodiversity) since much research and fire management strategies have focused on effects of fire frequencies or intensities within usually the dormant season.

Soil C and N responses

The increase in soil C and N in the alternate-season fire treatment in 1994 may have been the result of a greater number of fires and fewer growing seasons after the last fire compared with the other treatments on this sample date. This difference disappeared by 2006, even though C₄ mid-grass biomass remained greater in this treatment. The increase in soil C and N from 1994 to 1996 in the repeated-summer transition fire treatment was due in part to low values in 1994. This may have been because the 1994 sampling occurred only 3 months after the summer 1994

fires and there may have been some temporary loss of C and N from combustion. This was the only treatment where one of the soil sample periods was at 0 yr post-fire. The subsequent increase in C and N from 1994 to 1996 may have been due to increased grass growth. Any temporary depletion in soil N in upper and lower soil depth increments caused by fire treatments disappeared by 1996 and 2006, respectively. The repeated-summer fire decreased soil N for 7 yr post-fire (to 2001). Similarly, repeated fires in a tropical savanna in South Africa with similar precipitation as our site showed little long-term effect on soil C and N (Pellegrini et al. 2020).

Soil $\delta^{13}\text{C}$ values in grassy areas between mesquite canopies ranged from -21 to -19‰ in the 0–10 cm depth, and 17.5 to -16.5‰ at 10–20 cm, consistent with previous measurements by Dai et al. (2006) at this same site and studies elsewhere (Wang et al. 2019). Since mean residence times of soil C increase with soil depth (Boutton et al. 1998, 2009), the high $\delta^{13}\text{C}$ values in the 10–20 cm depth indicate that this area had a heterogeneous landscape that included C₃ plants but was dominated by C₄ grasses. The lower $\delta^{13}\text{C}$ values in the 0–10 cm depth are consistent with the observed shift toward dominance by C₃ Texas wintergrass. We detected some evidence that the transition fire treatments changed soil $\delta^{13}\text{C}$ relative to changes in C₃ vs. C₄ grass composition over the 15-yr duration of the study. The increase in soil $\delta^{13}\text{C}$ in the repeated-summer fire treatment in both depth increments from 1996 to 2006 suggests a relative increase in soil C derived from C₄ grasses during that time interval, especially in the absence of livestock grazing (Connell et al. 2020). The increased $\delta^{13}\text{C}$ in the repeated-summer fire treatment may have been from the increase in C₄ short-grass cover. Conversely, the sharp decrease in $\delta^{13}\text{C}$ from 1996 to 2006 in the control and the repeated-winter fire treatment is consistent with the increase in C₃ Texas wintergrass cover in these two treatments during this period.

Soil $\delta^{15}\text{N}$ values showed few differences between fire treatments in either the 0–10 or 10–20 cm soil depths and generally lacked directional trends. Since soil $\delta^{15}\text{N}$ values are a consequence of the long-term net difference between $\delta^{15}\text{N}$ values of N inputs (e.g., fixation, deposition) and N outputs (e.g., gaseous losses,

leaching) (Pardo and Nadelhoffer 2010, Denk et al. 2017, Zhou et al. 2018), these data suggest that there were no major differences in N inputs vs. losses over the duration of this study. Lower values in the surface soil are likely because plant inputs are concentrated in the uppermost portion of the profile and have lower $\delta^{15}\text{N}$ than soil total N, and because atmospheric deposition has $\delta^{15}\text{N}$ values near 0‰. In addition, N isotope fractionation during soil organic matter decay results in higher $\delta^{15}\text{N}$ values in older and more highly decomposed organic matter located deeper in the profile compared with newer organic matter concentrated in the surface soil.

Ecological and management implications

The increase in woody plant abundance in grasslands and savannas throughout the world is among the most significant ecological changes occurring globally and has profound implications for the ecology, conservation, and management of these ecosystems. An understanding of global trends related to the role of fire in woody plant management in these biomes is critical (Bond et al. 2005, Eldridge et al. 2011). However, we emphasize in this study that management solutions must be addressed at local scales to determine ecological and economic costs and benefits of anthropogenic fire regimes (Bowman et al. 2011, Osborne et al. 2018). These solutions may encompass trade-offs related to multiple ecosystem service objectives specific to that region (Bond and Archibald 2003, Archer and Predick 2014, Werner and Peacock 2019). In this study, our objective was to provide a sustainable brush management solution to limit or reduce C_3 woody and grass encroachment into what was once mostly C_4 grassland using only prescribed fire. Alternative treatments including application of brush specific herbicides and various mechanical treatments have been studied, but more research on combining these treatments with prescribed fire regimes is needed. Future management options may also include pyric herbivory or training of livestock to browse and maintain suppression of invasive woody plants.

We demonstrated that prescribed “transition fires” that alternated between summer and winter seasons (pyrodiversity) enhanced mesquite top-kill and facilitated a long-term increase in C_4 mid-grass biomass and percent of total perennial

grass cover. Repeated-summer transition fires also affected a change in the grass community as this was the only treatment that caused a long-term reduction in C_3 Texas wintergrass biomass, a grass species that has increased with mesquite invasion. However, the ecological “cost” of this transition fire treatment was that it did not increase C_4 mid-grass biomass relative to the control, and it slowed the rate of litter accumulation. We also found that grass restoration through fire alone must include subsequent fires to maintain mesquite suppression since very few mesquite plants are root-killed by fire. Mesquite post-fire regrowth without a decline in plant density may yield negative outcomes if maintenance fires are not regularly applied after top-killing fires have shifted arborescent old-growth to a coppice physiognomy.

The general resistance of the present *Prosopis/Nassella* association to a concentrated series of “transition” prescribed fires suggests that this plant community may be an alternate stable state that cannot be forced back to the original C_4 grassland configuration only through the re-introduction of fire (Briske et al. 2003, Briggs et al. 2005). We hypothesize that the apparent stability of the *Prosopis/Nassella* community is likely attributable to: (1) the inherent biological and ecological attributes of these species that confer strong competitive ability vs. the original C_4 -dominated grassland communities, and (2) significant changes in atmospheric CO_2 concentration, temperature, and rainfall regimes during the past 100 yr may be shifting the competitive balance in favor of C_3 vs. C_4 plants in this southern Great Plains and other grass-dominated regions around the world. Continued efforts are needed to explore and develop ecologically sound management approaches that can more effectively limit the potential for continued woody plant encroachment, reduce existing woody plant cover in areas that were once grass-dominated, and foster the continued viability of animal production systems.

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DATA AVAILABILITY

Data are available from Dryad: <https://doi.org/10.5061/dryad.612jm644k>

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: <http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3885/full>