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Key Points:

- We identified the longitudinal effects of 30 years of contrasting burning and grazing regimes on soil C and N in tallgrass prairie
- Increased soil C concentrations were associated with increased woody plants under fire suppression or C₄ grasses under annual burning
- Soil N concentration increased over time when fire was suppressed; N was recycled less tightly if bison were present

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Three Decades of Divergent Land Use and Plant Community Change Alters Soil C and N Content in Tallgrass Prairie

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Abstract Frequent fire and grazing by megafauna are important determinants of tallgrass prairie plant community structure. However, fire suppression and removal of native grazers have altered these natural disturbance regimes and changed grassland plant communities with potential long-term consequences for soil carbon (C) and nitrogen (N) storage. We investigated multidecade changes in soil C and N pools in response to contrasting long-term burning and grazing treatments. Fire suppression with or without grazers and exclusion of grazers in annually burned prairie increased soil C content and shifted the $\delta^{13}\text{C}$ signature of soil C over time, concomitant with changes in plant community composition. Soil $\delta^{13}\text{C}$ values indicated that increased soil C content was associated with an increased contribution from plants using a C₃ photosynthetic pathway (i.e., woody shrubs) under fire suppression. Soil N content also increased when fire was suppressed, relative to frequently burned grassland, but the rate of increase was slower when grazers were present. Additionally, changes in $\delta^{15}\text{N}$ values suggested that grazing increased the openness of the N cycle, presumably due to greater N losses. By coupling long-term fire and grazing treatments with plant community data and soil samples archived over three decades, we demonstrate that human-caused changes to natural disturbance regimes in a tallgrass prairie significantly alter soil C and N cycles through belowground changes associated with shifts in the plant community. Since natural disturbance regimes have been altered in grasslands across the world, our results are relevant for understanding the long-term biogeochemical consequences of these ongoing land use changes.

Plain Language Summary The tallgrass prairie of the central United States was historically maintained by frequent fire and bison grazing. However, human-caused fire suppression and bison removal has altered the plant community composition with cascading effects on elemental cycling. By analyzing soil collected over a 30-year period, we investigated the long-term effects of contrasting fire and grazing regimes on soil carbon and nitrogen. Soil carbon content increased over time if fire was suppressed and/or bison were absent. Soil nitrogen content increased only if fire was suppressed. Plant community data and soil isotopic evidence indicated that soil carbon was highest when woody plants were contributing more carbon to the soil in the fire suppression treatments. On the other hand, soil carbon was highest in the annually burned and grazed treatment when warm-season grasses were contributing more carbon to the soil. Since many grasslands around the world are subject to similar land use changes, our results are important for predicting the long-term effects of those changes on the ecosystem. Additionally, while our results indicate that the encroachment of woody plants into grasslands may increase soil carbon, land managers should consider the negative trade-offs of woody encroachment on grassland diversity and ecosystem services.

1. Introduction

A major goal of ecosystem ecology is to identify the mechanisms regulating pools and transformations of organic matter in order to predict how global changes will alter carbon (C) storage over time. Processes that influence the movement and storage of soil organic matter (SOM) are especially important because SOM is the major reservoir for C in most terrestrial ecosystems, and changes in SOM can impact the ecosystem services on which humanity relies: plant productivity, air quality, climate moderation, and water quality (Lal, 2004). Approximately 30% of the North American land surface is covered by grassland ecosystems (Pendall et al., 2018). Because grasses allocate 40–80% of their net primary productivity (NPP)

belowground (tallgrass prairie, in particular, allocates 75%; Hui & Jackson, 2006), grasslands have great potential to sequester large quantities of C in the SOM pool (Dermer & Schuman, 2007).

Land management decisions influence many of the factors that control the inputs and processing of SOM, especially in grasslands (Conant et al., 2017; Pendall et al., 2018). For example, in more mesic grasslands like tallgrass prairie, decisions about the timing and frequency of prescribed fires can alter the productivity and relative dominance of perennial C₄ grasses and shift the balance of herbaceous and woody plant cover (Collins & Smith, 2006; Collins et al., 1998; Heisler et al., 2003; Spasojevic et al., 2010). The natural fire and grazing disturbance regimes that created and maintain North American tallgrass prairie (Anderson, 2006) have been altered through human activity, notably through fire suppression and replacement of native ungulate grazers with managed domestic grazers (Anderson, 2006; Collins, 1990). In addition, fire and grazing disturbance regimes that are believed to have been sufficient to maintain grass dominance in these ecosystems in the past may not be sufficient to do so under current conditions (Bond & Midgley, 2000; Briggs, Knapp, et al., 2002; Suding et al., 2004). Human alteration of these natural disturbance regimes coupled with other global change drivers has important implications for both plant and soil processes in grasslands (Allred et al., 2012; Fynn et al., 2003), though the net effects on SOM accumulation remain unresolved.

The frequency of fires in tallgrass prairie affects pools and fluxes of both C and nitrogen (N). If tallgrass prairie remains unburned over multiple growing seasons, accumulated plant litter alters the soil microenvironment, decreases light availability, and reduces both aboveground NPP (Knapp & Seastedt, 1986) and belowground plant biomass (Kitchen et al., 2009). Fire oxidizes aboveground biomass and accumulated detritus, which increases light availability and creates an environment favorable for new shoot growth (Knapp & Seastedt, 1986). Fire also volatilizes N in plant biomass and litter, so that combustion losses of N in frequently burned prairie, rather than denitrification or leaching, is the primary pathway of N loss in ungrazed tallgrass prairie (Blair et al., 1998). Therefore, frequent fires induce greater N limitation on plant productivity (Blair, 1997). Belowground, frequent fires stimulate root biomass production of grasses (fine and total) but decrease root N concentrations (Johnson & Matchett, 2001; Kitchen et al., 2009). Because frequent fire widens the C:N ratio of aboveground and belowground plant material, this can have cascading effects on its decomposability and subsequent transfer to the SOM pool.

Before European colonization of the U.S. central Great Plains, the dominant grazers of tallgrass prairie were American bison (*Bison bison*), but they were nearly extirpated due to overhunting in the late nineteenth century (Flores, 2016). Although their numbers have rebounded, bison do not serve the ecological role in modern grasslands that they once did (Freese et al., 2007), and in many grasslands they have been replaced by cattle as the dominant ungulate herbivore. Ungulate grazing alters plant community composition, aboveground and belowground plant productivity, and N cycling processes (Johnson & Matchett, 2001; Pineiro et al., 2010; Winter et al., 2015). Through urine and feces deposition, grazers can increase rates of N mineralization and nitrification (Frank & Groffman, 1998; Hobbs, 1996), and potential N losses through leaching, denitrification, and ammonia volatilization, which can in turn increase the $\delta^{15}\text{N}$ signature of the N that is retained in the soil.

Changes in grassland management, including prescribed fire and grazing regimes, have important effects on plant communities that can feedback to alter soil processes. Fire suppression has been implicated in the recent expansion of woody vegetation into tallgrass prairie (Briggs et al., 2005; Fuhlendorf et al., 2008; Taylor et al., 2012). Increased cover of clonal C₃ woody shrubs such as *Cornus drummondii* and *Rhus glabra* is a common response to reduced fire frequency in tallgrass prairie (Ratajczak, Nippert, & Ocheltree, 2014). Fire suppression allows for woody shrub recruitment and as woody plants become established, they become more tolerant of fire disturbance and can reduce fine fuels and the intensity of fires when they do occur. Therefore, returning to a frequent fire interval may not be sufficient to reverse the transition from grassland to shrubland (Ratajczak, Nippert, & Ocheltree, 2014). Due to the differences in their physiology, resource allocation, and litter quality, a shift from a graminoid to a shrub dominated plant community has important implications for belowground C and N cycling. Grazers also alter plant community composition (Hickman et al., 2004). In the tallgrass prairie, plant diversity increases in response to grazing (Collins & Calabrese, 2012). In the absence of grazers, tallgrass prairie plant communities are typically dominated by a few species of C₄ grasses (e.g., *Andropogon gerardii*), particularly when frequently burned. Bison

preferentially feed on the dominant grasses, which changes the competitive relationships between C_4 grasses and C_3 forbs on the landscape, decreasing the dominance and cover of C_4 grasses and increasing the abundance of C_3 forbs (Knapp et al., 1999).

The ecosystem-level impacts of woody plant encroachment are still an open question, and there have been mixed results from previous studies regarding how the increased prevalence of woody species in grasslands affects carbon storage belowground (Barger et al., 2011; Eldridge et al., 2011; Lett et al., 2004). There are concerns that woody encroachment into grasslands will lead to overall losses in stored C, particularly in mesic grasslands (Jackson et al., 2002); however, it is difficult to assess the overall influence of woody encroachment since woody plant occupancy does not occur uniformly across the landscape, and it can have opposing impacts on different C pools. Previous approaches have used changes in aboveground NPP (Knapp et al., 2008), changes in soil C content associated with soil depth (Smith & Johnson, 2003), and satellite data (Asner et al., 2003) to assess the changes in C storage with woody plant encroachment over time. Key questions regarding ecosystem effects of different types of land management include the magnitude of those effects and the timeframe over which those effects develop. In this study, we analyzed selected properties of soil samples taken across a period of three decades from the same locations within watersheds under contrasting fire and grazing regimes to infer the impacts of fire and grazing on soil C and N dynamics. To our knowledge, this is the first study to harness multidecadal samples and associated data to document changes in soil C and N content associated with altered fire and grazing regimes and associated plant community changes, including woody encroachment.

In addition to assessing changes in total soil C and N content over time, we also examined changes in natural abundance C and N isotopic signatures in soil. Stable isotopes are an excellent tool for assessing the differential impacts of land management on soil C and N dynamics because C isotopes can reveal differences in the contributions of plant functional groups that use different photosynthetic pathways to the soil C pool as a result of community shifts, such as a shift in relative abundance of C_4 grasses and C_3 forbs or a C_4 grass to C_3 woody plant transition. Similarly, changes in natural abundance N isotopes in soil can be used to infer changes in N cycling processes under contrasting fire and grazing regimes, though identifying the specific processes driving altered ^{15}N isotopic signatures may be difficult. By tracking changes in both total C and N content and changes in the C and N isotopic signatures of soil over several decades, we aim to infer the impacts of multiple drivers on soil C and N content in the tallgrass prairie.

The overarching goal of this research was to assess the impacts of divergent disturbance regimes and associated changes in plant community composition on belowground C and N dynamics in a tallgrass prairie ecosystem. We sought to test three hypotheses: (1) Soil C and N concentrations will change over time as a function of divergent fire and grazing treatments and associated shifts in plant community composition. Specifically, we hypothesized that surface soil C and N concentrations would increase with fire suppression, but the magnitude of this effect would vary depending on presence or absence of grazers; (2) Because both fire suppression and the addition of native grazers shift plant community composition from C_4 grass dominance to greater abundance of C_3 plants (i.e., forbs and woody species), we hypothesized that soil $\delta^{13}\text{C}$ values would reflect changes in sources of organic inputs and would differentially correlate with soil C content under different treatments. Specifically, we expected a positive relationship between soil $\delta^{13}\text{C}$ values and soil C content in frequently burned, ungrazed prairie where C_4 grasses contribute proportionally more C below ground and a negative relationship in unburned and/or grazed prairie if C_3 plants contribute proportionally more C belowground; and (3) We hypothesized that changes in total soil N would generally track changes in total C, but divergent long-term grazing and burning regimes would alter N cycling processes and the openness of the N cycle, which would be reflected by changes in the soil $\delta^{15}\text{N}$ signature. A greater $\delta^{15}\text{N}$ value for soil N would indicate a more open N cycle with more N being lost from the system, potentially via increased denitrification, leaching, or ammonia volatilization in the presence of grazers.

2. Materials and Methods

2.1. Study Site

To assess changes in soil C and N pools and isotopic signatures and relate these to changes in plant communities under contrasting fire and grazing regimes, we analyzed archived soil samples and used plant

Table 1
Burning and Grazing History of Konza Watershed-Level Treatments Used for This Study

Treatments	Konza LTER watershed ID	Years burned	Notes
UG1	001D	1978–2015	
G1	N01B	1988–2015	Bison added 1992
UG20	020B	1991	Wildfire
G20	N20B	1980, 1991, and 1996	Wildfires in 1991 and 1996 and Bison added 1992

Note. All prescribed burns were conducted in the spring. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, and G20 = grazed, burned every 20 years.

composition data collected as part of the Long-Term Ecological Research (LTER) program at the Konza Prairie Biological Station (KPBS). Konza Prairie is located in the Flint Hills ecoregion of northeast Kansas, USA. The 34.87-km² KPBS site consists primarily of unplowed tallgrass prairie on a heterogeneous landscape consisting of rolling hills that divide the area into a series of small watersheds or catchments that include distinct uplands, lowlands, and slopes. The KPBS site includes watershed-level manipulations of prescribed spring burning and grazing by native ungulates (*Bison bison*) that were established in phases between 1972 and 1992. For this study, we used data from four experimental watersheds (001D, 020B, N01B, and N20B) ranging in size from 24 to 122 ha, each with comparable management histories and initial conditions, but with subse-

quent divergent fire and grazing treatments imposed over time. In this paper, we refer to the treatments as follows: UG1 = ungrazed, annually burned; UG20 = ungrazed, burned every 20 years; G1 = grazed, annually burned; G20 = grazed, burned every 20 years. All prescribed burns were performed in the spring. The full history of the burning and grazing regimes for each watershed, including any unplanned wildfires, is provided in Table 1.

2.2. Archived Soil Collection

Archived soil samples were available for selected dates spanning more than three decades. All soil samples were collected along four permanent sampling transects established within the lowland region of each watershed. Each transect was 50 m long, and the four transects were dispersed to maximize coverage of the watershed area and increase spatial independence. For this study, we considered each transect to be an independent replicate. Our analyses focused on exploring temporal dynamics of change in soil C and N pools in watersheds under divergent fire and grazing regimes and linking these changes to observed plant responses. However, because fire and grazing treatments were not replicated independently across multiple watersheds, we advise caution in extrapolating these results more broadly. Soils in the lowland regions of these watersheds are part of the Tully series which are nonrocky, silty clay loams that are deeper than other soils at KPBS (Collins & Calabrese, 2012; Ransom et al., 1998) which is why we focused solely on lowlands for this study. Four 2-cm-diameter soil cores were taken to a depth of 25 cm along each transect and composited into a single sample. All soil was passed through a 4-mm sieve to remove large rocks and roots, and the sieved soil was hand-picked to remove small rocks, root fragments, and any coarse organic debris. Samples were dried to constant weight at 60°C and finely ground with a ball grinder before being archived and stored at Kansas State University of Agriculture and Applied Science (Manhattan, KS, USA). For this study, we analyzed samples collected in 1982, 1987, 2002, 2010, and 2015. Bulk density was not measured when the samples were collected, so we present our C and N results as concentrations throughout the manuscript. Samples collected during the 1990s were not archived, so we were unable to include them in our analyses.

2.3. Plant Community Data

Plant community composition was assessed annually in permanent plots located along 50-m transects adjacent to the same transects from which the soil cores were collected (Hartnett et al., 2020). Five permanent circular plant sampling plots of 10-m² each were evenly spaced along each transect. The identity and percent foliar cover of all plants within each plot was recorded annually in both spring and autumn. Briefly, cover of each species was visually estimated using modified Daubenmire cover class categories and the maximum spring or fall value for that species was used to create annual cover values for each species in each plot. Further details on the methods used to generate plant species cover values are provided in Collins and Calabrese (2012). For this study, we converted Daubenmire cover class category to percent cover by using the midpoint in each cover class. We grouped plant species by photosynthetic pathway (C₃ or C₄) and summed total percent cover of all C₃ plants and all C₄ plants in each transect. To calculate proportional cover of C₃ or C₄ plants in each transect we divided those sums by the grand total of plant cover in each transect. We also grouped plant species by growth form (C₃ grass, C₄ grass, forb, woody, sedge, or nongrass monocot) and calculated the proportional cover of each growth form in each transect.

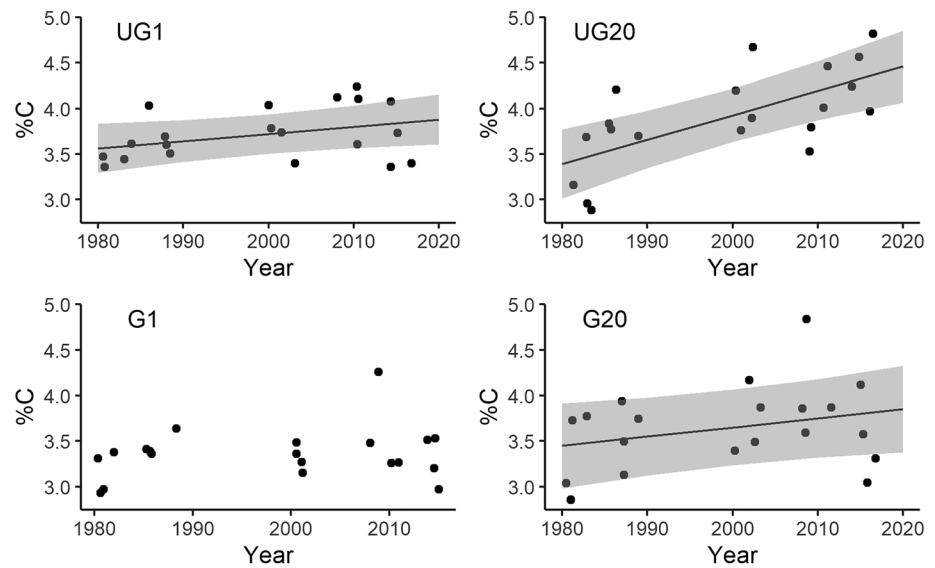


Figure 1. Change in soil C concentration over time in four watersheds at the Konza Prairie LTER site from five sample dates (1982, 1987, 2002, 2010, and 2015). Points have been jittered to minimize visual overlap. Shaded areas represent 95% confidence interval for predicted values. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, and G20 = grazed, burned every 20 years.

2.4. Isotope and Nutrient Analysis

In the fall of 2016, we analyzed a subsample of each archived soil sample at the Stable Isotope Mass Spectrometry Lab at Kansas State University of Agriculture and Applied Science. Dried soil samples were ground and homogenized using a Wig-L-Bug amalgamator. Samples were packed at 4 mg in pressed tin capsules and analyzed via continuous flow on a ThermoFinnigan Delta Plus isotope ratio mass spectrometer via a ConFlo II interface with a CE 1110 elemental analyzer. The isotopic ratio of samples was calculated using delta notation as

$$\delta \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) * 1,000 \right]$$

where R is the ratio of the heavy to light isotope for the sample and standard, respectively. For carbon, the laboratory working standards were calibrated relative to the international standard Vienna Pee Dee Belemnite (VPDB), while for nitrogen, the laboratory working standards were calibrated relative to the international standard atmospheric air. The within-run variability estimated as the standard deviation of working standards was always $<0.05\text{‰}$ for carbon and $<0.10\text{‰}$ for nitrogen, and the between-run variability, estimated as the difference between the measured value of a working standard and its long-term calibrated value, was always $<0.05\text{‰}$ for carbon and $<0.15\text{‰}$ for nitrogen.

2.5. Isotope Mixing Model

To calculate the proportional contribution of C_3 plants to belowground C, we used the following isotopic mixing model:

$$C_3 = (\delta^{13}C_{\text{soil}} - \delta^{13}C_{C_4}) / \delta^{13}C_{C_3}$$

In the above equation, C_3 represents the proportion of belowground C attributable to the contribution of C_3 plants, $\delta^{13}C_{\text{soil}}$ represents the measured $\delta^{13}C$ value of the soil, $\delta^{13}C_{C_4}$ represents the average $\delta^{13}C$ value of C_4 plant biomass, and $\delta^{13}C_{C_3}$ represents the average $\delta^{13}C$ value of C_3 plant biomass. In our equation we used $\delta^{13}C_{C_4} = -13\text{‰}$ and $\delta^{13}C_{C_3} = -28\text{‰}$ (Peterson & Fry, 1987).

Table 2
Equations, 95% Confidence Intervals (CIs) for Slope Estimates, and *p* Values for All Linear Mixed Models

Response	Equation	95% CI for slope	<i>p</i>
<i>UG1</i>			
% C	0.0079x – 12	(–0.00047, 0.016)	0.062
$\delta^{13}\text{C}_{\text{soil}}$	0.019x – 52	(0.0091, 0.029)	0.001
% N	0.00059x – 0.89	(–0.00025, 0.0014)	0.156
$\delta^{15}\text{N}$	–0.017x + 38	(–0.054, 0.020)	0.344
% C versus $\delta^{13}\text{C}$	0.37x + 9.0	(0.086, 0.65)	0.014
% N versus $\delta^{15}\text{N}$	–0.015x + 0.35	(–0.024, –0.0054)	0.004
C ₃ cover	–0.0051x + 10	(–0.0084, –0.0018)	0.003
C ₃ contribution	–0.0013x + 2.6	(–0.0019, –0.00061)	<0.001
<i>G1</i>			
% C	0.0045x – 5.8	(–0.0036, 0.013)	0.251
$\delta^{13}\text{C}_{\text{soil}}$	–0.48x – 1.5x ² – 15	x: (–1.8, 0.86) x ² : (–2.9, –0.21)	0.034
% N	0.00063x – 0.97	(–0.00023, 0.0015)	0.139
$\delta^{15}\text{N}$	–2.2x + 3.2x ² + 3.9	x: (–3.9, –0.35) x ² : (1.4, 5.0)	<0.001
% C versus $\delta^{13}\text{C}$	–0.053x + 2.5	(–0.22, 0.11)	0.505
% N versus $\delta^{15}\text{N}$	–0.014x + 0.35	(–0.021, –0.0074)	<0.001
C ₃ cover	0.012x – 24	(0.0088, 0.15)	<0.001
C ₃ contribution	0.032x + 0.10x ² + 0.16	x: (–0.057, 0.12) x ² : (0.014, 0.19)	0.034
<i>UG20</i>			
% C	0.027x – 49	(0.014, 0.039)	<0.001
$\delta^{13}\text{C}_{\text{soil}}$	–0.075x + 130	(–0.098, –0.052)	<0.001
% N	0.0030x – 5.6	(0.0014, 0.0046)	0.001
$\delta^{15}\text{N}$	–4.8x – 1.6x ² + 3.1	x: (–6.5, –3.1) x ² : (–3.3, 0.081)	<0.001
% C versus $\delta^{13}\text{C}$	–0.33x – 1.2	(–0.46, –0.20)	<0.001
% N versus $\delta^{15}\text{N}$	–0.029x + 0.40	(–0.037, –0.021)	<0.001
C ₃ cover	0.018x – 35	(0.014, 0.022)	<0.001
C ₃ contribution	0.0050x – 9.8	(0.0035, 0.0066)	<0.001
<i>G20</i>			
% C	0.010x – 16	(–0.0012, 0.021)	0.077
$\delta^{13}\text{C}_{\text{soil}}$	–0.046x + 76	(–0.066, –0.026)	<0.001
% N	0.00094x – 1.6	(0.00010, 0.0018)	0.030
$\delta^{15}\text{N}$	–3.2x + 2.3x ² + 3.7	x: (–4.2, –2.3) x ² : (1.3, 3.2)	<0.001
% C versus $\delta^{13}\text{C}$	–0.29x – 0.90	(–0.44, –0.14)	<0.001
% N versus $\delta^{15}\text{N}$	–0.019x + 0.37	(–0.027, –0.012)	<0.001
C ₃ cover	0.013x – 25	(0.0085, 0.017)	<0.001
C ₃ contribution	0.0031x – 5.9	(0.0017, 0.0044)	<0.001

Note. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, and G20 = grazed, burned every 20 years.

2.6. Data Analysis

First, linear mixed models were used to explore directional changes over time in soil and plant response variables and to investigate correlations between the selected soil variables (e.g., % soil C and $\delta^{13}\text{C}$) using samples from all available dates (1982–2015). Comparisons of slopes were done by calculating the 95% confidence intervals (CIs) for slope estimates. If the CI did not overlap, we considered the slopes to be statistically different. Second, to determine the effects of the divergent burning and grazing regimes, linear mixed models were used to analyze the independent and interactive effects of burning and grazing on % soil C, % soil N, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$. For these analyses, we used only data from 2002, 2010, and 2015 since the combined

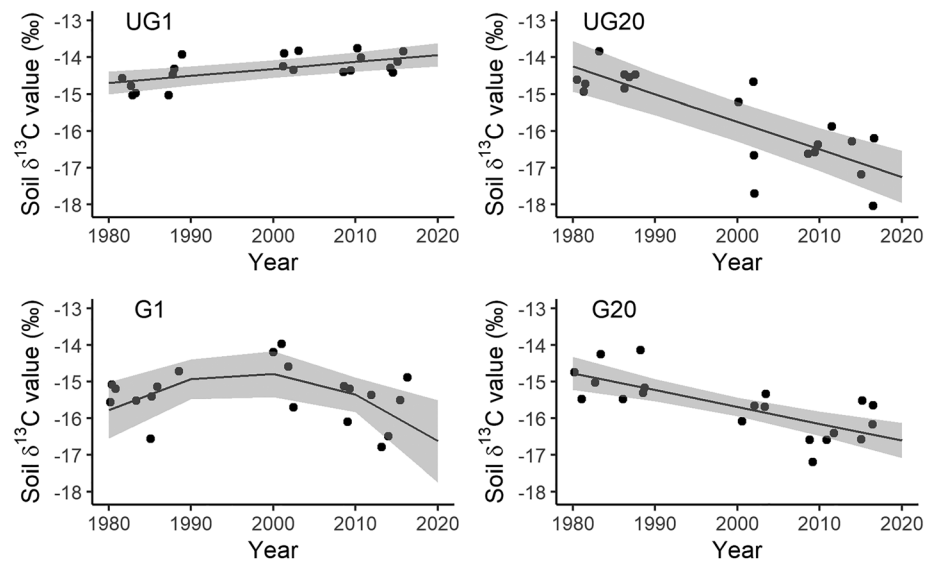


Figure 2. Change in the soil $\delta^{13}\text{C}$ signature over time in four watersheds at the Konza Prairie LTER site from five sample dates (1982, 1987, 2002, 2010, and 2015). Points have been jittered to minimize visual overlap. Shaded areas represent 95% confidence interval for predicted values. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, and G20 = grazed, burned every 20 years.

burning and grazing treatments were not fully established until 1992 (Table 1). We used Tukey's Honestly Significant Difference Test of least squares means to detect statistically significant differences in means. For all analyses, transect was treated as a random effect and temporal autocorrelation for repeated measures of the transects was accounted for. All models were tested to ensure that they did not violate model assumptions of normality and homoscedasticity. For all analyses of $\delta^{15}\text{N}$, this required dropping one outlier data point (UG20: Year = 2002, $\delta^{15}\text{N} = 13.57$). All analyses were conducted in R (R Core Team, 2019) with the packages *nlme*, *lsmeans*, and *multcomp* (Hothorn et al., 2008; Lenth, 2016; Pinheiro et al., 2019). All figures were created using the packages *tidyverse* (Wickham, 2017) and *cowplot* (Wilke, 2019).

3. Results

3.1. Trajectories of Change in Soil Carbon

Percent soil C increased over time under every treatment except the annually burned, grazed treatment (G1; Figure 1). Although the 95% CIs of the estimated slopes slightly overlapped, of the treatments that experienced an increase in soil C concentration, the increase was slowest in the annually burned, ungrazed treatment (UG1) at $0.008\% \text{ year}^{-1}$ and most rapid in the infrequently burned, ungrazed treatment (UG20) at $0.027\% \text{ year}^{-1}$ suggesting an effect of fire frequency on rates of soil C accrual (Table 2). The $\delta^{13}\text{C}$ value of soil C also changed over time in all treatments (Figure 2), though the direction and trajectory varied. In the infrequently burned treatments, soil $\delta^{13}\text{C}$ values decreased over time regardless of grazing treatment (UG20: $0.075\text{‰} \text{ year}^{-1}$; G20: $0.046\text{‰} \text{ year}^{-1}$). In contrast, soil $\delta^{13}\text{C}$ values increased in the annually burned, ungrazed treatment (UG1) at $0.019\text{‰} \text{ year}^{-1}$. In the annually burned, grazed treatment (G1), soil $\delta^{13}\text{C}$ values also increased initially until 2002, and then decreased between 2002 and 2015.

3.2. Trajectories of Change in Soil Nitrogen

Soil N concentration increased over time in the infrequently burned treatments regardless of presence or absence of grazers, but not in the annually burned treatments (Figure 3). However, the rate of N accumulation was significantly more rapid, by a factor of 3, in the infrequently burned treatment without grazers (UG20: $0.003\% \text{ year}^{-1}$) compared to the grazed treatment (G20: $0.00094\% \text{ year}^{-1}$) (Table 2). Soil $\delta^{15}\text{N}$ values changed nonlinearly with time in the treatments G1, G20, and UG20 (Figure 4). In both of the grazed treatments, $\delta^{15}\text{N}$ values decreased until 2002, 10 years after the bison introduction, and then increased between

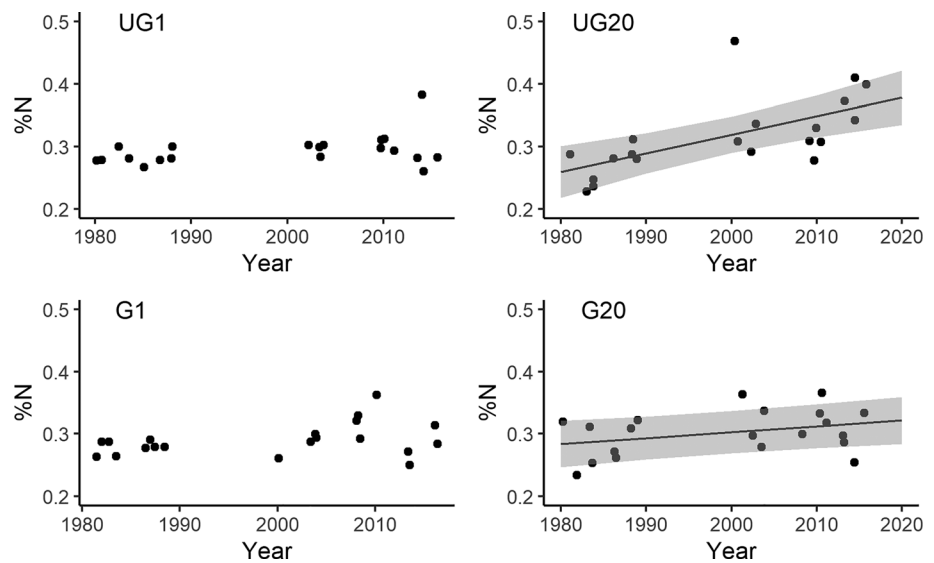


Figure 3. Change in soil N concentration over time in four watersheds at the Konza Prairie LTER site from five sample dates (1982, 1987, 2002, 2010, and 2015). Points have been jittered to minimize visual overlap. Shaded areas represent 95% confidence interval for predicted values. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, and G20 = grazed, burned every 20 years.

2002 and 2015. Soil $\delta^{15}\text{N}$ values ultimately decreased over time in the unburned, ungrazed treatment (UG20).

3.3. Cumulative Effects of Burning and Grazing on Soil C and N

Mean soil C and N concentrations averaged over the latter part of the temporal sequence (2002–2015) were impacted by the divergent burning and grazing regimes. On average, soil C content was 10% higher in the infrequently burned versus annually burned treatments and 10% lower in grazed versus ungrazed

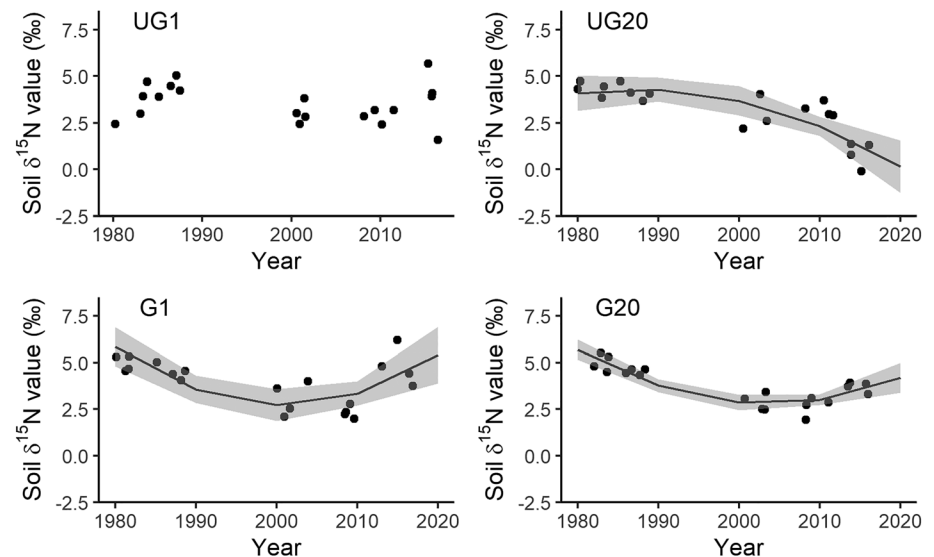


Figure 4. Change in soil $\delta^{15}\text{N}$ signature over time in four watersheds at the Konza Prairie LTER site from five sample dates (1982, 1987, 2002, 2010, and 2015). Points have been jittered to minimize visual overlap. Shaded areas represent 95% confidence interval for predicted values. There was an outlier removed in UG20 (Year = 2002, $\delta^{15}\text{N} = 13.57$). UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, and G20 = grazed, burned every 20 years.

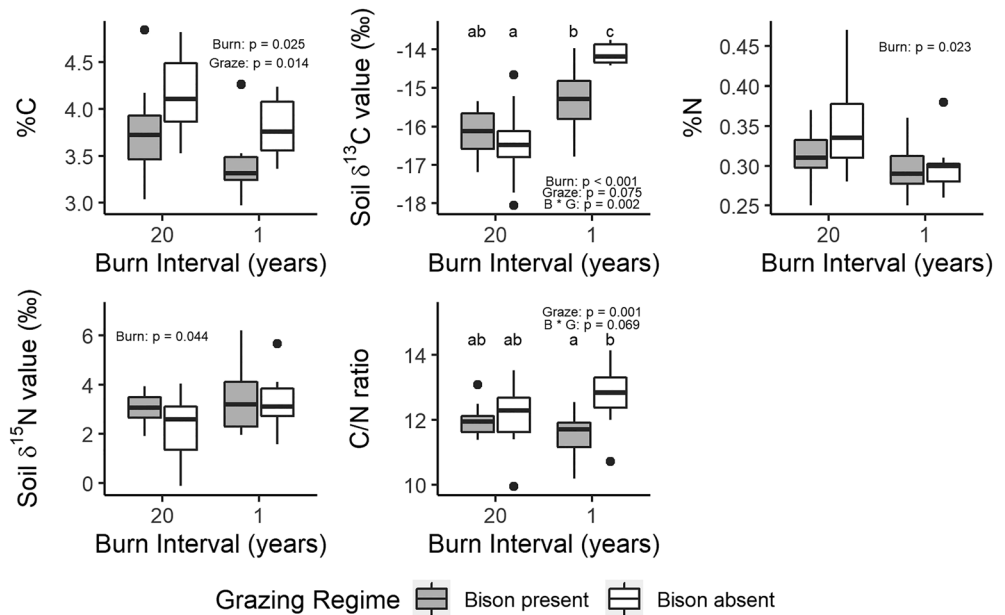


Figure 5. Cumulative effects of divergent burning and grazing regimes on soil % C, $\delta^{13}\text{C}$ values, % N, $\delta^{15}\text{N}$ values, and C/N ratio. For the analysis of $\delta^{15}\text{N}$ values, there was an outlier removed in UG20 ($\delta^{15}\text{N} = 13.57$). Only data from 2002–2015 were used in this analysis. If $p < 0.1$ for the interaction between burn and graze regime, letters denote significant differences according to Tukey post hoc pairwise comparisons.

treatments (Figure 5). Mean soil $\delta^{13}\text{C}$ values also diverged in response to burning, grazing, and their interaction (Figure 5). Soil $\delta^{13}\text{C}$ values were highest (-14.1‰) in the annually burned, ungrazed treatment (UG1) and lowest (-16.5‰) in the infrequently burned, ungrazed treatment (UG20). Soil N content and $\delta^{15}\text{N}$ values were also impacted by burn regime (Figure 5). On average, annually burned treatments had 10% lower soil N content and a soil $\delta^{15}\text{N}$ value that was 24.3% higher than infrequently burned treatments. Grazing decreased average soil C/N ratios by 10.4% (from 12.7 to 11.5) but only in the annually burned treatment, which also had the lowest C/N ratio (Figure 5).

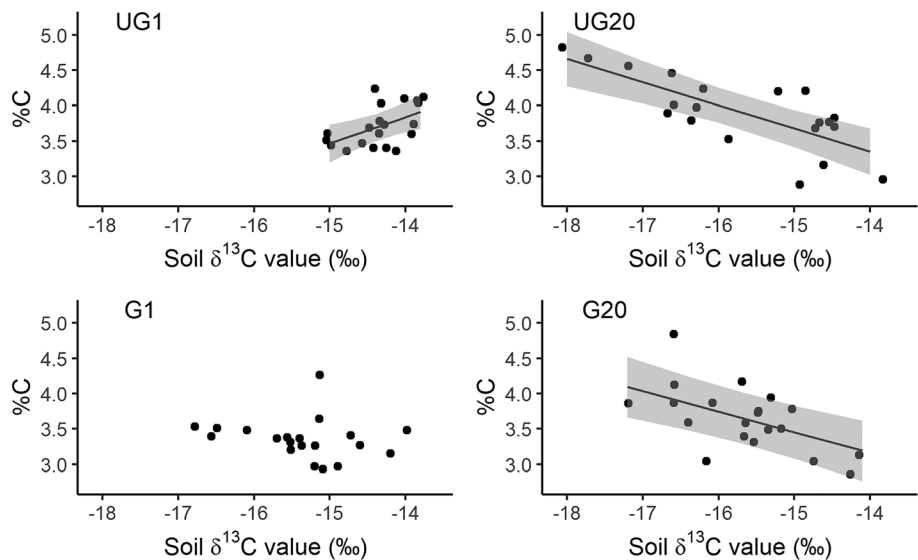


Figure 6. Relationship between the soil $\delta^{13}\text{C}$ values and soil C content in four treatments at the Konza Prairie LTER site. Shaded areas represent 95% confidence interval for predicted values. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, and G20 = grazed, burned every 20 years.

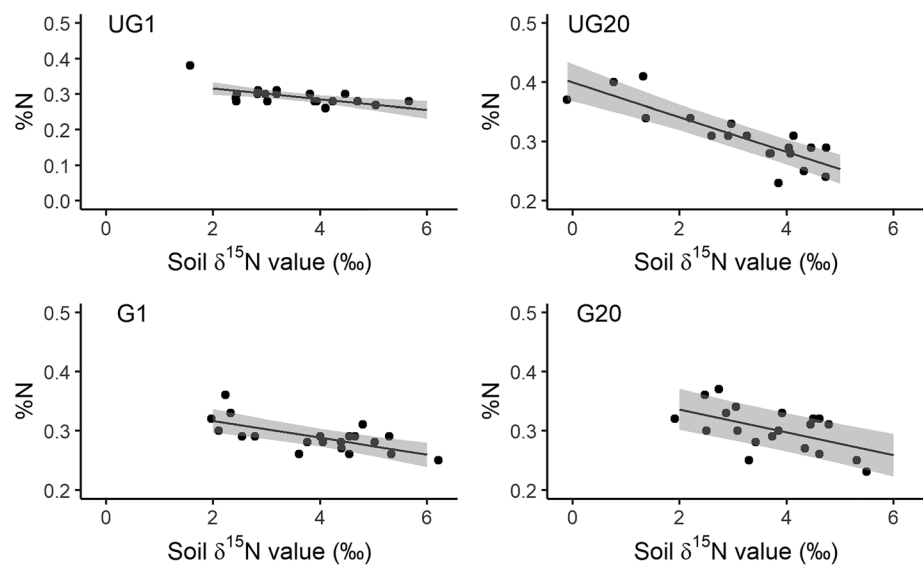


Figure 7. Relationship between the soil $\delta^{15}\text{N}$ signature soil N content across four watersheds at the Konza Prairie LTER site. Shaded areas represent 95% confidence interval for predicted values. There was an outlier removed in UG20 ($\delta^{15}\text{N} = 13.57$, % N = 0.47). UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, and G20 = grazed, burned every 20 years.

3.4. Relationship Between Soil Isotopes and Nutrient Concentrations

Soil $\delta^{13}\text{C}$ values and soil C content were correlated across soil samples from all dates in treatments UG1, UG20, and G20, but the nature of the relationship varied with treatment (Figure 6). In the annually burned, ungrazed treatment (UG1), this relationship was positive ($0.37\% \text{ C } \text{‰}^{-1}$). However, in the infrequently burned treatments, the relationship was negative (G20: $-0.29\% \text{ C } \text{‰}^{-1}$; UG20: $-0.33\% \text{ C } \text{‰}^{-1}$). Soil N content was negatively correlated with soil $\delta^{15}\text{N}$ values across all treatments (Figure 7). The slope of the negative relationship between soil N content and $\delta^{15}\text{N}$ values was steepest in the infrequently burned,

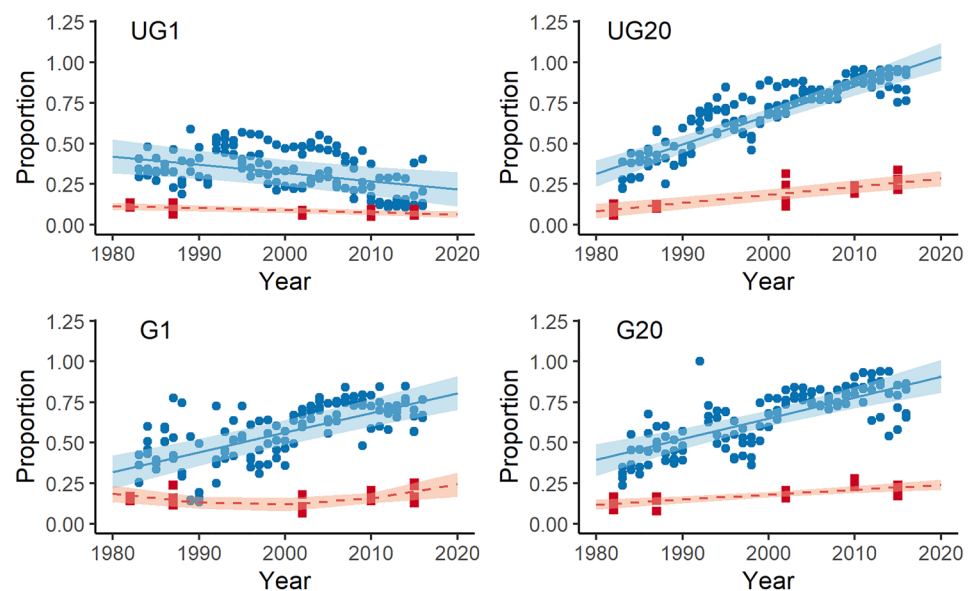


Figure 8. Changes in the proportional cover of C_3 plants of the plant community according to plant community composition data (blue circles and solid line) and changes in proportional contribution of C_3 plants to soil C according to our isotopic mixing model (red squares and dashed line) in four treatments at the Konza Prairie LTER site from 1982–2015. Shaded areas represent 95% confidence interval for predicted values. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, and G20 = grazed, burned every 20 years.

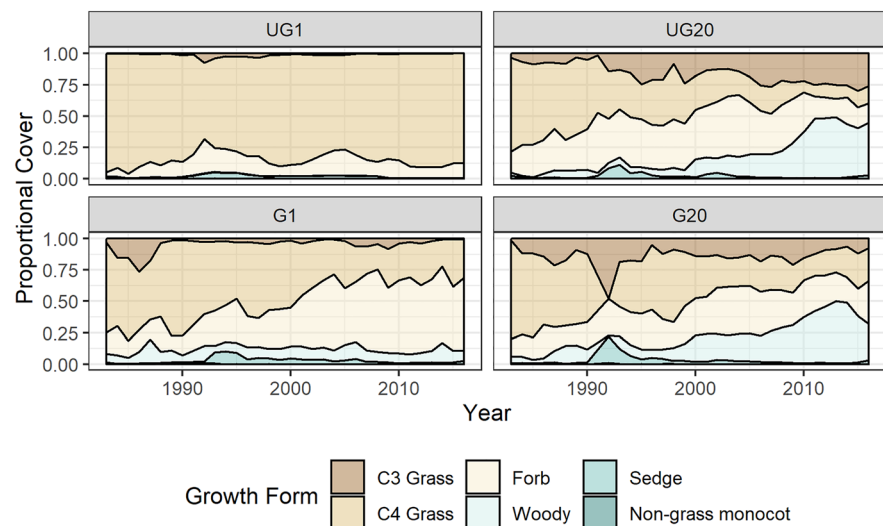


Figure 9. Change in proportional cover of six categories of growth form in plant communities according to data from four treatments at the Konza Prairie LTER site from 1982–2015. UG1 = ungrazed, annually burned, UG20 = ungrazed, burned every 20 years, G1 = grazed, burned annually, and G20 = grazed, burned every 20 years.

ungrazed treatment (UG20; $-0.029\% \text{ N } \%^{-1}$) and shallowest in the annually burned, grazed treatment (G1; $-0.014\% \text{ N } \%^{-1}$).

3.5. Plant Community Trajectories and the Contribution of C₃ Plants to Soil C Over Time

The proportional cover of C₃ plants relative to the cover of C₄ plants increased over time in all treatment combinations except the annually burned, ungrazed treatment (UG1; Figure 8), where the cover of C₃ plants declined over time. The change in proportional cover of C₃ plants was most rapid under low fire frequency and in the absence of grazers (UG20; 0.018 year^{-1}) and most gradual in the annually burned, ungrazed treatment (UG1; $-0.0051 \text{ year}^{-1}$). The rate of change in relative cover of plants using C₃ and C₄ photosynthetic pathways was significantly slower in the annually burned, ungrazed treatment compared to the other three (Table 2). The shifts in relative cover of plants using C₃ and C₄ pathways were driven by changes in cover of major plant growth forms (Figure 9), which varied under different treatment combinations. In annually burned treatments, C₄ grasses remained dominant when grazers were absent, but C₄ grass dominance decreased and relative cover of C₃ forbs increased when grazers were present. In infrequently burned treatments, C₄ grasses became less dominant as C₃ woody plants became more abundant (Figure 9).

Based on our two end-member mixing model, the proportional contribution of C₃ plants to soil C changed over time in all treatments (Figure 8). In the infrequently burned treatments (UG20 and G20), the proportional contribution of C₃ plants to soil C increased over time, albeit at a significantly slower rate than the change in C₃ plant cover (Table 2). In contrast, in the annually burned treatment without grazers (UG1), the proportional contribution of C₃ plants to soil C decreased over time, and this rate was not significantly different from the change in C₃ plant cover (Table 2). The proportional contribution of C₃ plants to soil C changed nonlinearly over time in the annually burned treatment where grazers were reintroduced (G1), such that it decreased at the beginning of the study period, similar to burned treatment without grazers, but increased after 2002. Finally, the contribution of C₃ plants to soil C changed significantly more rapidly in the infrequently burned and ungrazed treatment (UG20) than the annually burned and ungrazed treatment (UG1; Table 2).

4. Discussion

4.1. Hypothesis 1: Soil C and N Concentrations Will Change Over Time as a Function of Divergent Fire and Grazing Treatments and Associated Shifts in Plant Community Composition

Soil C content increased in three out of the four fire and grazing treatment combinations over the 33-year period encompassed by our study (Figure 1), and the mean C content of soils was affected by

manipulations of both burning and grazing. By the later portion of the study (2002–2015), mean soil C content was lowest in soils under disturbance regimes that included frequent burning and/or or grazing (Figure 5). Additionally, the increase in soil C content over time was greatest in treatments in which fire was suppressed and grazers were absent (Figure 1). In the absence of major disturbances, it appears that the C content of tallgrass prairie soils can increase over time, which may contribute to additional C sequestration (Barger et al., 2011) although in the case of greatly reduced fire frequency, the increase in soil C may come at the expense of loss of tallgrass prairie vegetation to encroachment by woody species and associated losses of consumer species that require native prairie habitat. For example, in another study in this region, an increase in soil organic C content in the absence of prescribed burning was associated with encroachment by the woody species, *Juniperus virginiana* (McKinley & Blair, 2008) and concomitant loss of native herbaceous plant species (Briggs, Hoch, et al., 2002). Such replacement of tallgrass prairie vegetation by woody species is associated with both ecological (e.g., loss of native biodiversity) and economic (e.g., loss of grazable rangelands) impacts that offset the potential benefits of increased soil C storage with reduced fire frequency.

Analysis of archived soil samples suggests that contrasting fire treatments had a larger effect on N dynamics than did presence or absence of grazers (Figure 5). Cumulatively, mean soil N content was higher in the infrequently burned treatments (Figure 5), reflecting the gradual increase in soil N accumulation over time in the absence of frequent fires (Figure 3). Burning increases N limitation in mesic grasslands through combustion and volatilization of N in aboveground litter (Blair, 1997; Fynn et al., 2003), widening of C/N ratios in organic inputs, and an increase in the N immobilization potential of soils (Dell et al., 2005). Our results are consistent with frequent fire as a mechanism for maintaining chronic N limitation in grasslands. Grazers did not significantly influence total soil N content in this study. Ungulate grazers amplify the magnitude and spatial heterogeneity of N mineralization and nitrification rates (Frank & Groffman, 1998; Johnson & Matchett, 2001) through the deposition of labile N in urine and feces (Hobbs, 1996). Additionally, through the consumption of aboveground biomass and deposition of more labile forms of N, ungulate grazers often decrease the C/N ratio of plant tissue, which could subsequently increase the quality of SOM. Grazing did decrease the C/N ratio of SOM in our study but only in the annually burned treatment, which resulted in the lowest C/N ratio we observed (Figure 5). Based on deposition of urea in ungulate urine and enhanced rates of N transformation, one might predict that grazed grasslands could be more prone to losses via ammonia volatilization, leaching, and denitrification pathways compared to ungrazed grasslands (Frank & Evans, 1997). However, our results indicate no change in total soil N content with grazing in the annually burned treatment and an increase in N content with or without grazers in the infrequently burned treatments (Figure 3). In the grazed and burned treatment, any enhanced losses of N due to enhanced microbial transformation may have been offset by the reduced volatilization of N during fire (i.e., due to reduced aboveground biomass and litter in the presence of grazers) and conversion of organic N in plant biomass to inputs of bison dung. In the grazed and infrequently burned watershed, N increased over time, but the rate of increase was lower than in the absence of grazers (Figure 3), potentially reflecting greater N losses due to microbial activity in the presence of grazers when fire is infrequent (see Hypothesis 3). Since bison at KPBS have the ability to freely move between watersheds of different burn regimes, the effect of grazers on N loss might have been weaker in the infrequently burned treatment than the annually burned treatment because bison preferentially forage in areas that have been recently burned (Raynor et al., 2017). In total, our results suggest that the effect of grazers on soil N is dependent on fire regime.

4.2. Hypothesis 2: Soil $\delta^{13}\text{C}$ Values Reflect Changes in Sources of Organic Inputs and Will Differentially Correlate With Soil C Content Under Different Treatments

Plants are the conduit through which C moves from the atmosphere to the soil, and a change in plant community composition can influence the magnitude of that flux (Yang et al., 2019) especially if there are large shifts in the proportions of different plant functional groups (Fornara & Tilman, 2008). In all treatments except for UG1, the proportional cover of C_3 plants increased over time (Figure 8). In the rarely burned treatments, the increase in C_3 plant cover and associated change in the $\delta^{13}\text{C}$ signature of the soil were likely driven, in large part, by encroachment of C_3 woody plants in the absence of regular fire disturbance (Figure 9). Fire increases total aboveground NPP and flowering culm productivity of the dominant C_4 grasses (La Pierre et al., 2011), whereas fire suppression allows for establishment and expansion of woody shrubs and trees, which outcompete grasses for light as they grow in size (Ratajczak et al., 2011; Ratajczak, Nippert, Briggs,

et al., 2014). Ungulate grazers also alter the relative abundance of different plant growth forms. Preferential grazing of C_4 grasses by bison reduces the cover of C_4 plants and allows C_3 forbs to increase in abundance (Raynor et al., 2017), even with frequent burning, offsetting the effects of annually burning alone (Collins et al., 1998), as observed in the annually burned and grazed treatment in this study.

After change in the relative proportion of plants using different photosynthetic pathways, a temporal change in the $\delta^{13}C$ value of soil C is indicative of SOM derived from the previous plant community turning over and being replaced by the organic inputs of the new plant community (Boutton et al., 1998). In such cases, isotopic mixing models can be used to estimate the relative contributions of C_3 versus C_4 plants to the soil C pool over time. In this study, the overall contribution of C_3 plants to soil C increased over time in response to fire suppression and to grazing following the reintroduction of bison in the annually burned treatment (Figure 8) as indicated by the decreasing $\delta^{13}C$ values of the soil (Figure 2). During the same time, the proportional contribution of C_3 plants to soil C decreased in the annually burned treatment with no grazers present (UG1; Figure 8). This decrease in the contribution of C_3 plants with annual burning in the absence of grazers is likely due to turnover of older C_3 -derived organic matter that accumulated as a result of the site's history of cattle grazing, which would have favored a higher abundance of C_3 forbs than are currently supported in that treatment. In the annually burned and grazed treatment, soil $\delta^{13}C$ values also initially increased between 1982 and 2002, a period during which grazers were absent for the first decade, and then decreased after 2002, presumably in response to the competitive release of C_3 forbs as a result of bison reintroduction starting in 1992 (Figure 9). The lag between bison introduction and shifts in soil $\delta^{13}C$ signatures likely reflects both the time it takes for plant communities to change following the addition of a new driver and the time required for those changes to subsequently impact soil C.

The calculated contribution of C_3 plants to soil C increased at a slower rate than the increase in cover of C_3 plants in the infrequently burned treatments (Table 2), and soil $\delta^{13}C$ values remained much closer to the C_4 plant endpoint ($\delta^{13}C = -13\%$) in all treatments. These results reflect the large accumulations of C_4 -derived C that is typical of native tallgrass prairie soils and suggest a potential lag between gradual plant community changes and change in soil C isotopic composition. The turnover rate of SOM is affected by many ecological factors (Schmidt et al., 2011). Mineral sorption (Cotrufo et al., 2013; Kleber et al., 2007) and physical protection from microbial attack (Dungait et al., 2012; Tisdall & Oades, 1982) can allow for SOM to persist for decades to centuries. This leads us to hypothesize that the changes in soil $\delta^{13}C$ values and C concentrations are likely due to recent organic inputs from the new plant communities that resulted from three decades of divergent burning and grazing treatments.

In the infrequently burned treatments, soil C content was higher when the stable isotope signature indicated a higher proportional contribution of C_3 plants (Figure 6). However, in the annually burned and ungrazed treatment, we observed the opposite pattern—greater soil C content when the isotopic signature indicated more C_4 -derived organic matter. These conflicting patterns indicate that soil C content is higher when land management selects for a particular plant growth form (i.e., C_3 woody shrubs or C_4 grasses; Pellegrini et al., 2020; Wigley et al., 2020), especially if that growth form contributes proportionally more C belowground. The one exception to this was in the annually burned, grazed treatment (G1) where the plant community became increasingly more dominated by C_3 forbs over time with no subsequent increase in soil C content. It seems that in the case of combined annual burning and grazing by a species that reduces the abundance of the dominant C_4 grasses, grazing may offset the positive effects of burning on C_4 grass production and its contribution to soil C content, while annual burning maintains relatively low cover of woody vegetation and litter inputs that would otherwise contribute to increased soil C concentration, as occurred in the grazed and rarely burned treatment. Therefore, it appears that the plant community changes driven by divergent burning and grazing regimes (i.e., Figures 8 and 9) may have altered belowground C concentrations with potential long-term impacts on belowground C storage in tallgrass prairie.

4.3. Hypothesis 3: Long-Term Grazing and Burning Regimes Will Alter Multiple N Cycling Processes and, Consequently, the Soil $\delta^{15}N$ Signature

The soil $\delta^{15}N$ signature is often used as an indicator of the openness of the nitrogen cycle (i.e., a higher $\delta^{15}N$ value indicates N cycling is more rapid and N is more prone to loss through denitrification, volatilization, or leaching, whereas a lower $\delta^{15}N$ value indicates N is cycling more slowly and being retained within the

system; Austin & Vitousek, 1998). Accordingly, soil $\delta^{15}\text{N}$ values suggest that N is cycled more tightly when fire is suppressed (Figure 5), albeit interactions with grazing affected the temporal trajectory of soil $\delta^{15}\text{N}$ values (Figure 4). The N cycle appears to have become more closed over time in the infrequently burned, ungrazed treatment (UG20), while in the grazed treatments, there was no clear linear trend (Figure 4). In the grazed treatments, soil $\delta^{15}\text{N}$ values exhibited a curvilinear change over time, which may be due to a lag following reintroducing bison in 1992. There was also a clear negative relationship between soil $\delta^{15}\text{N}$ values and soil N content (Figure 7) across all dates and in all treatment combinations, which could indicate that when the rate of N cycling slows, organic soil N concentration increases.

Fire, grazing, and their interaction affect how N is lost in tallgrass prairie. Fire volatilizes organic N via combustion, but grazers reduce the amount of organic material that can be burned (Hobbs et al., 1991; Holdo et al., 2007). Soil $\delta^{15}\text{N}$ values are positively related to the residence time of different pools of SOM within grasslands (Liao et al., 2006). Since soil $\delta^{15}\text{N}$ values were significantly higher in the annually burned treatments (Figure 5), this could indicate that in addition to frequent N volatilization discriminating against the ^{15}N isotope, a higher proportion of organic N is immobilized by soil microbes (Dell et al., 2005; Ojima et al., 1994) and/or is physically protected within aggregates (Liao et al., 2006) in frequently burned prairie. Whether large grazers accelerate or decelerate N cycling is not consistent across all grasslands (Singer & Schoenecker, 2003) indicating that the factors that control N cycling are complex and dependent on local factors. For example, in Yellowstone National Park, ungulate grazers simultaneously stimulated rates of N mineralization and nitrification while promoting N retention such that soil $\delta^{15}\text{N}$ values were lower than expected (Frank et al., 2000). On the other hand, cattle exclusion increased rates of N mineralization in a European grassland due to enhanced herbivory by small mammals (Bakker et al., 2004). Overall, we suggest that bison accelerated N cycling in our system since soil $\delta^{15}\text{N}$ values increased over time after they were introduced, but the specific processes that contribute to this acceleration cannot be determined based on isotope data alone.

5. Conclusions

This research demonstrates that contrasting burning and grazing regimes have divergent effects on soil C and N pools and, through their impacts on the plant community, have shifted the relative contributions of different plant growth forms to the soil C and N pool. Changes in soil C and N content were associated with three decades of land management favoring certain plant growth forms (C_3 woody encroachment vs. C_4 grass dominance) in the tallgrass prairie ecosystem. Through stable isotope analysis, we demonstrated that in heavily encroached grasslands, more C is stored belowground when woody plants have contributed a larger proportion of C to that pool (Pellegrini et al., 2020). In addition, this research has demonstrated the unique potential for using long-term soil collections to more fully understand the biogeochemical impacts of human-altered disturbance regimes in grasslands.

Although our results suggest that woody encroachment may increase soil C and N content with potential benefits with respect to some selected ecosystem services, woody encroachment has other negative ecological consequences that should be taken into consideration (Archer et al., 2017). The tallgrass prairie is among the most endangered ecosystems in the world (Samson & Knopf, 1994), and woody encroachment threatens many plant species (Ratajczak et al., 2012) and decreases the amount of habitat available for grassland-dependent animals (Archer et al., 2017). Woody encroachment can also decrease the economically important ecosystem services that grasslands provide. For example, modest increases in woody plant cover decrease livestock production (Anadón et al., 2014). Finally, while our results show that soil C content can increase over time in response to divergent burning and grazing regimes, observed rates of increase were lower than $0.4\% \text{ year}^{-1}$, the recommended sequestration rate proposed by the UN Climate Action Program (Minasny et al., 2017).

Data Availability Statement

All data are archived at <http://lter.konza.ksu.edu/data> (Connell et al., 2020; Hartnett et al., 2020).

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