RESEARCH ARTICLE

Frequent burning causes large losses of carbon from deep soil layers in a temperate savanna

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Abstract

1. Fire activity is changing dramatically across the globe, with uncertain effects on ecosystem processes, especially below-ground. Fire-driven losses of soil carbon (C) are often assumed to occur primarily in the upper soil layers because the repeated combustion of above-ground biomass limits organic matter inputs into surface soil. However, C losses from deeper soil may occur if frequent burning reduces root biomass inputs of C into deep soil layers or stimulates losses of C via leaching and priming.

2. To assess the effects of fire on soil C, we sampled 12 plots in a 51-year-long fire frequency manipulation experiment in a temperate oak savanna, where variation in prescribed burning frequency has created a gradient in vegetation structure from closed-canopy forest in unburned plots to open-canopy savanna in frequently burned plots.

3. Soil C stocks were nonlinearly related to fire frequency, with soil C peaking in savanna plots burned at an intermediate fire frequency and declining in the most frequently burned plots. Losses from deep soil pools were significant, with the absolute difference between intermediately burned plots versus most frequently burned plots more than doubling when the full 1 m sample was considered rather than the top 0–20 cm alone (losses of 98.5 Mg C/ha [−76%] and 42.3 Mg C/ha [−68%] in the full 1 m and 0–20 cm layers respectively). Compared to unburned forested plots, the most frequently burned plots had 65.8 Mg C/ha (−58%) less C in the full 1 m sample. Root biomass below the top 20 cm also declined by 39% with more frequent burning. Concurrent fire-driven losses of nitrogen and gains in calcium and phosphorus suggest that burning may increase nitrogen limitation and play a key role in the calcium and phosphorus cycles in temperate savannas.

4. *Synthesis.* Our results illustrate that fire-driven losses in soil C and root biomass in deep soil layers may be critical factors regulating the net effect of shifting fire regimes on ecosystem C in forest-savanna transitions. Projected changes in soil C with shifting fire frequencies in savannas may be 50% too low if they only consider changes in the topsoil.

KEYWORDS

fire, global change ecology, repeated burning, savanna, soil carbon, soil nutrients, tree cover

1 | INTRODUCTION

Soils are the largest carbon (C) storage pool on earth, but the response of soil C to global change remains uncertain (Jackson et al., 2017). The response of deep soil C is especially important, as shifts in deep soil C caused by global change factors such as climate change and woody encroachment can lead to large changes in total soil C (Fontaine et al., 2007; Hicks Pries, Castanha, Porras, & Torn, 2017; Jackson, Banner, Jobbágy, Pockman, & Wall, 2002; Koven, Lawrence, & Riley, 2015). In contrast, potential effects of changes in fire regimes on deeper soil C (e.g. >20 cm) have received less attention because deeper soils are less prone to heating, and combustion by fire is assumed to primarily decrease above-ground biomass C inputs to surface soils (Certini, 2005; Johnson & Curtis, 2001; Nave, Vance, Swanston, & Curtis, 2011; Pellegrini et al., 2018; Pellegrini, Hoffmann, & Franco, 2014; Reich, Peterson, Wedin, & Wrage, 2001; Wan, Hui, & Luo, 2001). However, in many fire-prone ecosystems, such as savannas, a major amount of soil and root C is stored in deeper layers (Jackson et al., 2000; Jobbagy & Jackson, 2000; Jobbágy & Jackson, 2001). Consequently, even a minor relative effect of fire on the deeper C pool could result in a large absolute change in total soil C stocks; but such effects depend on several factors such as changes in plant biomass and composition. Addressing these uncertainties is critical given the dramatic changes in fire activity across the globe, especially in savannas, which are some of the most frequently burned ecosystems and contribute the majority of fire-driven greenhouse gas emissions to the atmosphere (Andela et al., 2017).

Fire-driven changes in both above-ground and especially below-ground C inputs could lead to shifts in deep soil C. Combustion losses of above-ground C (Kauffman, Cummings, & Ward, 1994) and long-term declines in plant biomass and leaf litter production (Peterson & Reich, 2001; Wardle, Hörnberg, Zackrisson, Kalela-Brundin, & Coomes, 2003) can decrease above-ground C inputs, and also limit vertical leaching of dissolved C (Shibata, Petrone, Hinzman, & Boone, 2003) that forms a source of mineral-associated C in deeper soil (Kalbitz & Kaiser, 2008). Changes in below-ground inputs via root biomass could also have more direct but highly variable effects on deep soil C. For example, fire can increase root biomass (Johnson & Matchett, 2001), decrease it slightly (Pellegrini et al., 2014) or leave total biomass unchanged (Oliveras et al., 2013). Past studies have illustrated that changes in root biomass across gradients in tree cover positively correlated with the changes in soil C (Jackson et al., 2002). Consequently, the degree to which fire changes root biomass may contribute to whether frequent burning reduces deep soil C.

Fire effects on plant community composition, especially the relative cover of grasses and trees, is a potentially important factor determining the changes in root biomass and productivity as well as soil C. Frequent burning tends to promote the colonization of productive and deep-rooted shrubs and grasses (Brockway & Lewis, 1997), in some cases offsetting the losses of tree root biomass (Coetsee, Bond, & February, 2010). Past studies have found that deep roots and large allocation of biomass below-ground in grasses can sustain C inputs despite a reduction in above-ground tree biomass (Jackson et al., 2002). However, frequent burning can shift the distribution of roots and increase the relative biomass in shallower soil layers (Oliveras et al., 2013). Consequently, changes in the amount and vertical distribution of root biomass may be important in explaining the depths over which soil C pools change.

Whether fire-driven reductions in soil organic matter pools throughout the entire horizon are due to lower plant productivity and inputs can be partially evaluated using soil $\delta^{13}C$, spatially stratified measurements and soil C:N ratios. Changes in $\delta^{13}C$ can be used to evaluate the role of shifts in inputs resulting from tree biomass losses and C_4 grass biomass increases: higher inputs from C_4 grasses (high $\delta^{13}C$) relative to C_3 tree (low $\delta^{13}C$) inputs can increase soil $\delta^{13}C$ (Jackson et al., 2002; Lloyd et al., 2008; Tieszen, Senyimba, Imbamba, & Troughton, 1979), helping to identify how changes in soil C depend on shifts in grass versus tree biomass inputs. Furthermore, in savanna systems, trees can have localized effects on soils under their canopy (e.g. higher soil C), often assumed to result from higher litterfall inputs, and localized effects can sum to be significant at the landscape scale (Holdo, Mack, & Arnold, 2012). Soil C:N can provide another line of evidence of shifting inputs because new plant biomass inputs in savannas tend to have higher C:N than microbial biomass and residual soil organic matter (C:N ratios for grass leaf litter: 141, tree leaf litter: 55, microbial biomass: 6.0, soil organic matter: 16, all values sampled in the same ecosystem, Norris, 2008; Norris & Reich, 2009). As organic matter becomes increasingly processed by microbes, it is expected that the C:N ratio becomes more similar to microbial biomass. Consequently, the C:N ratio might be an indicator

of whether below-ground biomass inputs might be changing—with lower inputs reflected in lower C:N ratios, and higher inputs reflected in higher C:N ratios.

In addition to considering changes in C stocks, changes in soil nutrients essential for plant and microbial growth can also be important for determining the response of C to repeated burning. Low soil nitrogen (N) can limit plant productivity, especially in fire-prone savannas (LeBauer & Treseder, 2008; Norris, Avis, Reich, & Hobbie, 2013), and is often reduced (in both total and inorganic forms) by repeated burning (Dijkstra, Wrage, Hobbie, & Reich, 2006; Pellegrini et al., 2018; Reich et al., 2001). Consequently, the degree of N loss with repeated fires may regulate ecosystem C recovery by limiting primary productivity (Reich et al., 2001) and production of biomass that may serve as an input to soil pools (e.g. litter and roots). However, N losses may also stimulate root production (Johnson & Matchett, 2001) and/or lead to greater allocation to roots in deeper soil (Mueller, Tilman, Fornara, & Hobbie, 2013).

Other nutrients, such as calcium (Ca) and phosphorus (P), are also essential for plant growth and microbial decomposition (Fay et al., 2015; Hobbie et al., 2006), but may respond to fire in fundamentally different ways because they can be resupplied from weathering (Vitousek, Porder, Houlton, & Chadwick, 2010) and their high oxidation temperatures reduce their potential to be lost in a fire (Grier, 1975). Subsequently, Ca and P concentrations tend to increase in residual ash and the surface soil after a fire (Gray & Dighton, 2006; Grier, 1975; McKee, 1982; Raison, Khanna, & Woods, 1985), and become enriched in the biomass of regenerating vegetation (Reich, Abrams, Ellsworth, Kruger, & Tabone, 1990). However, whether Ca and P become depleted by leaching or erosion as fires recur for several decades remains unclear (DeBano & Conrad, 1978; Pellegrini et al., 2018; Pivello et al., 2010).

Here, we evaluated a 51-year-long fire frequency manipulation experiment to test how altered fire frequencies changed soil C and nutrients throughout a 1-m soil profile in a temperate oak woodland ecosystem spanning a large gradient in grass and tree cover, from open oak savanna in a C_4 grass matrix in frequently burned sites to closed-canopy oak forest in unburned sites. Specifically, we tested the hypotheses that (a) repeated burning reduces soil C via reductions in the inputs of plant biomass and that losses of C primarily occur in the top soil; (b) increases in grass biomass result in greater allocation below-ground to roots, especially in deeper soil layers, which could increase C inputs to deeper layers; and (c) fire causes concurrent changes in soil nutrients, characterized by depletion of N but enrichment of Ca and P.

2 | MATERIALS AND METHODS

2.1 | Study site

Cedar Creek Ecosystem Science Reserve is a Long-Term Ecological Research Site in Minnesota, USA (latitude: 45.40°, longitude: −93.19°; spanning 22 km^2 and established in 1942), which is in the relatively northern part of the Midwest of the USA, c. 440 km from the Canadian Border. Cedar Creek contains a mosaic of prairie/grassland, savanna, woodland and forest ecosystems (Figure 1). Terrain of the area is relatively flat, with a total relief of less than 10 m. Soils are fine to medium sands (<3% clay) largely derived from glacial outwash and are excessively drained; major soil associations include the Sartell and Zimmerman soil series (Grigal, Chamberlain, Finney, Wroblewski, & Gross, 1974). Parent material is dominated by quartzite, as well as other weatherable material including amphibole, feldspar and pyroxene. Cedar Creek has received 780 mm/year in precipitation and experienced a mean annual temperature of 6.72°C on average from 1987 to 2016 (weather station in the reserve).

In May 2016 we sampled 12 plots from a fire frequency manipulation experiment that was established in 1964, where plots several hectares in size separated by fire breaks were burned during the spring (ranging from April to May depending on conditions) at different frequencies ranging from every 3 of 4 years to not at all (Figure 1; Peterson & Reich, 2001). Historically, fires are thought to occur every 1–3 years in open oak savannas, but have varied substantially across the region over the past century (Grimm, 1984; Leys, Griffin, Larson, & McLauchlan, 2019). Within Cedar Creek, it is likely that fires recurred intermittently every 1–3 years until ≈1920 when fire exclusion policies almost completely stopped fire for ≈40 years until the establishment of the experiment. Burns are conducted in the spring when fire intensities are low (<1 m flame heights), although it is likely fire intensities are highest in the savanna plots given the increase in herbaceous biomass fuel and its relationship with fire intensity at Cedar Creek (Wragg, Mielke, & Tilman, 2018). Analyses of aerial photographs from the 1930s to 1960s demonstrated that the landscapes were all savanna or open oak woodlands at the onset of the experiment (ranging from 17% to 39% tree canopy cover with an extensive herbaceous layer; Faber-Langendoen & Davis, 1995). Comparisons of tree cover pre-experiment and after 20 years illustrated that although there were no apparent differences in tree cover among plots at the onset of the experiment, tree cover significantly increased in fire-protected plots but remained roughly unchanged in the most frequently burned plots for the first two decades (Faber-Langendoen & Davis, 1995) and declined modestly thereafter in the most frequently burned plots (Peterson & Reich, 2001, P. Reich, unpublished data). Thus, fire frequency was well-correlated (r^2 = .54) with tree canopy cover c. 30 years after the onset of the experiment (Reich et al., 2001). Consequently, heterogeneity in vegetation structure is mainly a result of fire treatments rather than underlying edaphic and/or historical land use variability among plots.

More specifically, measurements made in 2010 (Cavender-Bares & Reich, 2012 and unpublished data) determined that unburned plots were mostly closed-canopy forest dominated by *Quercus ellipsoidalis* (trees >1.5 m in height; basal area: 16–29 m^2/ha; abundance: 472–1,042 indiv./ha), triennial burning resulted in an open savanna maintaining substantial tree and shrub cover of *Corylus americana* and *Q. ellipsoidalis* resprouts (basal area: 13 m^2/ha; abundance: 168–184 indiv./ha), and the most frequently burned plots (3 of every 4 years) consisted of extensive grassy areas with few large trees
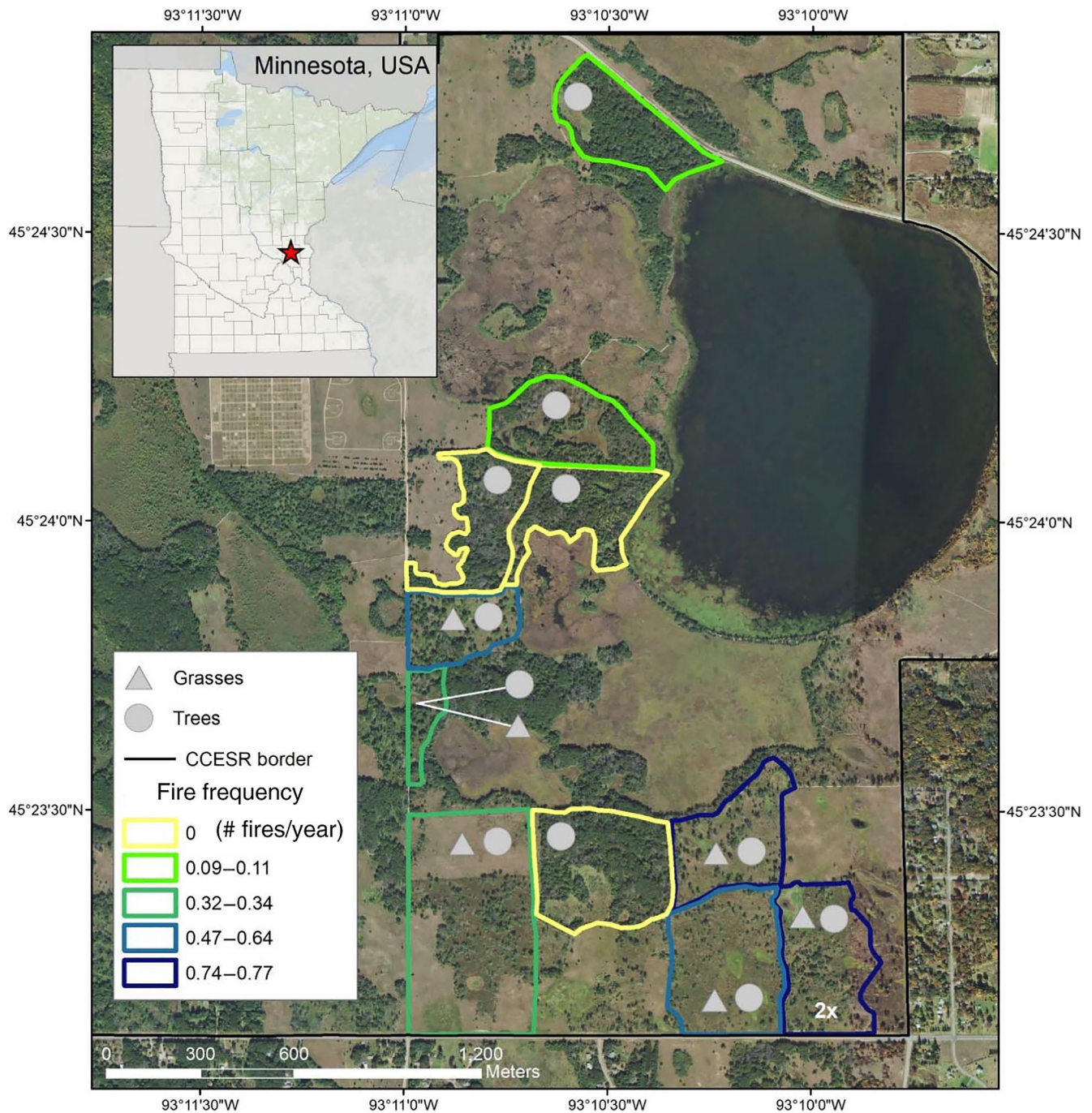
FIGURE 1 Aerial imagery (taken in 2017) of fire plots across the Cedar Creek reserve from the National Agriculture Imagery Program from the Farm Service Agency. Plots are outlined with a colour corresponding to their fire frequencies expressed in terms of number of fires per year (e.g. 0.33 is one fire every 3 years). Shapes within the plots indicate whether soil samples were taken from under grasses (triangles) or trees (circles) and do not represent actual sampling locations. The '2×' indicates that there were two plots within a single burn unit located in different areas of the unit. Within each plot and vegetation type, $n = 6$ for soil cores for the 0–5, 5–10 and 10–20 cm layers, and $n = 1$ for the 20–60 and 60–100 cm layers (Table 1). Consequently, in a plot with both grasses and trees, there are 12 sampling points (six under trees and six under grasses) at the 0–5, 5–10 and 10–20 cm layers, and two sampling points at the 20–60 and 60–100 cm layers

dominated by *Q. macrocarpa* (basal area: 5.3–5.9 m^2/ha; abundance: 80–85 indiv./ha) that are largely fire resistant (consistent with previous measurements from 1995; Peterson & Reich, 2001). Tree species in the frequently burned plots tend to have thicker leaves, lower foliar N content and higher N resorption with generally lower litter decomposition rates than tree species in the less frequently burned plots

(Cavender-Bares & Reich, 2012; Norris & Reich, 2009). Both of the oak species are ectomycorrhizal (Dickie, Dentinger, Avis, McLaughlin, & Reich, 2009).

In the savanna plots, the understorey and grassy areas between trees contain an extensive C$_4$ grass layer, with *Andropogon gerardii*, *Schizachyrium scoparium* and *Sorghastrum nutans* as the dominant

C_4 species (Peterson & Reich, 2007). Surveys conducted every 5 years from 1985 to 2015 have demonstrated that in the savanna plots, C_4 grasses cover 38% of ground area whereas C_3 grasses cover 17% (P. Reich, unpublished data), which is largely consistent with previous surveys of the herbaceous community (Peterson & Reich, 2007).

2.2 | Field sampling and processing of soils

We sampled soils across 12 plots, which we defined as a landscape unit that received a single fire frequency treatment (Figure 1), that cover a range of fire frequencies with the end members being plots burning every 3 of 4 years to not at all. Sampling was performed in a hierarchical manner such that within a plot, soil cores were collected in patches under different vegetation types: under six different tree patches and, when extant, six different grass patches that were outside tree canopies. Underneath each tree or grassy patch, the litter layer was removed, and mineral soils were sampled at 0–5, 5–10 and 10–20 cm increments (total of 114 patches with these depths); depths from 20–60 to 60–100 cm were also sampled but only underneath one tree and one grass patch within each plot (total of 19 patches with these depths). In five of the plots with the lowest fire frequencies (three unburned and two burned roughly once every 10 years), forests had developed over much of the plot area (Figure 2a), leaving little grassy area away from tree canopies; consequently, we only sampled soils underneath trees in the forested plots (Figure 1, Table 1). To avoid confounding effects of different tree sizes in the different fire frequency plots, we focused on sampling underneath large trees of approximately equivalent size in each plot.

All samples were passed through a 2 mm sieve and either (a) dried at 110°C to calculate gravimetric soil moisture, (b) air-dried to constant weight before analysis for total elemental concentrations or (c) extracted for inorganic nitrogen (N; see below).

2.3 | Laboratory analysis

Total C and N and $\delta^{13}C$ of the air-dried samples were measured using standard techniques (Nelson, Hu, Tian, Stefanova, & Brown, 2004) on a Carlo Erba NC2500 elemental analyser (CE Instruments) interfaced with a ThermoFinnigan Delta V+ isotope ratio mass spectrometer (ThermoFisher Scientific) at the Central Appalachians Stable Isotope Facility located at the University of Maryland Center for Environmental Science Appalachian Laboratory, Frostburg, Maryland, USA. The $\delta^{13}C$ data were normalized to the VPDB (Vienna Pee Dee Belemnite) scale using normalization curves with laboratory standards calibrated against USGS40 and USGS41. The long-term analytical precision of an internal soil standard that was analysed alongside the samples is 0.3‰ for $\delta^{13}C$. The %C and %N of each sample were calculated using atropine standards.

Concentrations of Ca and P were measured using wavelength-dispersive XRF spectrometry with a Bruker Tracer 5i handheld X-ray fluorescence analyser (Bruker/Tracer 5i) on dried soil samples using the 'Soil Nutrients' calibration module. XRF-based measurements for this method are well-correlated ($r^2 > .95$ for Ca and P) with concentrations determined via ICP-MS (Pompeani, McLauchlan, Chileen, Wolf, & Higuera, 2018); repeatability of this method is high, with a 2% analytical error on lacustrine sedimentary samples (K. McLauchlan, unpublished data).
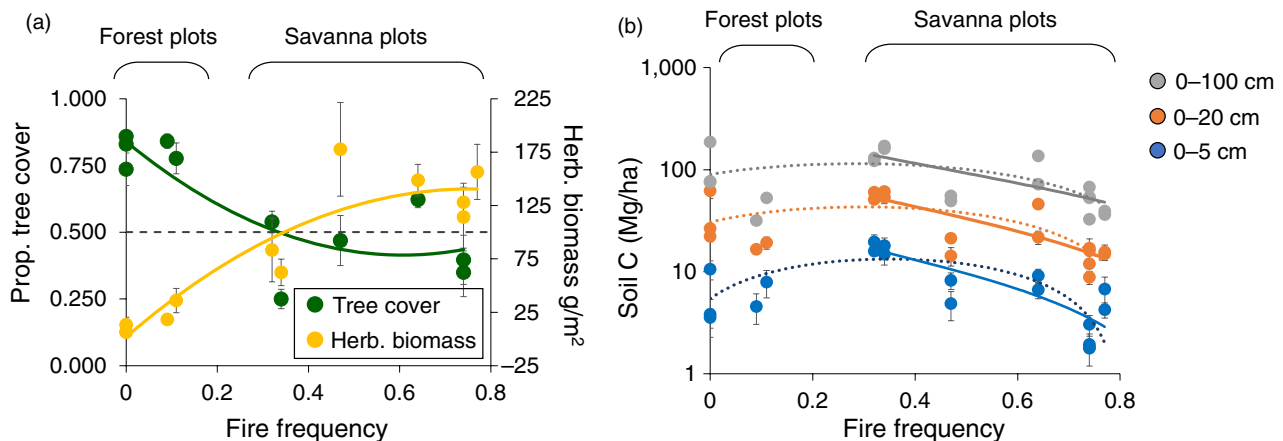
FIGURE 2 Frequent burning created an ecosystem type transition between savanna and forest, with nonlinear effects on soil carbon (C) stocks. (a) Tree cover expressed as a proportion of land area on the primary axis and herbaceous biomass on the secondary axis. Dashed line illustrates 50% tree cover which is the threshold between forest and savanna plots. Each point is an average within the plot ($n = 12$ plots) with standard errors across sampling points ($n = 12$ for canopy cover and $n = 8$ for herbaceous biomass) within plots (data are in Table S2). (b) Regressions between fire frequency and soil C stocks (on a log scale) in three different depth layers. Dashed lines indicate the polynomial regression across the entire fire frequency gradient, solid line is the linear regression only within the savanna plots, delineated by a fire frequency >0.30. Models include a vegetation patch effect and interaction term with frequency. Statistics for vegetation trends are in the text and those for soil C are in Table 1 and Table S2. Fire frequency is expressed as number of fires/year

TABLE 1 Study design and vegetation structure across the replicate plots giving the fire frequencies, vegetation types sampled (T = tree, G = grass), the number of location points a soil core was taken and the total number of soil samples at the different points for the depths

Plot	Fire frequency	Veg types sampled	Soil points	Total soil samples 0–5, 5–10 and 10–20	Total soil samples 20–60 and 60–100	Herbaceous biomass	Tree cover	SEM herb	SEM TC
110	0	T	6	18	2	6.4	0.859	2.5	0.023
209	0	T	6	18	2	13.4	0.736	7.0	0.061
301	0	T	6	18	2	6.8	0.830	2.8	0.006
113	0.09	T	6	18	2	18.2	0.841	3.4	0.027
111	0.11	T	6	18	2	36.0	0.776	11.4	0.058
107	0.32	T&G	12	36	4	83.4	0.538	29.8	0.041
108	0.34	T&G	12	36	4	62.4	0.250	12.3	0.036
105	0.47	T&G	12	36	4	177.6	0.469	43.9	0.094
106	0.64	T&G	12	36	4	148.7	0.622	14.8	0.030
104 N	0.74	T&G	12	36	4	114.2	0.397	31.6	0.092
104 S	0.74	T&G	12	36	4	128.0	0.350	14.1	0.091
103	0.77	T&G	12	36	4	156.5	0.454	25.7	0.104

Note: Fire frequency is expressed as # fires/year. Vegetation structure is broken down into herbaceous biomass (g/m^2) and tree cover (TC) expressed as the proportion of plot area where light is blocked by tree leaf area. Errors given are standard errors (*SEM*), based off subsamples within each plot (*n* = 12 for tree cover sampled in 2010, *n* = 8 for herbaceous biomass averaged across annual samples from 1999 to 2002).

Soil inorganic N (IN) was extracted within 48 hr of collection by shaking 10 g fresh soil in 50 ml of 1 M KCl for 1 hr, followed by filtering through pre-leached Whatman 41 filters, standard for analyses of IN (Reich et al., 2001). The extract was analysed for ammonium (indophenol blue method) and nitrate and nitrite (cadmium reduction method) on an automated spectrophotometer WestCo SmartChem 200 (Westco) discrete analyser at Stanford University, Stanford, California, USA.

2.4 | Root biomass sampling

We incorporated previously unpublished data on root biomass that have been collected periodically since 1999. Root biomass was sampled annually from 1999 to 2007 and then again in 2010 at eight fixed points within the plots using a 5.08 cm diameter PVC pipe. Cores were taken at two different depths: 0–20 and 20–40 cm. The soil from each single depth was washed and the roots sorted according to fine roots (<1 mm), coarse roots (>1 mm) and crowns. In the cases where part of a root was classified as coarse and part of it was classified as fine, the root was broken in two parts. Crowns were the points at or below the soil surface where the root joins the stem; crowns were generally characterized by (a) having several roots radiating out (the radiating roots were cut off and classified as either coarse or fine) from a small area and/or (b) being connected to above-ground biomass (anything green is removed). Roots were dried at 40°C and weighed. Here, we analysed only coarse and fine root biomass. We incorporated year as a random effect for the statistical analyses (see below). Excavating roots had minimal effects on plot integrity given the large size of these plots (several hectares) and the small area sampled by the cores.

2.5 | Tree cover and herbaceous biomass surveys

To delineate the plots as being either forest or woodland/savanna (hereafter called savanna), based on the relative amount of tree versus grass cover, we analysed previously collected data on tree canopy cover (rather than basal area or abundance), using a 50% tree cover threshold, which has been used to delineate savanna from forest (Staver, Archibald, & Levin, 2011). Given the high variability in leaf area allometries for the tree species in the plots that could be applied to basal area and abundance data, we utilize direct measurements of leaf area cover rather than another measure of woody plant population size. Tree canopy cover was assessed in 2010, which is the closest to our soil sampling date, at 12 sample points within each plot in the same area where herbaceous biomass was sampled. Using a pair of LICOR LAI-2000 sensors, eight sensor readings were taken at each sample point using a 270° lens cap with the sensor placed above the tall shrub layer, which was compared to a corresponding reading from an 'above' sensor placed in an open field without any tree canopy coverage, and the average light penetration was used to calculate tree cover within each plot (*n* = 12 sample points in each of the 12 plots).

To test how fire changed potential organic matter inputs to soil via the herbaceous layer, we utilized surveys of grass cover and herbaceous biomass. Herbaceous and shrub biomass were measured annually from 1999 to 2002. All vegetation except for large trees were clipped a centimetre above the soil within a 0.25 m^2 area that was replicated eight times across each plot to produce a plot-level

average (*n* = 8 clipping points in each of the 12 plots). The biomass was sorted into herbaceous and shrub species, dried and weighed. In this study we analysed the herbaceous biomass.

We also analysed the percent cover of grasses from a survey conducted in 2015 (a continuation of surveys conducted every 5 years since 1985) to verify that the relationship between fire and grass cover did not qualitatively change from c. 2000 to 2015. We verified cover was a representative metric via a regression from data in 2000 when cover and biomass were both measured (r^2 = .84, $F_{1,9}$ = 54.48, p < .0001).

2.6 | Statistical analyses

Because of the hierarchical sampling design (multiple depths within a patch and multiple patches within a plot), we used mixed effects models to test for significant effects of fire, vegetation type (samples taken under vs. away from a tree canopy) and their interactions on soil variables. Models were constructed such that sampling patches (i.e. individual tree or grass patch within a plot) were nested within replicate plots that were modelled as random intercepts (package LME4, Bates, Mächler, Bolker, & Walker, 2015). For example,

$$\text{soil C in } 0-5\,\text{cm} \sim \text{fire frequency} + \text{vegetation type} + (1|\text{replicate plot}).$$

All analyses were performed in R (R Development Core Team, 2010).

Fire frequency was modelled as a continuous variable. When testing for significant effects of fire, vegetation type and depth, we performed model selection using a threshold AIC value of 2 to determine variable inclusion. We then performed an ANOVA using Satterthwaite's degrees of freedom to test for significance (package LMERTEST, Kuznetsova, Brockhoff, & Christensen, 2017). Total N was not detectable in the 60–100 cm samples in two of the high fire frequency plots and thus evaluation of fire effects on N at this depth is limited.

In the plots under fire exclusion or burned every 10 years, there was relatively little area not underneath a tree canopy, and thus we only sampled underneath trees (Figure 1, Table 1). Consequently, we evaluated the potential vegetation type effect in models that only included the plots where both grass and trees co-occurred and were sampled, which had fire frequencies >0.30 (units are number of fires per-year) and were savannas (Figure 2). To analyse the effect of fire across the entire fire gradient, we performed our analyses on soils taken from underneath both grasses and trees, and disregarded the vegetation type effect because the trees and grasses did not co-occur on all the plots (where both grasses and trees did co-occur, their relative cover should be included in estimates of whole plot attributes if local effects are found to be significant). We distinguish these two analyses by referring to tests as conducted either only in savannas or across the entire fire gradient.

We tested for potential nonlinear relationships between fire and soil chemistry (as has been found previously in Reich et al., 2001) by comparing the AIC values of models with fire frequency fit as either a linear function or second order polynomial followed by an ANOVA

on the top model. We also evaluated the potential fit using a logarithmic function for the potentially saturating relationships (e.g. fire frequency and tree cover). We added a constant to all values (in this case +0.0001) in order to log-transform fire frequency of the unburned plots (frequency of 0 fires/year). The logarithmic curve tended to be a poorer fit than the polynomial, largely because it overestimated the rate of change in the lower fire frequency plots.

We also tested for how soils varied across different depths, and whether the effects of vegetation and fire varied across soil depth. Because the 20–60 and 60–100 cm increments were only measured in one patch within five of the plots that did not contain grass patches, tests that evaluated the effect of fire or vegetation type within these layers could not use a mixed effects structure; in these cases, models were fit as either a linear function or second order polynomial and were evaluated for significance using an ANOVA.

Total stocks of elements in the soil were calculated using bulk-density values that were averaged across the six sampling points taken within a vegetation type within a plot. For bulk density measurements, coarse roots were removed, but fine roots were not, which likely has indistinguishable effects on bulk-density calculations given that fine root biomass is a marginal fraction (0.1%) of total soil sample mass (determined via destructive harvesting). In order to account for the variability in the 0–20 cm samples in our calculations of soil C in the full 1 m, we added the C stocks from the 20–100 cm layers to all the separate unique measurements on cores from 0 to 20 cm because cores taken from 20 to 60 and 60 to 100 cm were collected from only one point in each vegetation type in each plot. We then repeated the statistical analyses of testing fire and vegetation type effects on C. We tested for how the magnitude of fire's effects on stocks changed with depth by (a) testing for depth–fire interactions in the statistical models and (b) cumulatively adding the stocks within layers and repeating the statistical tests of fire effects.

We used δ^{13}C to infer the contribution of C_3 versus C_4 plant biomass inputs to soil organic matter, a common approach in savannas (Boutton, Archer, Midwood, Zitzer, & Bol, 1998; Tieszen et al., 1979). This indicator is based on the observation that C_3 species discriminate more strongly than C_4 species against δ^{13}C during photosynthesis (Farquhar, 1983), and that the isotopic fractionation during decomposition is minimal relative to the isotopic differences between tissue types (Wedin, Tieszen, Dewey, & and, 1995). Previous studies in the Cedar Creek region have used δ^{13}C values of –27‰ for C_3 and –13‰ for C_4 biomass (Nelson et al., 2004) to reconstruct plant species composition in sediments. Although C_3 grasses occur at Cedar Creek, analyses of herbaceous community composition surveys conducted every 5 years from 1985 to 2015 illustrated that they are in lower abundance than C_4 grasses (percent cover of 17% and 38% respectively; P. Reich, unpublished data). As an additional test that soil δ^{13}C is indicative of C_4 biomass inputs, we compared standing C_4 biomass (calculated by weighting harvested biomass by the percent cover of C_4 species) and our measurements of soil δ^{13}C in the plots.

Effects of fire on root biomass were analysed using mixed effects models on root biomass averaged across individual points within a replicate plot with year sampled as a random intercept.

3 | RESULTS

3.1 | Frequent burning reduced tree cover and increased herbaceous biomass

Fire frequency treatments created a gradient in vegetation structure across the plots, with tree cover declining from 81% in the unburned plots to 40% in the plots burned most frequently (3 of 4 years or frequency ≈0.75 fires/year; Figure 2a, Table 1). The relationship was nonlinear, such that plots burned at frequencies >0.30 were savanna (i.e. <50% tree cover), and those burned at frequencies <0.11 were forest (there were no plots with treatments in between these two frequencies; models fit to the replicate plot means, $n = 12$, polynomial model fit displayed in figure: $r^2 = .69$, exponential model fit, not shown: $r^2 = .49$, logarithmic model fit: $r^2 = .49$; Figure 2a, Table 1).

Herbaceous biomass followed the opposite trend of tree cover, increasing from 8.9 g/m^2 in the unburned plots to 133 g/m^2 in the plots burned most frequently (Figure 2a, Table 1). The effect of fire frequency on herbaceous biomass was nonlinear, such that biomass increased with fire frequency at low burn frequencies but then decreased in the most frequently burned plots (polynomial model fit: $r^2 = .86$, logarithmic model fit: $r^2 = .59$; Figure 2a, Table 1). A more recent survey of the herbaceous community in 2015, which only measured percent cover (percent cover is highly correlated with biomass, $r^2 = .84$, $F_{1,9} = 54.48$, $p < .0001$), confirmed the biomass results: percent cover of grasses increased with more frequent burning ($F_{1,9} = 18.9$, $p = .002$, $r^2 = .64$) a trend that and did not change through time since 2000 ($F_{1,40} = 1.3$, $p = .26$, surveys in 2000, 2005, 2010 and 2015). Consequently, the trend that herbaceous cover and biomass increases with more frequent burning is robust to different measurements and sampling dates. Given these nonlinear trends, we evaluated the effects of fire frequency both across the entire fire gradient and within the savanna sites alone.

3.2 | Frequent burning depleted soil carbon throughout the soil profile

We first evaluated the uppermost soil depth with the expectation that it would show the strongest response to fire frequency. Across the entire fire frequency gradient, total soil C stocks in the top 0–5 cm were nonlinearly related to fire frequency (second order polynomial fit: $p = .005$, dotted regression in Figure 2b; Tables S1 and S2, logarithmic fit: $r^2 < .01$). For example, underneath trees, soil stocks were 6.0 Mg C/ha in unburned forests and 3.4 Mg C/ha in the most frequently burned savannas (fire frequency ≈0.75 fires/year), but peaked at 15.3 Mg C/ha in savanna plots that burned with a frequency of ≈0.30 fires/year, an intermediate fire frequency, which was also the point where forest transitioned into savanna (Figure 2a; Table S1). Within the savanna plots (fire frequencies >0.30 fires/year), more frequent burning caused a strong monotonic decline in soil C stocks (regression including both grass and tree patches, solid line in Figure 2b; Tables S1 and S2).

In deeper soil, frequent burning reduced total soil C stocks throughout the entire soil profile within savannas. First, there was no depth-by-fire interaction ($p > .5$) when depth, vegetation type and fire were included in a model; in other words, fire effects on total soil C were statistically indistinguishable across the different soil layers (Figure 2b). Furthermore, fire effects on soil C in the deep (>20 cm) soil layers were important contributors to the magnitude of changes in C stocks (Figure 2b). A comparison between unburned and most frequently burned plots (defined by frequency ≈0.75 fires/year) illustrated that unburned plots had 22.8 Mg C/ha (+163%) more C in the top 0–20 cm than the most frequently burned plots (values averaged underneath grasses and trees in frequently burned plots; tree canopies only differed from grasses when %C was analysed (not total C stocks) in the top 0–5 cm and thus we do not stratify our analyses, Figure 3a; Table S2). The difference in absolute magnitude of soil C stocks nearly
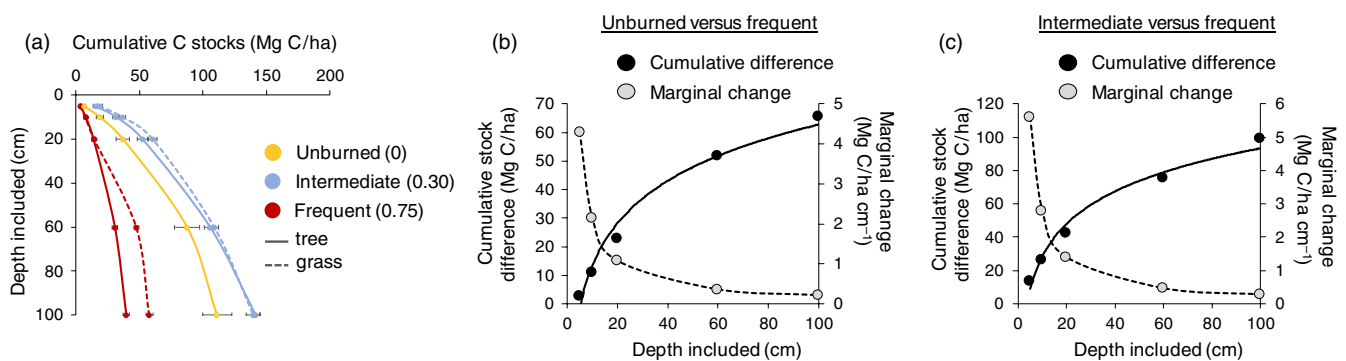
FIGURE 3 Fire-depleted total soil carbon (C) across all depth increments in unburned versus frequently (≈0.75 fires/year) burned plots and intermediate (≈0.30 fires/year) versus frequently (≈0.75 fires/year) burned plots. (a) Cumulative stock changes averaged within fire treatments. Error bars represent the standard error of the mean across the different fire frequency categories ($n = 3$ for frequent, $n = 2$ for intermediate, $n = 3$ for unburned) and the dashed versus solid lines delineate soils underneath grasses versus trees respectively (data in Table S2). Panels (b) and (c): Cumulative difference in total soil C stocks between the (b) unburned versus frequently burned plots and (c) intermediately versus frequently burned plots with increasing soil depth (primary axis, solid line with black circles). The marginal change in soil C stock differences with each additional cm depth sampled is represented on the secondary axis (dashed line with grey circles) which is the derivative of the curve on the primary axis. (b) and (c) have fewer points because they represent the differences between means of two different pairs of plots: unburned versus frequent (mean of $n = 3$ in both) and intermediate ($n = 2$) versus frequent ($n = 3$)

tripled to +65.8 Mg C/ha (+140%) when the full 0–100 cm profile was considered (Figure 3a; Tables S2). Consequently, the change in soil C below the top 20 cm was a key contributor to the total changes in soil C stocks. We estimated that increased fire frequencies from exclusion to ≈0.75 fires/year produced soil C declines to 100 cm depth of 1.22 Mg C ha^{-1} year^{-1} when changes were averaged over the entire 51 years.

The observed peak of soil C stocks in the plots burned at intermediate frequencies (defined by frequency ≈0.30 fires/year) illustrated that both intermediate and high frequencies of burning had large but divergent effects on soil C within savannas (Figure 3). A comparison between savanna plots burned at intermediate frequencies (≈0.30 fires/year) with those burned most frequently (≈0.75 fires/year) indicated that intermediately burned plots had 42.3 Mg C/ha (+301%) more C in the top 0–20 cm than the most frequently burned plots (values averaged underneath grasses and trees; Figure 3a). The difference in soil C stocks more than doubled to +98.5 Mg C/ha (+210%) when the full 0–100 cm profile was considered (Figure 3a; Table S2). We estimated that increased fire frequencies from ≈0.30 to ≈0.75 fires/year produced soil C declines to 100 cm depth of 1.88 Mg C ha^{-1} year^{-1} when changes were averaged over the length of the experiment (51 years).

Despite significant effects of fire on absolute soil C stocks at all soil depths, the differences between fire treatments became less apparent when the deeper soil layers were considered. For example, the plots burned at intermediate frequency had on average 35% more C in the top 0–20 cm but 21% more C in the full 1 m than the unburned plots. We quantified marginal (per-cm) changes in soil C stocks to evaluate how soil C changed as a function of depth while controlling for differences in sampling intervals among depths. The decline in relative changes was determined by the logarithmic (and therefore saturating) relationship between the depth layers included and the cumulative stock difference between the fire frequency treatments (solid lines in Figure 3b,c). We fit a saturating curve because comparisons between the depth profiles for the different treatments (Figure 3a) suggest differences approach an asymptote. Analyses within the individual soil layers supported this trend because the effect of fire on soil C was largest in the top layers when the entire gradient was analysed (0–5 cm: $F_{2,13.3} = 8.7$, $p = .004$; 5–10 cm: $F_{2,13.3} = 7.2$, $p = .008$; 10–20 cm: $F_{2,13.8} = 8.6 = 0.004$; 20–60 cm: $F_{1,17} = 2.1$, $p = .16$; 60–100 cm: $F_{1,17} = 0.59$, $p = .45$; Figure S1). When savanna plots alone were analysed, we found significant effects of fire at all depths (0–5 cm: $F_{1,5} = 20.4$, $p = .006$; 5–10 cm: $F_{1,5} = 19.5$, $p = .007$; 10–20 cm: $F_{1,5} = 8.5$, $p = .033$; 20–60 cm: $F_{1,12} = 7.0$, $p = .021$; 60–100 cm: $F_{1,12} = 17.8$, $p = .001$; Figure S1). Given the asymptotic relationship in Figure 3b,c, the derivative of the logarithmic fit represents the marginal change in the difference between stocks with increasing sampling depth. These results illustrate that marginal changes in soil C were largest at the surface and declined 20-fold across the entire 100 cm profile, with declines in marginal soil C differences observed at 20 cm depth (dotted line in Figure 3b,c), due to both a reduction in fire effects as well as lower C concentrations (Table S3). Importantly, the differences between fire treatments continue to grow, albeit diminishingly, with increasing soil depth incorporated into the calculations (Figure 3b,c).

3.3 | Vegetation type only influenced soil carbon in the upper soil layers

Effects of tree cover and fire on soil δ^{13}C were limited to the upper soil layers, where δ^{13}C in soil below trees was lower than soil below grasses, and fire, on average, increased soil δ^{13}C values (Figure 4; Table S4). Several lines of evidence illustrated that fire and tree cover effects attenuated with depth. First, there was a
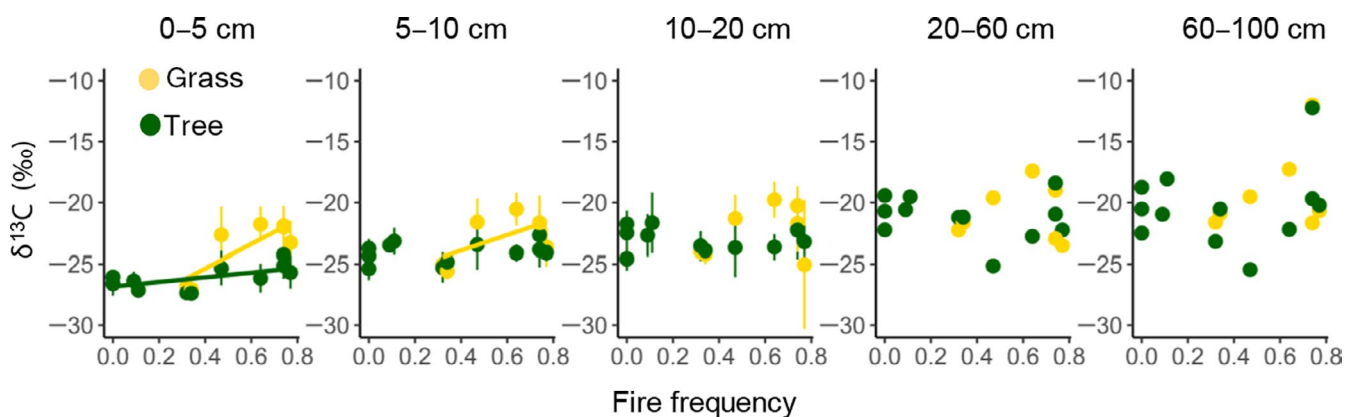
FIGURE 4 Effects of fire and grass patches on soil δ^{13}C in different depth layers. Panels indicate separate analyses across the depth layers, with each point representing the mean value underneath a vegetation type within a plot. Regressions were run independently for each vegetation type and only the significant relationships are displayed as solid lines. Statistics are given in Table S2 and the lines are plotted separately because of the significant fire-vegetation interaction effects. Regressions were determined via mixed effects models that incorporated within-site variability in soil cores for 0–5, 5–10 and 10–20 cm samples which are expressed as standard errors around the mean in the plots. We used linear models on the 20–60 and 60–100 cm depths because we only collected single samples underneath the vegetation types at these depths. Fire frequency is expressed as number of fires/year

depth–fire interaction effect across the entire fire gradient and in the savanna plots alone ($F_{4,219}$ = 2.5, p = .04 for frequencies 0–0.75 fires/year; and $F_{4,254}$ = 2.82, p = .026 for frequencies 0.30–0.75 fires/year respectively). Separate analyses within each layer illustrated that the depth–fire interaction was due to an attenuation of fire effects on $\delta^{13}C$ in the deeper soil layers (Figure 4; Table S4). Fire effects on $\delta^{13}C$ were only significant at depths of 0–5 cm underneath both grasses and trees and 5–10 cm underneath grasses (Figure 4; Table S4). Second, although there was no depth-vegetation type interaction within the savanna plots, analyses within layers illustrated that soil $\delta^{13}C$ underneath trees and grasses differed only in the top 0–5 cm layer: grass patches had 2.6‰ higher values (in the most frequently burned plots: soils under trees −25.0‰ vs. grasses −22.4‰). Consequently, $\delta^{13}C$ support the hypothesis that a greater amount of soil C is derived from C_4 grass biomass in the more frequently burned plots, but that the effect is only apparent in the uppermost layers (0–10 cm). Furthermore, the potential enrichment effect of tree canopies on soil C was only significant for C concentrations, not stocks, and was also constrained to the top 0–5 cm. Consequently, the localized effects of trees on soils were concentrated in the upper soil layer, and thus fire-driven changes in tree contributions to soils were not apparent in deep layers soils.

Although the presence of C_3 grasses limits our ability to use $\delta^{13}C$ to completely segregate grass versus tree inputs, the isotopic changes are likely to be an indicator of C_4 grass versus C_3 tree biomass inputs for three reasons. First, C_3 grasses and herbaceous biomass in general were almost entirely absent in the most fire-protected plots (c. 10% cover and <0.1 Mg/ha, Figure 2, Table 1, and P. Reich, unpublished data), suggesting the decline in $\delta^{13}C$ in the unburned plots were most likely due to increases in tree biomass inputs. Second, when we weighted above-ground herbaceous biomass by C_4 grass percent cover, we found a positive relationship between above-ground C_4 grass biomass and soil $\delta^{13}C$ (0–5 cm: $F_{1,10}$ = 12.2, p = .006; 5–10 cm: $F_{1,10}$ = 4.7, p = .055, not significant in other depths). Third, previously published data, discussed below, found that several C_3 grass species only allocate c. 1% of their rooting biomass >30 cm deep. Consequently, higher inputs from woody plants likely contributed to changes in soil C in the topsoil, but the

changes in the deeper layers were likely due to changes in biomass derived from both C_4 and C_3 plants.

The generally higher $\delta^{13}C$ in deeper soil (beneath both tree and grass patches) indicates a greater C_4 signature with depth (−24.9‰ in the top 0–5 cm and −20.1‰ in the bottom 60–100 cm), consistent with the low tree cover and high grass cover documented at the onset of the experiment (Faber-Langendoen & Davis, 1995).

3.4 | Declines in root biomass with repeated burning

Fire frequency changed root biomass, but whether biomass increased or decreased differed between the size of roots (fine vs. coarse) and the soil depth (0–20 cm or 20–40 cm). In the deeper layer (20–40 cm), total root biomass declined from 4.3 Mg/ha in the unburned plot to 3.7 Mg/ha in the intermediate burned plots to 2.4 Mg/ha in the most frequently burned plots. The primary cause of this decline was the loss of coarse root biomass, declining from 3.5 to 1.1 Mg/ha across the gradient ($F_{1,10.0}$ = 11.6, p = .007; Figure 5; Table S5). Fine root biomass in the 20–40 cm layer, in contrast, increased with fire frequency from 0.8 to 1.3 Mg/ha across the gradient ($F_{1,10.0}$ = 9.1, p = .013; Figure 5; Table S5).

In the 0–20 cm layer, fine root biomass was higher than in the 20–40 cm layer, and increased with more frequent burning ($F_{1,10}$ = 14.7, p = .003; Table S5). The gains in fine root biomass in the 0–20 cm layer, but losses of coarse root biomass in the deeper 20–40 cm layer, with more frequent burning resulted in a lower proportion of root biomass in the deeper layers (e.g. 38% in unburned vs. 25% in most frequently burned plots respectively, Table S5). In other words, fire caused larger relative losses of root biomass from the deeper (20–40 cm) than shallower depths (0–20 cm; Figure S2).

3.5 | Repeated burning reduced total and inorganic nitrogen

Across the savanna plots, frequent burning reduced total soil N (all layers significant except 0–5 cm where p = .09, Tables S6 and S7).

(a) 0–20 cm   (b) 20–40 cm
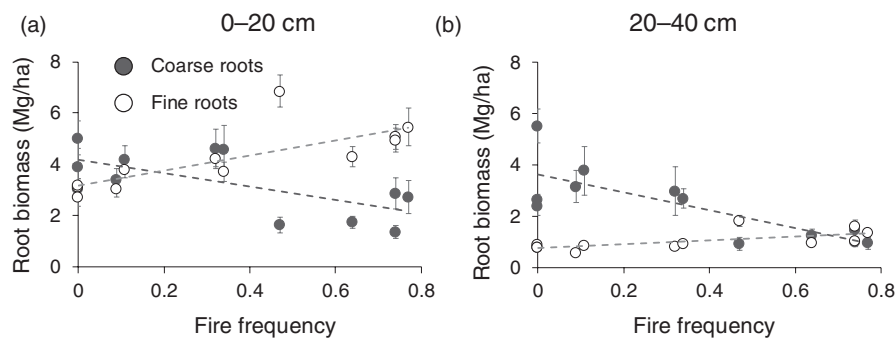
FIGURE 5 Root biomass partitioned between fine and coarse fractions across the fire frequency gradient in the 0–20 and 20–40 cm depth layers. Both dashed lines indicate significant trends (statistics in text). Root biomass was sampled only in the 0–20 and 20–40 cm layers at random throughout the plots. These error bars represent the standard errors within the plots (n = 8 cores per-year at 10 time periods within each of the 12 plots). Model included year and plot as random effects. Data are in Table S5
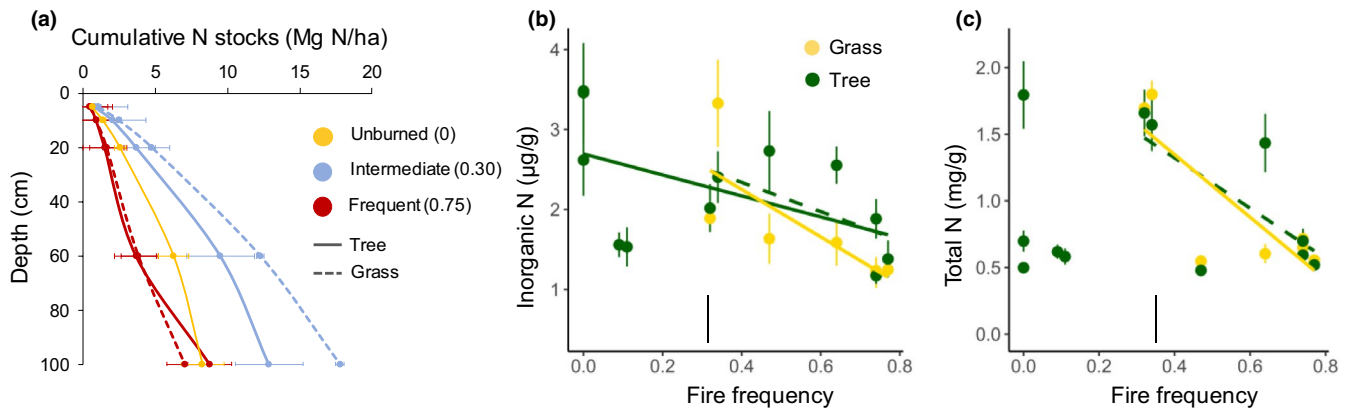
FIGURE 6 Total cumulative soil nitrogen (N) stocks with depth below the surface and the relationship between fire frequency (a) and inorganic nitrogen (b) and total N (c) concentrations in the top 0–20 cm layer. Lines fitted in (b) and (c) are significant relationships determined by using mixed effects models that consider trends either across the entire gradient (solid line) or within savannas alone (dashed lines, fire frequency >0.30). Vertical lines slightly above the x-axis indicate the transition from forest to savanna. Extractions were performed on cores sampled to 20 cm depth only, consequently we re-scaled the soil N values to that depth in panel c using bulk-density weighted averages to aggregate the 0–5 cm, 5–10 cm and 10–20 cm cores analysed for bulk N. Error bars are standard errors. For (a) this is across the replicate plots in the different fire levels (n = 3 for unburned, n = 2 for intermediate, n = 3 for frequent). For (b) and (c), these are the replicate soil samples in each plot within a vegetation type (n = 6 for total N; n = 5 for inorganic N except plot 105 which had n = 6)

Within the savannas, total N stocks in the top 20 cm of the soil were strongly affected by fire; for example, frequently burned plots had 1.8-fold less total soil N than the plots burned at intermediate frequency (2.3 Mg N/ha vs. 4.2 Mg N/ha respectively; Figure 6; Tables S6 and S7). When the entire soil profile was considered, the difference increased, with frequently burned plots having 2.6-fold lower total soil N stocks than infrequently burned plots (2.4 Mg N/ha vs. 6.1 Mg N/ha respectively; Figure 6; Tables S6 and S7). Comparisons between unburned forest plots versus frequently burned savanna plots yielded qualitatively similar results, albeit with a smaller magnitude: unburned plots had 1.6-fold higher total soil N stocks in the top 0–20 cm (2.6 Mg N/ha vs. 1.6 Mg N/ha) and 1.2-fold higher total soil N stocks in the 1 m sample (8.3 Mg N/ha vs. 7.9 Mg N/ha) than the frequently burned plots (Figure 6; Tables S6 and S7). However, when the entire fire frequency gradient was analysed, fire frequency did not have a clear effect on soil total N largely due to low soil N in forest plots burned at frequencies ≈0.10 (Figure 6c; Table S8).

Changes in soil inorganic N (IN) were mostly consistent with total soil N. The concentration of IN declined linearly with more frequent burning ($F_{1,4.7}$ = 13.7, p = .015), with similar fire effects underneath trees and grasses (Figure 6). However, unlike total N concentrations and stocks, which declined primarily from intermediate to frequently burned plots, the negative effect of fire frequency on IN was apparent across the entire fire frequency gradient (Figure 6).

Comparisons between soil C and N illustrated that fire shifted the balance between C and N storage. Frequent burning in savannas tended to reduce soil C:N (by mass) ratios when the entire depth profile was considered ($F_{1,56}$ = 4.7, p = .03); for example, in the savannas, soil C:N underneath trees declined from 20.4 (±3.8 SEM) in the intermediate burned plots to 10.8 (±1.6 SEM) in the most frequently burned plots in the top 0–5 cm (Figure S3). There was also a significant depth–fire interaction, such that the differences in C:N among

fire treatments declined with increasing soil depth, and the declines in soil C:N with depth were lowest in the most frequently burned plots ($F_{4,56}$ = 3.8, p = .008; Figure S3). Model selection demonstrated no significant effects of vegetation type on soil C:N in the full model (not included in the top model).

3.6 | Frequent fire increased phosphorus and calcium in the topsoil

Frequent burning increased the concentrations of Ca and P in the topsoil, but only when the entire fire frequency gradient was considered (Figure S4). The increase in P concentrations with more frequent burning was significant in the top 0–5 cm ($F_{1,7.7}$ = 25.0, p = .0012), and tended to be enriched in the 5–10 cm and 10–20 cm layers although these were not significant (p = .07 for both). Fire also increased Ca concentrations in the top 0–5 cm, 5–10 cm and 10–20 cm layers ($F_{2,6.4}$ = 8.7, p = .015; $F_{2,6.7}$ = 6.1, p = .031; $F_{1,7.2}$ = 9.8, p = .016 respectively). However, the increases in soil Ca and P were only evident when the entire gradient was analysed; across the savanna plots, there were little changes in soil Ca and P with increasing fire frequency from ≈0.30 to ≈0.75 fires/year. Consequently, fire increased Ca and P when burned plots were compared to unburned plots, but greater fire frequency within the burned plots did not continue to increase Ca and P (Figure S4).

4 | DISCUSSION

Repeated burning caused large changes in soil C, with more frequent burning within the savanna plots causing monotonic declines in soil C at all soil depths. Considering changes in total soil C in deeper soil

layers provided several insights into how fire affected soil C storage. Changes in total soil C in soils >20 cm deep (Figure 2), a depth often assumed to be relatively unresponsive to fires (Certini, 2005; Pellegrini et al., 2014; Wan et al., 2001), more than doubled our estimates of losses of soil C from frequent fire. Fire also caused total root biomass to decline by 43% in soil >20 cm deep, which was primarily due to a 68% loss of coarse root biomass, despite gains in fine root biomass. Contrastingly, total root biomass remained unchanged with fire in the upper soil layers because increases in fine root biomass compensated for losses of coarse root biomass with more frequent burning, illustrating that changes in root biomass alone cannot explain the entirety of soil C responses to fire at different depths.

Our finding that frequent burning-depleted soil C down to 1 m is, to our knowledge, the first observation that deep soil C is sensitive to multi-decadal changes in fire frequencies. Other studies of repeated burning in savannas rarely measured soils below 20 cm, limiting our understanding of patterns in deeper layers (Coetsee et al., 2010; Holdo et al., 2012; Kitchen, Blair, & Callaham, 2009; Pellegrini, Hedin, Staver, Govender, & Henry, 2015; Turner, Blair, Schartz, & Neel, 1997). Consequently, a recent meta-analysis of fire effects on soils only considered changes within the top 20 cm (Pellegrini et al., 2018), and therefore likely underestimated the already large reductions in soil C caused by fire in savannas. However, our observed losses of soil C from the deeper soil horizons contrast with a previous study in Brazilian tropical savanna-forest ecotone (receiving 1,400 mm/year in precipitation), which found that frequent burning for >50 years did not change soil C past 15 cm (sampled down to 100 cm) despite an order of magnitude change in vegetation biomass (Pellegrini et al., 2014). The differences between the two studies may be due to the low clay content in the Cedar Creek soils (3%) relative to the Brazilian soils (50%–75%) because low clay content can allow for more rapid turnover of organic matter (Oades, 1984) and potentially the manifestation of fire effects. Frequent burning for c. 50 years in a South African savanna also did not change soil C in deep soils despite having a low clay content, potentially because of the lack of change in root biomass (Coetsee et al., 2010).

The largest declines in soil C were found in the most frequently burned plots (c. 0.77 fires/year), which had 76% less total soil C to 1 m in depth than the plots burned at intermediate frequencies (c. 0.33 fires/year). We evaluated the sensitivity of deep soil C using two methods: (a) sequentially adding deeper soil layers and repeating the statistical comparisons of total soil C stocks across fire treatments and (b) comparing stocks within the individual soil sampling layers (e.g. 0–5 cm or the 20–60 cm layer). The sequential addition demonstrated that fire effects on absolute soil C stocks grew with increasing soil depths, but the relative effect tended to decline (Figure 3). The decline in relative change in deep soil C was likely in part due to lower soil C concentrations in deeper soil layers (e.g. 3.8% C in the top 0–5 cm vs. 0.45% C in the bottom 60–100 cm layer for the intermediate fire frequency plots), although the concurrent increase in bulk density with depth (0.95 g/cm^3 vs. 1.84 g/cm^3 in the 0–5 cm and 60–100 cm layers respectively) resulted in the deeper

soils still comprising a large C pool. Comparisons of stocks within individual sampling layers revealed that large variability in the deeper soil stocks in the forests resulted in no clear effect of fire on the 20–60 cm and 60–100 cm layers; contrastingly, the fire effects were very consistent within the savanna plots, which exhibited significant fire effects at all soil depths. Importantly, in the case of the forest comparison, although there were not continuous fire frequency effects, comparisons between the forests and most frequently burned plots (≈0.75 fires/year) illustrated that losses of soil C at all depths contributed to the losses of soil C (Figure 3).

4.1 | Fire alters rooting biomass, depth and plant–soil relationships

Lower root biomass in the frequently burned plots is consistent with the hypothesis that fire-driven reductions in below-ground inputs contribute to losses of deep soil C. Only coarse root biomass declined with repeated burning, but the large magnitude of declines in the 20–40 cm depth more than offset the gains in fine root biomass (Figure 5). It is unlikely that changes in the leaching of dissolved organic matter were responsible for deep soil C changes. Previous work in these systems found that dissolved N leaching losses were not increased by fire (Dijkstra et al., 2006), which suggests that C losses are also not increased by fire given the high correlation between dissolved organic N and C at Cedar Creek (F. Dijkstra, unpublished data). Consequently, we propose that lower inputs of root C into the deep soil C pools is the primary contributor to lower soil C stocks with increased burning although further work is necessary to evaluate the role of changes in decomposition.

Our results demonstrate that frequent burning reduced total root biomass in the soil, but the trends differed according to root size and soil depth. Fine root biomass was highest in the top 0–20 cm and increased with more frequent burning, supporting previous studies in other ecosystems (Johnson & Matchett, 2001; Oliveras et al., 2013). The gains in fine root biomass throughout the 0–40 cm profile were correlated with increasing above-ground grass cover and biomass despite losses of tree biomass and cover with more frequent burning. Our results support previous studies that found a shift from trees to grasses resulted in increased fine root biomass even in the deep layers (Jackson et al., 2002). In our study, the two dominant C$_4$ grasses that increased with fire were *A. gerardi* and *S. nutans* ($p < .0001$ for both, P. Reich, unpublished data). In another experiment at Cedar Creek without fire manipulation, both grass species allocated 29% and 28% of their fine root biomass deeper than 20 cm, amounting to 233 g/m^2 and 110 g/m^2 respectively. Moreover, *A. gerardi* and *S. nutans* allocated 86% and 81% of their total root biomass to fine roots. Consequently, increasing grass cover was the likely cause for gains in fine root biomass, even in the deep soil layers.

However, in our study, the fire-induced gains in fine root biomass in the deep soil did not compensate for the losses of coarse

root biomass. Losses of coarse root biomass potentially occurred because of losses of tree biomass, but further work is needed to distinguish the contributions of herbaceous versus woody species to root biomass. Total root biomass is only part of the equation connecting biomass stocks to soil C inputs given that turnover and decomposition rates of root biomass can vary substantially across herbaceous species (Fornara, Tilman, & Hobbie, 2009), potentially influencing soil C formation (Fornara & Tilman, 2008). Consequently, improved understanding of the physiological traits of the roots as well as interspecific root strategies could help improve our understanding of the role of fine versus coarse root biomass and herbaceous species composition in the formation of soil C across the fire frequency gradient.

Our observed changes in rooting depth were slightly less intuitive given theories arising from previous studies in the same ecosystem. For one, herbaceous species composition is thought to influence rooting depth. For example, some herbaceous legumes species in this ecosystem tend to allocate a greater amount of root biomass to deep soil (Mueller et al., 2013), leading to the hypothesis that the increased abundance of herbaceous legumes with more frequent burning observed previously (Peterson, Reich, Wrage, & Franklin, 2007) would increase relative biomass allocation to deeper layers. For example, *Lupinus perennis*, *Amorpha canescens* and *Lespedeza capitata*, all herbaceous legumes, allocate 79%, 78% and 64% of their total root biomass to depths >20 cm (P. Reich, unpublished data). Yet, we found evidence that frequently burned plots had a lower proportion of root biomass in the deeper soil (38% in unburned vs. 25% in most frequently burned plots), suggesting the colonization of herbaceous legumes could not drive the trends in root biomass. Moreover, the increases in relative root biomass in the topsoil despite losses of inorganic N disagree with previous studies in the same ecosystem that have proposed N losses stimulate greater rooting depths and biomass (Mueller et al., 2013). Finally, fire appears to have altered the relationships between plant species composition and soil C formation. For example, a different experiment at Cedar Creek, where fire frequency was not manipulated, demonstrated that the presence of C_4 grasses and forbs increased the rates of soil C formation (Fornara et al., 2009). Yet in our study, we observe the opposite trend. All these counter-intuitive results highlight the large effect that variability in fire regimes can have on plant–soil interactions despite similar underlying soil types, species pools and climates.

4.2 | Fire effects on plant biomass inputs differed according to depth and plant type

In contrast to fire effects on soil C, the localized effects of tree canopies leading to increased soil C were apparent only in the top 0–5 cm, and only for C concentrations, not total C stocks. Moreover, lower $\delta^{13}C$ underneath tree canopies, indicating a higher contribution of woody plant biomass to soil C, was only apparent in the top 0–10 cm (Figure 4; Table S4). Consequently, higher above-ground litter inputs

from woody plants (Reich et al., 2001) were likely responsible for the higher surface soil C pools under trees and in less frequently burned plots. In deeper layers, soils underneath trees did not have higher C or lower $\delta^{13}C$ than soils under grasses (Figure 4; Table S4), suggesting that the root inputs of the two vegetation types were more equivalent.

The lack of changes in $\delta^{13}C$ with more frequent burning in the deeper soils does not support our hypothesis that fire increases the contribution of C_4 grasses to deep soil C. However, our inferences about the relative contribution of grasses versus trees based on $\delta^{13}C$ are limited by the concurrent increase of C_3 grasses (Peterson et al., 2007) which share a similar $\delta^{13}C$ signature to trees; consequently, increases in $\delta^{13}C$ likely underestimate the grass contribution to soil C because C_3 grass inputs would deplete $\delta^{13}C$.

Moreover, certain C_3 herbaceous legumes, which also have lower $\delta^{13}C$, are deep-rooted. In our experiment, species of these herbaceous legumes that increased in relative cover with more frequent burning include *A. canescens* (p = .002) and *L. capitata* (p = .011), which allocate 79% and 64% of their total root biomass to depths >20 cm. Previous studies have demonstrated that leguminous forbs can contribute substantially to soil C (Fornara & Tilman, 2008; Mueller et al., 2013). Consequently, the low $\delta^{13}C$ measured in grass patches away from tree canopies (where tree inputs are presumably lower) or the lack of change in $\delta^{13}C$ in deep soil layers may partly be a result of inputs by C_3 herbaceous legumes. Furthermore, the lack of fire-driven changes in $\delta^{13}C$ in the deeper soil layers may be a result of herbaceous legumes compensating for lower C_3 tree inputs. Further work is needed to establish the potential compensatory effects different herbaceous species may have on soil C, such as identifying roots from individual species and/or isotopic labelling of plant biomass.

Lower soil C:N ratios in the frequently burned plots and deep soil layers provided insight into fire-driven shifts in inputs and losses (Figure S3). The decline in soil C:N ratios with more frequent burning is consistent with trends expected from a shift from ratios found in plant biomass (e.g. 48 in leaf litter and 56 in fine roots) towards ratios found in microbial biomass (e.g. 6) at these sites (Norris, 2008; Norris & Reich, 2009). Fire-driven changes in plant species composition could not explain these declines, because leaf litter C:N of grass species that dominate in frequently burned plots tends to be higher than that of leaf litter of trees (e.g. 141 vs. 48; Norris et al., 2013; Peterson et al., 2007), and there were no significant effects of vegetation type on soil C:N. Furthermore, analyses of root C:N demonstrate that fire actually increased C:N in root biomass across the plots (Reich et al., 2001), in contrast to the trends in the soils. Instead, the declines in soil C:N likely represent a fire-driven decrease in fresh plant inputs into C and N pools, causing a shift towards the C:N signature of microbial biomass and decomposed soil organic matter. Furthermore, depth–fire interactions demonstrate that soil depth diluted fire's effect on C:N ratios, consistent with the hypothesis that the inputs of roots with higher C:N ratios partially offset the declines in C:N due to lower inputs and/or that deeper C may be less responsive to fire because it turns over more slowly.

Repeated burning caused declines in both total and dissolved inorganic N within the savanna (Figure 6), suggesting that fire-driven N losses may influence both productivity and rooting depths (Mueller et al., 2013; Oliveras et al., 2013). In contrast, fire enriched the concentration of Ca and P in the soil, consistent with the higher retention of Ca and P relative to N after fire due to processes such as pyromineralization (Raison, 1979; Raison et al., 1985). The enrichment was evident only when comparing the unburned versus burned plots, however, and not when comparing the different fire frequencies within the burned plots in the savanna. This result suggests that the enrichment of Ca and P due to repeated burning is of a similar magnitude irrespective of fire frequency, which is different from the continuous decline in soil N with greater fire frequencies. Taken together, it is likely that in these temperate savanna-forest ecotones, repeated burning promotes N limitation. The sensitivities of soil Ca and P, which are often assumed to be determined by bottom-up constraints via rock weathering processes, suggest that fire is an important process modifying their availability to plants, illustrating the need for fire's effects to be included in global models of N and P (and eventually Ca) dynamics.

5 | CONCLUSIONS

By sampling one of the longest running fire frequency manipulation experiments, we demonstrated that changes in deep soil C were critical to determining the full effects of fire on total soil C. Whereas C inputs from trees only influenced surface soil C (0–10 cm), we detected significant effects of fire on soil C down to 1 m depth. Furthermore, changes in the deep soil C appeared to result from shifting inputs of root biomass within the deep layers, illustrating the need to consider processes regulating fire effects below the uppermost surface soil layers. The different responses within savannas versus across the forest-savanna ecotone has interesting management implications. The estimated historical fire frequency for oak savannas is around 0.33 fires/year (Grimm, 1984; Leys et al., 2019), suggesting that increased fire frequency from a historical baseline for several decades could result in large losses of soil C from these ecosystems. However, intermediate frequencies of burning did not cause any soil C losses relative to the unburned forest plots, suggesting little below-ground C cost to restoring savanna in areas encroached by forest. Overall, the large changes in below-ground C stocks suggest that current estimates of fire effects on C cycling based only on above-ground biomass losses could underestimate the long-term effects of repeated burning. Consequently, future studies that seek to evaluate the effects of fire on ecosystems should consider deeper soil layers to capture the full effects of fire on ecosystem C storage and nutrient dynamics.

AUTHORS' CONTRIBUTIONS
A.F.A.P. and K.K.M. conceived of the manuscript. K.K.M. was the lead PI on the Novus Research Coordination Network grant. P.B.R. and S.E.H. are lead PIs on the fire manipulation experiment at Cedar Creek. A.F.A.P., K.K.M., S.E.H., M.C.M., A.L.M., D.M.M., S.P., P.B.R. and K.W. assisted in sample collection and analysis. A.F.A.P. wrote the first draft of the manuscript and all authors provided feedback.

DATA AVAILABILITY STATEMENT
Data available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.02v6wwq07 (Pellegrini et al., 2020).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.