Carbon and Nitrogen Cycling are Resistant to Fire in Nutrient-Poor Grassland

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We used a long-term experiment with four different fire frequencies, annual burns (B1), biennial burns (B2), burn every 4 yr (B4), and no burn (BC) over a 27-yr period. We quantified temporal changes in vegetation dynamics, aboveground and belowground carbon (C) and nitrogen (N) pools, and we examined the cumulative effects of fires on N cycling in a nutrient-poor, old field grassland at Cedar Creek, MN. Compared with fires in fertile grasslands with high productivity, fire in this nutrient-poor and low-productivity old field grassland caused only minor shifts in plant functional groups and did not change net primary productivity (NPP) or N cycling rates. We also found that fire frequency did not affect ecosystem C pools or N pools, and the soil C was accumulating at 28 times of the rate of N accumulation for the period 2000 to 2010. This N accumulation in the soil, combined with the low-productivity and the dominant C_4 grasses, which have relative low litter N concentration and thereby low fire-induced N losses, makes this successional grassland resistant to fire-induced N cycling and C and N pool changes for at least decades after agricultural abandonment.

Abbreviations: NPP, net primary productivity; SOM, soil organic matter.

Fire is a common disturbance and can have significant effects on plant community structure, composition, dynamics (Collins and Wallace, 1990, Magnan et al., 2012), NPP, and carbon (C) and nitrogen (N) cycling (Ojima et al., 1994, Reich et al., 2001, Wardle et al., 2003). Fire frequency and intensity are increasing with global warming and with more frequent extreme climate events worldwide (Piñol et al., 1998). However, in many fire adapted grasslands like the North American prairies, fire frequency has decreased, because of human fire suppression and the fragmented nature of the remaining prairies (Collins and Wallace, 1990).

We used a 27-yr experiment in a grassland at Cedar Creek Ecosystem Science Reserve in Minnesota, USA, with four different fire frequencies to examine the impacts of fire frequency on the structure and functioning of this nutrient-poor old field ecosystem. Several approaches, such as manipulated fire experiments (Shay et al., 2001), observational or gradient studies (Reich et al., 2001), repeated sampling from permanent plots (Dijkstra et al., 2006, Knops, 2006, Li et al., 2013), and modeling studies (Ojima et al., 1994, Thornley and Cannell, 2004) have been used to study ecosystem fire effects. However, ecosystem effects are inconsistent and have shown increases in N mineralization and NPP with decreases in fire frequency (Buis et al., 2009), or no changes in N mineralization (Blair, 1997, Turner et al., 1997, Zhou et al., 2009). Frequent fire can result in lower C and N pools due to elevated N losses (Fynn et al., 2003). However, a meta study showed

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no significant changes in total N pools in response to fire (Wan et al., 2001). Additionally, fire increased root production (Johnson and Matchett, 2001), and increased microbial and soil organic (SOM) matter N immobilization can offset any N losses caused by fire (Dell et al., 2005). In higher productivity grasslands (e.g., tallgrass prairie) fire often can change species composition, vegetation structure, and NPP (Abrams et al., 1986, Briggs and Knapp, 1995, Knapp et al., 1998). The few studies in unfertile grasslands have shown that fire has fewer short-term effects on species and productivity (Valone et al., 2002) and no significant effects on total soil N and C pools (Coetsee et al., 2010). Thus, fire-induced ecosystem changes or resilience may depend on habitat productivity and fertility (Wang et al., 2009).

Previously we have reported that fire frequencies in this study induced minor vegetation changes (Knops, 2006, Li et al., 2013). Increased fire frequency lowered C_3 grass abundance, and after 17 yr, high fire frequency promoted the establishment and growth of the legume species *Lespedeza capitata*, which may lead to ecosystem changes in the long term. However, we found no changes in the relative abundance of the dominant species *Schizachyrium scoparium* (C_4 grass) (Li et al., 2013). Here, we used a 27-yr experiment to (i) quantify fire-induced changes in vegetation NPP and (ii) quantify fire-induced changes in N cycling and C and N ecosystem pools. We hypothesize that (i) fire-induced N losses are minimal, because in low-nutrient soils of old fields the majority of the ecosystem N pool and NPP are belowground and therefore not susceptible to loss through fire, and the fire-induced aboveground litter losses have a low N content. If fire-induced N losses are minimal, we hypothesize that (ii) this successional grassland is resistant to shifts in NPP, N cycling, and C and N pools in fire frequency.

METHODS

Study Area

This experiment was performed in an old field, abandoned in 1957 at Cedar Creek Ecosystem Science Reserve (hereafter Cedar Creek) in Minnesota, USA (45°24′ N, 93°12′ W), where N is the primary resource limiting NPP (Tilman, 1984). This region has a midcontinental climate with hot, humid summers and cold winters. The warmest month, July, averages 28.6°C (range 13.9–39.5); the coldest month, January, averages −5.7°C (range −27 to 12.8). Average annual rainfall was 1367 mm during the 27 yr after plot establishment (from 1983 to 2010) and fluctuated from 754 to 2143 mm among the sampling years (858 mm 1983, 754 mm 1987, 1516 mm 1991, 1656 mm 2000, and 2063 mm 2010, respectively). The soil of this field is classified as a Zimmerman association and consists of rapidly permeable fine sand (93% sand, 3% silt, 4% clay) formed in sandy outwash sediments (Grigal et al., 1974) and is nutrient-poor. Agriculture caused the loss of approximately 80% of the soil organic matter in the top 10 cm (Knops and Tilman, 2000). Soil %N in 2010 at 0- to 10-cm depth varied from 0.030% to 0.093%, with a mean of 0.056%.

Experiment Design

Plots were established in 1983. Beginning in 1984, fire was applied in four different treatments: annual burns (B1), biennial burns (B2), burn every 4 yr (B4), and no burn (BC). Each treatment has six replicates (8 × 8 m) placed randomly in 24 plots, with a 2-m buffer between them. Burning was done by using a strip head fire technique each spring in March or April, depending on snow melt. In 1995, a prescribed fire escaped and burned the entire experiment. For a detailed description of the fire procedure see Li et al. (2013).

Sampling Methods

At the peak of standing biomass, vegetation was sampled by clipping a 10-cm by 3-m long strip at ground level within each plot in mid-July of 1983, 1987, 1991, 2000, and 2010. All clipped samples were sorted to individual plant species and litter. Vascular plants were divided into five functional groups: cool season C_3 grasses; warm season C_4 grasses; legumes (N fixing broadleaves); forbs (non-N fixing broadleaves); and sedges. For more details on the vegetation sampling methods refer to Knops, (2006) and Li et al. (2013).

In 2000 (17 yr after plot establishment) and 2010 (27 yr after plot establishment), C and N pools were estimated to measure changes across time. Soil and root samples were collected in mid-July of 2000 and 2010. Soil samples of the depth intervals 0 to 10, 10 to 20, 20 to 40, 40 to 60, 60 to 80, and 80 to 100 cm from each plot were taken by a 5-cm-diameter soil core. The soils were dried and sifted through a 2-mm sieve to remove roots. A 5-cm-diameter corer was used to collect one soil sample at the same depths within each plot. These samples were dried and weighed to determine bulk density.

Roots were collected using three 5-cm-diameter cores from each plot: 0 to 20, 20 to 40, 40 to 60, and 60 to 100 cm. Roots were washed and handpicked over a 1-mm screen to remove all soil, pebbles, and debris and then dried and weighed.

For soil gross and net N cycling measurements (N fluxes), we resampled soils from 0 to 10 cm by a 5-cm-diameter core at each plot in mid-July of 2010. Each sample was immediately sieved (2 mm), then mixed in a plastic bag and kept on ice during transport to the laboratory until soil N cycling measurements were taken.

Carbon and Nitrogen Analyses

All soil, root, and plant biomass samples were dried to a constant mass at 60°C. The soil was ground in a coffee mill and the roots, shoots, and litter were ground using a Wiley mill (Thomas Scientific, Swedesboro, NJ). Samples were analyzed on a Costech ECS 4010 (Costech Analytical Technologies, Valencia, CA) for % C and N. Soil % C and % N were converted to g C and N m^{-2} by the corresponding soil bulk density. Plant and root % C and % N were converted to g C and N m^{-2} by the corresponding biomass.

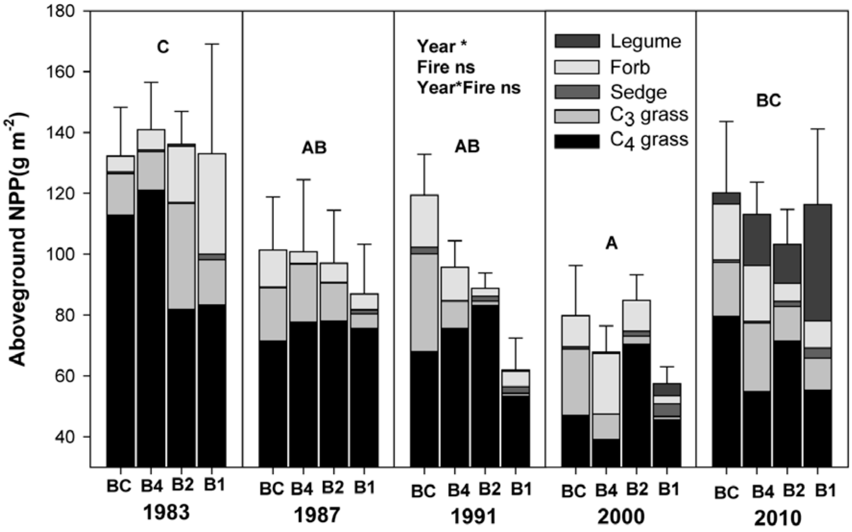

Fig. 1. Dynamics of aboveground net primary productivity (NPP) in plant functional groups under the four different burn frequencies from 1983 to 2010 over 27-yr period: annual burns (B1), biennial burns (B2), burn every 4 yr (B4), and no burn (BC). Plant functional groups include C_3 grass, C_4 grass, sedge, forb, and legume. All variables were examined using a repeated measure ANOVA. Error bars represent ± standard error of total standing biomass. Significant differences for total standing biomass among years are indicated by capital letters (*P* < 0.05 of a Tukey post hoc test); ns *P* > 0.05, **P* < 0.05. The results of repeated measures analysis of all plant functional groups are shown in Table 1.

Soil Nitrogen Cycling

We measured gross ammonification and gross nitrification rates using ^{15}N isotopic-pool dilution (Hart et al., 1994) in the surface soil, 0 to 10 cm deep. To determine gross ammonification rates, a ~250 g (fresh weight) homogenized and sieved soil sample was injected with 10 mL of 0.147 mmol L^{-1} solution of $(^{15}NH_4)_2SO_4$ (99% atom). To determine gross nitrification rates, a ~250 g (f.w) homogenized and sieved soil sample was injected with 10 mL of 0.301 mmol L^{-1} solution of $K^{15}NO_3$ (99% atom). Each sample was mixed in a resealable plastic bag to ensure homogenous distribution of ^{15}N solution. After ^{15}N addition one soil sample [~50 g of soil (f.w.)] of each labeled pair was extracted immediately with 75 mL of 2 mol L^{-1} KCl [time 0 samples (T0)] to determine initial NH_4 and NO_3 pool size, respectively. Another remaining labeled soil was resealed in the bag to stop the loss of any moisture from the sample and was incubated in the dark at room temperature (22°C) for 24 h. After the 24-h incubation period, time 1 samples (T1) were extracted in the same way that the initial samples were extracted. To determine ^{15}N enrichment levels at both T0 and T1, N from soil extracts was concentrated by diffusion onto paper discs following Hart et al. (1994) and subsequently analyzed for $^{15}N/^{14}N$ by a stable isotope mass spectrometer at the University of California, Davis, Stable Isotope Facility (Europa Integra). Gross N cycling rates were calculated using T0 and T1 following Eq. [1] (Takahashi, 2001): where m_N is the gross ammonification or nitrification rate (mg N kg^{-1} soil d^{-1}); Pool_1 NH_4 or NO_3 concentration at T1 and Pool_0 NH_4 or NO_3 concentration at T0; APE_1 is the atom percent ^{15}N excess of NH_4 or NO_3 pool at T1 and APE_0 is the atom percent ^{15}N excess of NH_4 or NO_3 pool at T0 (Hart et al., 1994).

$$m_N = \frac{\left[\mathrm{Pool}_1 - \left(\mathrm{Pool}_0 \frac{\mathrm{APE}_1 \times \mathrm{Pool}_1}{\mathrm{APE}_0 \times \mathrm{Pool}_0}\right)\right] \times -\mathrm{LN}\left(\frac{\mathrm{APE}_1 \times \mathrm{Pool}_1}{\mathrm{APE}_0 \times \mathrm{Pool}_0}\right)}{1 - \frac{\mathrm{APE}_1 \times \mathrm{Pool}_1}{\mathrm{APE}_0 \times \mathrm{Pool}_0}} \qquad [1]$$

Net ammonification and nitrification were calculated as the difference between Pool_1 and Pool_0 for NH_4 and NO_3, respectively. Microbial N consumption rates were calculated as the difference between gross N production and net rates for both NH_4 and NO_3. Immobilization of NH_4 was calculated as the difference between NH_4 consumption and gross nitrification (Davidson et al., 1991).

Statistical Analysis

The temporal effects of fire frequency on aboveground NNP (ANPP) were analyzed with a repeated measure ANOVA, using a Tukey post hoc test ($P < 0.05$) to determine significant differences among years. The differences in soil N cycling rates (0–10 cm) under the four different burn frequencies were analyzed with a one-way ANOVA. We found that the response of most ecosystem C and N variables to burning frequency were never statistically significant; consequently we used a one-way ANOVA ($n = 24$) to examine differences in ecosystem C and N pools among sampling years (2000 and 2010). The root and soil C pools and N pools at the different depths between 2000 and 2010 were compared with paired *t* tests. All statistical analysis was done with SPSS 20.0. For each analysis, we assessed normality of data and an appropriate transformation was applied when necessary.

RESULTS

Ecosystem and Functional Group Net Primary Productivity

Net primary productivity fluctuated between 80 and 140 g m^{-2}; but fire frequency had no significant effect ($F_{3,20} = 0.364$, $P = 0.780$; Fig. 1, Table 1). Over the 27 yr, NPP of the C_4 grasses dominated our site at each sampling date and accounted for 45 to 92% of total NPP (Fig. 1). Although NPP of the C_3 grasses (dominated by *Poa pratensis*), the C_4 grasses (dominated by *Schizachyrium scoparium*), and the sedges (dominated by *Carex pensylvanica*) fluctuated significantly over time ($F_{3,20} = 4.887$, $P < 0.05$), fire frequency had no significant effect (Table 1). C_4 grass biomass was significantly higher in the sampling of 1983, before the fire treatment started, but none of the other sampling years were significantly different (Fig. 1). C_3 grass biomass was significantly lower in 1991 and 2000, as compared with the other three sampling dates. Sedge biomass was significantly higher in

Table 1. Aboveground net primary productivity (NPP) and plant functional group productivity response to fire frequency from 1983 to 2010.†

	Year	Fire	Fire*Year
Total standing biomass	4.88*	0.36ns	0.08ns
C_3 grass	6.32*	2.50ns‡	2.20ns
C_4 grass	31.93***	0.50ns	2.83ns
Legume	19.51***	3.44*	3.59*
Forb	0.01ns	0.28ns	1.27ns
Sedge	8.90**	1.78ns	1.93ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Shown are the *F* values from a repeated measurement ANOVA with four different fire frequencies [annual burns (B1), biennial burns (B2), burn every 4 yr (B4) and no burn (BC)]. C_3 grass and sedge data were ln (x + 1) transformed to improve homogeneity of variances.

‡ ns, not significant.

the last three sampling times, as compared with the first two. Only the NPP of legumes (dominated by *Lespedeza capitata*) significantly increased with fire frequency and over time ($F_{3,20} = 19.513$, $P < 0.001$; Table 1, Fig. 1). Legumes first established in 2000 and contributed up to one third of the NPP in the most frequently burned treatment in 2010.

Table 2. Changes in ecosystem carbon and nitrogen pools in response to fire frequency for the period 2000–2010.†

	Carbon			Nitrogen		
	Year	Fire	Fire*Year	Year	Fire	Fire*Year
Total ecosystem	12.68**	0.28ns‡	1.35ns	2.91ns	0.30ns	1.09ns
Aboveground live plant	25.75***	0.21ns	0.95ns	14.74***	0.06ns	1.07ns
0–100 cm root	68.23***	1.71ns	0.57ns	27.44***	0.10ns	0.35ns
0–100 cm soil	6.91*	0.24ns	1.44ns	2.12ns	0.30ns	1.16ns
Litter	51.12***	30.68***	3.86*	24.65***	11.49***	5.12**
C_3 grass	34.90***	2.47ns	3.74*	4.76*	1.80ns	1.71ns
C_4 grass	4.15ns	1.35ns	0.82ns	0.39ns	1.53ns	0.61ns
Legume	59.41***	5.15**	2.19ns	22.16***	4.26*	3.45*
Forb	3.02ns	2.42ns	0.54ns	0.34ns	2.83ns	0.50ns
Sedge	0.87ns	1.93ns	0.31ns	0.15ns	2.06ns	0.28ns
0–10 cm soil	12.99**	0.18ns	0.24ns	10.49**	0.19ns	0.07ns
10–20 cm soil	38.17***	0.80ns	0.51ns	27.35***	0.61ns	0.73ns
20–40 cm soil	0.98ns	0.07ns	2.99ns	0.05ns	0.26ns	3.19ns
40–60 cm soil	0.03ns	1.28ns	2.30ns	0.86ns	1.16ns	1.55ns
60–80 cm soil	0.14ns	0.04ns	0.42ns	1.77ns	0.07ns	0.74ns
80–100 cm soil	0.00ns	0.97ns	0.61ns	3.16ns	1.27ns	0.89ns
0–20 cm root	56.28***	1.73ns	0.78ns	22.51***	0.04ns	0.12ns
20–40 cm root	40.90***	1.18ns	0.94ns	15.06***	1.28ns	1.60ns
40–60 cm root	0.26ns	1.65ns	0.08ns	2.59ns	0.75ns	0.64ns
60–100 cm root	9.78**	0.82ns	0.38ns	20.94***	0.74ns	0.40ns

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

† Shown are the *F* values from a repeated measurement ANOVA with four different fire frequencies (B1, B2, B4, BC). Data were ln (x + 1) transformed to improve homogeneity of variances. Note: Total ecosystem includes standing living plant, litter, roots down to 100 cm and soil down to 100 cm. The functional groups show aboveground stocks.

‡ ns, not significant.

Ecosystem Carbon and Nitrogen Pools

We found that fire frequency had no impact on the total ecosystem C pools ($F_{3,20} = 0.283$, $P = 0.837$) or N pools ($F_{3,20} = 0.301$, $P = 0.824$), and also had little influence on C and N in total aboveground plant, root, or soil C or N pools at any depths for the period 2000 to 2010 (Table 2). Increased fire frequency only significantly decreased the litter (C pools, $F_{3,20} = 30.684$, $P = 0.000$; N pools, $F_{3,20} = 11.494$, $P = 0.000$) and increased the legume (C pools, $F_{3,20} = 5.153$, $P = 0.008$; N pools, $F_{3,20} = 4.257$, $P = 0.018$) C and N pools (Table 2).

In contrast to the lack of a fire effect, we found a significant increase in total ecosystem C ($F_{1,46} = 16.184$, $P < 0.001$) and N pools ($F_{1,46} = 15.088$, $P < 0.001$) between 2000 and 2010 (Fig. 2). The soil 0- to 20-cm depth showed C significantly increasing from 1746 to 2244 g m^{-2} (Fig. 2a) and N increasing from 130 to 161 g m^{-2} (Fig. 2b). This corresponds with an annual C and N accumulation rate of 49.8 g C m^{-2} yr^{-1} and 3.1 g N m^{-2} yr^{-1} in the top 20-cm soil. In the entire 100-cm soil depth we found C increasing from 4032 to 4580 g m^{-2} and N increasing from 330 to 350 g m^{-2}, which corresponds with an annual accumulation rate of 54.8 g C m^{-2} yr^{-1} and 2.0 g N m^{-2} yr^{-1}. More than 75% of all roots (0- to 100-cm depth) are present in 0- to 20-cm depth (Fig. 3); thus, plant-derived C and N inputs in the soil are likely to be mainly at this depth, which corresponds to the significant increase in soil C and N in 0 to 20 cm and the absence of a significant increase in soil C at deeper depth. However, in contrast to the root biomass, only 50% of soil C and 40% of the soil N pool are present at 0 to 20 cm. The C to N ratio of the soil 0- to 20-cm depth also increased from 13.3 to 13.9 (paired *t* test, $t = -4.196$, $P = 0.000$); thus, C sequestration in this grassland corresponds with an increasing N pool and an increasing C to N ratio of the SOM. Overall there was no indication that fire affected C and N pools over time (Table 2).

Nitrogen Cycling Rates

We found no significant differences in gross ammonification ($F_{3,20} = 2.271$, $P = 0.111$), NH_4 immobilization ($F_{3,20} = 1.534$, $P = 0.236$), net ammonification ($F_{3,20} = 0.224$, $P = 0.879$), gross nitrification ($F_{3,20} = 0.620$, $P = 0.610$), NO_3 immobilization ($F_{3,20} = 0.594$, $P = 0.626$), net nitrification ($F_{3,20} = 0.951$, $P = 0.435$), and total net N mineralization ($F_{3,20} = 0.634$, $P = 0.602$) among the four fire frequencies

(Table 3). Thus, we have no indication that fire frequency has any impact on N cycling rates.

DISCUSSION

Fire

We found that fire frequency had no effect over 27 yr on NPP and N cycling rates. We also found that fire frequency had no effect on soil C pools, soil N pools, and plant aboveground biomass or plant belowground biomass for the period 2000 to 2010. Our results are consistent with other nutrient-poor grasslands where fire seems to have few short-term negative effects on species abundances (Valone et al., 2002) or soil C and N (Coetsee et al., 2010). In our site the standing biomass was low, 600–1400 kg ha^{-1}, as compared with many fertile grasslands (Reich et al., 2001, Towne and Kemp, 2003, Towne and Owensby, 1984). A recent meta-analysis of fire effects on ecosystem N dynamics also found that fire did not change total soil N (Wan et al., 2001). However, the lack of a fire-induced change in total ecosystem C and N in our study is not because we cannot detect ecosystem-level changes, as was shown by the significant increases in soil C and N for the period 2000 to 2010. The dominant species in our system are C_4 grasses, which have a majority of their biomass belowground, have low N concentrations, and retranslocate the majority of their aboveground N to belowground parts before abscising their leaves. The remaining aboveground litter in the spring, when burning takes place, likely has lost most easily leachable and decomposable C and N, and the high C to N ratio will prevent mineralization (Knops et al., 2002). Fire-induced volatilization losses of N have been estimated as 1.0 to 4.0 g m^{-2} yr^{-1} in productive grasslands (Ojima et al., 1994). However, volatilization losses in this low-productivity site would have been much lower because of the low N content of the aboveground litter (0.5 to 1.0 g N m^{-2}), suggesting that potential fire-induced N losses are low. Additionally, we also detected no significant fire effect on any of the N cycling rates or on ecosystem N pools. Therefore, the impact of fire on C and N pools may strongly depend on the dominant species traits and ecosystem fertility (Bond and Keeley, 2005, Li et al., 2013).

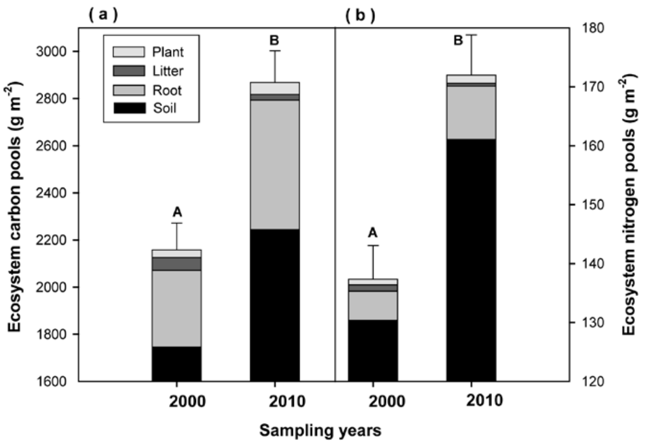

Fig. 2. Mean ecosystem carbon (a) and nitrogen (b) pools in plant and soil compartments in 2000 and 2010. Aboveground pools include standing living plant and litter. Belowground pools include roots down to 100 cm and soil down to 20 cm. Pools means between 2000 and 2010 were compared with one-way ANOVA (*n* = 24). Error bars represent ± standard error of total ecosystem carbon (a) and nitrogen (b) pools, and letters represent significant differences across year from post hoc Tukey tests ($P < 0.05$) for the change in the total ecosystem carbon (F = 16.184, $P < 0.001$) and nitrogen (F = 15.088, $P < 0.001$) pools.

We also examined the influence of precipitation variability on NPP and found that precipitation varied threefold over the five sampling periods but was not correlated with NPP (Regression, $F_{1,\ 118} = 2.37$, $P = 0.13$, $R^2 = 0.02$), and including precipitation as a covariable did not changes the results. However, this study only has five sampling dates. We also examined a different study at Cedar Creek that measures ANPP in four plots within 15 old fields since 1988 (Wilsey et al., 2005). This study showed a significant, but small, positive effect of precipitation on NPP (Regression, $F_{1,1429} = 32.3$, $P < 0.001$, $R^2 = 0.022$) but also showed no significant trend over time ($P > 0.5$).

Nitrogen Net Inputs, Pools, and Fluxes

For the period 2000 to 2010, 23.8 g N m^{-2} accumulated [30.7 g N m^{-2} in the SOM 0–20 cm, −10.9 g N m^{-2} in the SOM

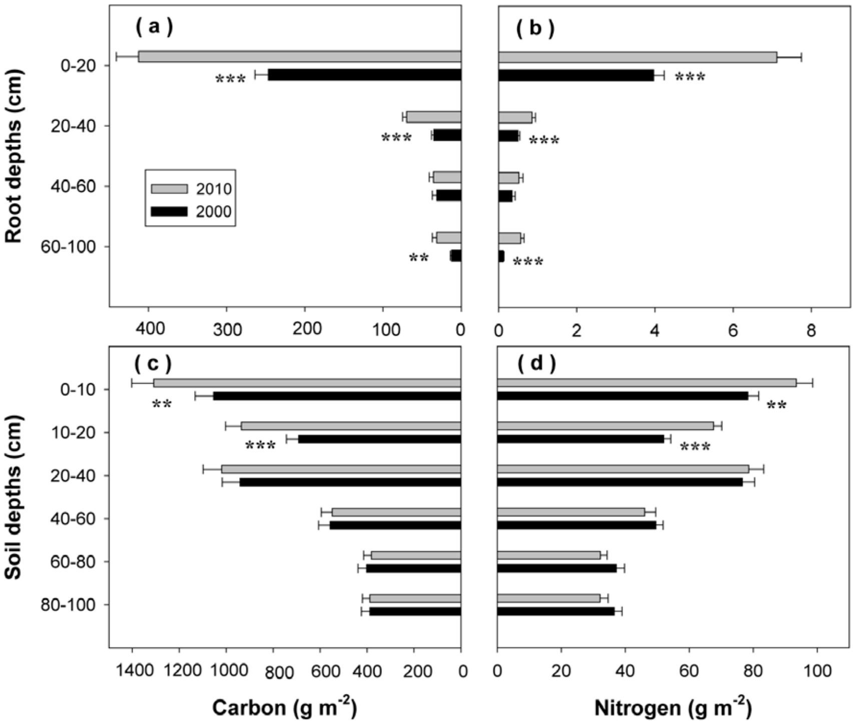

Fig. 3. Root carbon (a) and root nitrogen (b) and soil carbon (c) and soil nitrogen (d) distributions in the different soil depths in a series of different fire frequencies (mean + SE) in 2000 and 2010. The differences between 2000 and 2010 were compared with paired *t* test and significantly different are indicated by asterisks: $**P < 0.01$, $***P < 0.001$.

Table 3. Gross NH_4, NH_4 immobilization, net NH_4 mineralization, gross NO_3, NO_3 immobilization, net NO_3 mineralization, total net N mineralization (net NH_4 and NO_3 mineralization) in response to fire frequency.†

Category	1 yr	2 yr	4 yr	Control	*P* value
Gross NH_4	0.66 ± 0.06	0.78 ± 0.19	0.74 ± 0.11	1.20 ± 0.23	0.111
NH_4 immobilization	0.26 ± 0.15	0.39 ± 0.10	0.66 ± 0.17	0.79 ± 0.30	0.236
Net NH_4 mineralization	−0.44 ± 0.09	−0.45 ± 0.03	−0.49 ± 0.12	−0.37 ± 0.16	0.879
Gross NO_3	0.83 ± 0.13	0.85 ± 0.22	0.57 ± 0.13	0.77 ± 0.16	0.610
NO_3 immobilization	0.44 ± 0.25	0.65 ± 0.19	0.38 ± 0.09	0.32 ± 0.17	0.626
Net NO_3 mineralization	0.39 ± 0.17	0.20 ± 0.09	0.19 ± 0.13	0.45 ± 0.14	0.435
Total net N mineralization	−0.05 ± 0.23	−0.25 ± 0.11	−0.31 ± 0.24	−0.09 ± 0.29	0.602

† All rates are shown as mg N kg^{-1} soil d^{-1}. Values are mean ± 1SEs based, *n* = 6, *P* value are from a one-way ANOVA.

20–100 cm depth, 4.6 g N m^{-2} in plant (aboveground 0.5 and belowground 4.1) biomass and −0.6 g N m^{-2} in standing litter] (Fig. 4). This increase translates to an annual N accumulation of 2.38 g m^{-2} yr^{-1}, which is higher than the 1.23 g N m^{-2} yr^{-1} accumulation estimates based on an old field chronosequence at the same study site (Knops and Bradley, 2009; Knops and Tilman, 2000). Our higher annual N accumulation might be caused by the increasing legume abundance; however, we did not detect any significant correlation between plot differences in legume abundance and ecosystem N pools (data not shown). Other potential inputs like nonsymbiotic N fixation, foliar interception and uptake, animal movement (Knops et al., 2002), or retranslocation from soil below 100-cm depth might account for this. Longer-term, fire-induced legume abundance changes may increase N fixation and facilitate further ecosystem changes (Lambers et al., 2004, Reich et al., 2001).

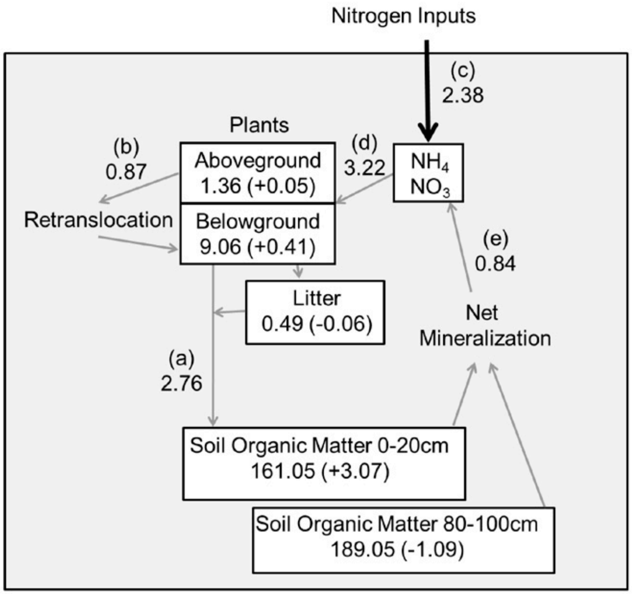

Fig. 4. Annual ecosystem nitrogen pools and fluxes in 2010. Pools are represented by boxes, all data in g N m^{-2}, and fluxes by lines, all data in g N m^{-2} yr^{-1}. Pool changes from 2000 to 2010 are shown as (+ increase) and (– decreases) in the brackets within the boxes. Plant nitrogen losses "(a)" are estimated to be 2.76 g m^{-2}, calculated from 0.49 g m^{-2} for aboveground litter and 2.27 g N m^{-2} for belowground roots turnover, based on 25% annual root turnover (Wedin and Tilman, 1996). Aboveground retranslocation "(b)" is calculated from the difference between the aboveground biomass and the litter. Nitrogen inputs "(c)" are estimated from the 2000 to 2010 total ecosystem N accumulation. Plant N uptake "(d)" and net mineralization "(e)" are calculated from the other annual fluxes and pool changes. Note the litter pool only represents the aboveground litter.

Linkage Between Net Primary Productivity and Nitrogen Cycling

We found no directional change in NPP, and NPP at this site is strongly limited by N. Thus, maintaining the NPP requires that the vegetation annually either (i) recycles N efficiently and/or (ii) replaces plant loss with N uptake from new N inputs, or SOM–litter turnover. The average aboveground biomass in 2010 contained 1.36 g N m^{-2}, whereas the litter contained 0.49 g N m^{-2} (Fig. 2); thus, N retranslocation is about 65%, which is typical for C_4 grasses (Wedin and Tilman, 1996). The belowground root pool was about 9.06 g N m^{-2}. If we assume that 25% of the roots turns over annually with no N retranslocation (Wedin and Tilman, 1996) and no losses of N occur through leaching or gaseous fluxes, the estimated belowground losses were 2.27 g N m^{-2} yr^{-1} (Fig. 4). Leaching losses of N are likely low because of the N limitation in this old field and the low soil mineral N concentrations. Similarly, gaseous losses also are likely low because of the sandy soil and the low mineral soil N concentrations. Although fire-mediated shifts in legume abundances occurred in this system, legume abundances in plots were not significantly correlated with ecosystem N pools. By combining the annual aboveground and belowground losses with the above and belowground N pool increase, we can estimate an annual N uptake of 3.23 g N m^{-2} (Fig. 4). The annual ecosystem N accumulation is 2.38 g N m^{-2}. If we assume no N losses, then atmospheric N deposition and other N inputs equal the ecosystem N accumulation. We estimated plant annual N uptake of 3.23 g N m^{-2}; deduct the annual N inputs of 2.38 g m^{-2} and we can estimate a net N mineralization of 1.31 g N m^{-2} yr^{-1}. Thus, the NPP in this grassland can be sustained by a combination of internal plant recycling (0.87 g N m^{-2} yr^{-1}), decomposition (1.31 g N m^{-2} yr^{-1}), and atmospheric N deposition and other N inputs (2.38 g N m^{-2} yr^{-1}) (Fig. 4), and net N mineralization at deeper soil depth may also be an important source of plant N.

Soil Organic Matter and N Inputs

In our study SOM is increasing over time. Abandoned agricultural fields have lost a large amount of SOM and often accumulate SOM at a rate proportional to N inputs (Knops and Tilman, 2000). This accumulation of N in the SOM pool proportional to N inputs, combined with the low aboveground productivity and the low N content of this C_4 dominated vegetation, might prevent any significant fire-induced N losses, and thereby might prevent any fire-induced N cycling changes.

Taken together, our experiment showed that fire frequency caused only shifts in legumes and did not influence grassland productivity or N dynamics. Thus, this old field grassland ecosystem may be resistant to fire in regards to N cycling and C and N pools for at least decades after agricultural abandonment. Any future fire-induced changes likely requires species changes in response to fire (Reich et al., 2001), especially an increase in legumes (Knops and Tilman, 2000).

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