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RESEARCH ARTICLE

Plant and microbial feedbacks maintain soil nitrogen legacies in burned and unburned grasslands

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Abstract

1. Nitrogen (N) availability is a well-known driver of ecosystem structure and function, but as air quality regulations continue to reduce atmospheric N deposition, there is a need to understand how managed and unmanaged ecosystems respond to widespread decreases in terrestrial N availability. Historical N eutrophication, from pollution or fertilisation, may continue to constrain contemporary responses to decreases in available N because of altered plant and microbial feedbacks. Thus, while certain management practices like prescribed fire remove N from grassland ecosystems, the role of fire supporting ecosystems recovering from chronic N input is unknown.
2. To address this knowledge gap, we ceased a 30-year N-fertilisation treatment at a field experiment in a tallgrass prairie ecosystem crossed with burned and fire-suppressed (unburned) treatments. We established subplots within each previously fertilised, recovering plot, fertilised at the same historical rate ($10\,g\,N\,m^{-2}\,year^{-1}$ as NH_4NO_3), to compare plant and soil properties in recovering plots with control (never-fertilised) and still-fertilised treatments within different fire regimes.
3. We document different N-fertilisation legacies among ecosystem properties in burned and unburned prairies recovering from N-fertilisation. Soil N availability, nitrification and denitrification potentials in recovering plots remained higher than controls for 3–5 years—indicative of positive legacies—in both burned and unburned prairies, but burning did not reduce this legacy. In burned prairies, however, a positive legacy in above-ground plant production persisted because a more productive grass species (switchgrass) replaced the previously dominant species (big bluestem) even though root C:N, but not soil C:N, increased to return back to control levels. Consequently, the main N loss pathways in burned and unburned prairies (pyrovolatilisation and microbially mediated processes, respectively) led to similar losses of soil total N ($20–28\,g\,N\,m^{-2}$) over 5 years.
4. *Synthesis*: Our results indicate that N eutrophication induces positive legacies of ecosystem functions that can persist for at least half a decade. N-induced legacies arise because of shifts in soil microbial N-cycling and plant functional traits. As a result, different management practices may elicit similar trajectories

of ecosystem recovery in terms of total and available soil N because of different plant and microbial feedbacks.

KEYWORDS
above-ground net primary productivity, denitrification, ecological memory, ecosystem recovery, fire, legacy, nitrification, tallgrass prairie

1 | INTRODUCTION

The widespread increase in bioavailable nitrogen (N) from modern industrialisation and fertiliser use has altered biogeochemical feedbacks that control terrestrial carbon (C) and nutrient budgets (Gruber & Galloway, 2008; Vitousek et al., 1997). This eutrophication has alleviated N-limiting conditions that constrain ecosystem functions globally, such as primary production (Gough et al., 2000), and led to N build-up in ecosystems as plant and soil pools slowly accrue this nutrient (Lovett & Goodale, 2011). While significant portions of the Earth's surface remain subject to N eutrophication from fertilisers or pollution relative to preindustrial levels (Chen et al., 2018; Fowler et al., 2013; Liu et al., 2013), decreases in relative N availability are evident in unfertilised terrestrial ecosystems (Mason et al., 2022; Olff et al., 2022) and rates of N emission and inorganic N deposition have declined in many regions with a history of higher N inputs (Ackerman et al., 2019). Accumulated exogenous N in an ecosystem represents a material legacy (Johnstone et al., 2016)—physical matter left over from a historical regime—that could be an important N source for future ecosystem functioning if it remains bioavailable. Yet, how long this material legacy persists or shapes ecosystem recovery from eutrophication is not well understood.

Nitrogen addition studies are crucial to understand how biotic and abiotic properties of ecosystems respond to exogenous N supply. Such studies have established that most ecosystems are sensitive to N-fertilisation as evident by altered C and N flux rates and pool sizes (Lu et al., 2011; Ouyang et al., 2018). However, the sensitivity (i.e. the magnitude of response) of ecosystem properties to elevated N availability varies because of differences in C and N pool quality, and differences in growth rates and N use physiology among different plant and microbial functional groups (Schimel, 1995; Yahdjian et al., 2014). Hence, plant and microbial populations with different life histories also have differential feedbacks on ecosystem responses to N-fertilisation (Hobbie, 2015), a mechanism that can be defined as an information legacy, due to the importance of traits that evolved in response to past environmental 'information' in determining responsivity to current or future conditions (Johnstone et al., 2016). As such, the persistence of altered functional traits in a population after a historical regime changes (e.g. decreased rates of N-fertilisation) may continue to regulate certain ecosystem processes. Therefore, differences in sensitivity among ecosystem N pools and functions, and among populations within plant and soil microbial communities, together modulate the form and quantity of available N, the net accumulation of N in the ecosystem and the mechanisms of N removal after ceasing fertilisation.

Critically, there are few studies to date that examine the fate of ecosystems recovering from N-fertilisation, though ceasing experimental fertilisation may reveal how ecosystems respond to decreases in N availability. Existing studies show that ecosystem pools and fluxes recover asynchronously in plants and soils (Gilliam et al., 2019; Stevens, 2016). For example, soil inorganic N can return to levels observed in untreated soils within a decade (Bredemeier et al., 1995; Stevens et al., 2012), but N in soil organic matter (SOM) and net N mineralisation rates often take longer to recover (Stevens, 2016), suggesting a material legacy in SOM could support higher N mineralisation rates. Also, leaf N content can decrease in as little as 2 years (Arróniz-Crespo et al., 2008), but higher above-ground plant production may persist (Isbell et al., 2013; Nieland et al., 2021; Storkey et al., 2015), constituting both material and information legacies, if certain populations are responsible for the persistence of function. As these examples show, the ecological memory of an ecosystem to past N-fertilisation is directed jointly by material and information legacies (Johnstone et al., 2016), and this memory can continue to support functions representative of the past fertilised condition. Slow quantitative recovery of N-cycling pools and rates towards levels observed in unfertilised controls is definitive of a positive legacy (Sala et al., 2012). However, our ability to predict future ecosystem functions from past increases to N availability is limited since the extent, duration and mechanisms of legacies are unknown.

Human intervention could ameliorate the effects of N eutrophication and facilitate ecosystem recovery through land management practices that support N removal. In many ecosystems, including grasslands, fire affects ecosystem structure and function (Bond, 2008). Grassy ecosystems, covering approximately 40% of the Earth's land surface (White et al., 2000), are historically maintained by periodic fire (Bond et al., 2005), and while a transient increase of soil ammonium level can occur post-fire (Wan et al., 2001), frequent fires sustain lower ecosystem N availability by combusting above-ground litter, preventing organic N return to the soil (Pellegrini et al., 2018). In contrast, fire suppression in grasslands lets above-ground litter decompose and increases biologically available N (Blair, 1997; Nieland et al., 2021; Ojima et al., 1994), but it can also allow the establishment of woody plants that directly and indirectly affect C and N cycles (Barger et al., 2011; Fynn et al., 2003; McCarron et al., 2003; Zhou et al., 2018). Thus, prescribed fire could drive ecosystem N loss and support faster ecosystem recovery after supplemental N is ceased (Isbell et al., 2013; Meyer et al., 2023; Storkey et al., 2015).

To assess the ecological memory of elevated N availability, we terminated a long-term N-fertilisation treatment at a field experiment in a tallgrass prairie ecosystem. Recent findings from this experiment showed that, after 30 years of N-fertilisation, annually burned prairies retained about half as much fertiliser-N in the SOM as unburned prairies, and had lower soil available N (Nieland et al., 2021). Because the pyrovolatilisation of dead plant litter limited long-term exogenous N retention, we hypothesised that annual burning could enhance ecosystem recovery from chronic fertilisation by further reducing this material legacy. We evaluated this hypothesis by measuring material legacies, in the form of available and total soil N, and information legacies, in the form of soil microbial (N-cycling potentials) and plant responses (productivity, tissue quality and community composition), for 5 years. Based on our hypothesis, we predicted that recovery of these selected ecosystem properties would be quicker, and greater in magnitude, in burned prairies than unburned prairies. Conversely, in unburned prairies, we predicted that potential for ecosystem N loss through soil microbial processes would be higher (specifically, nitrification, which promotes N leaching, and denitrification, which drives gaseous N loss to the atmosphere; Kuypers et al., 2018).

2 | MATERIALS AND METHODS

2.1 | Study site

Our study took place at the below-ground plots (BGP) experiment, located in an ungrazed area of the Konza Prairie Biological Station (KPBS) near Manhattan, KS, United States (39°05′ N, 96°35′ W). At this location, mean annual temperature at KPBS is 12.8°C, and mean annual precipitation (1986–2021) is 849 mm, most of which falls during the growing season (April–September). During the study period (2017–2021), interannual precipitation varied such that cumulative growing season precipitation was abnormally dry (one standard deviation (SD) under the mean) in 2018, abnormally wet (one SD over the mean) in 2019 and closer to average in other years (Figure S1). Soils at the BGP experiment are silty-clay loams (Irwin series: fine, mixed, mesic, pachic Argiustolls).

2.2 | Site and experiment description

The BGP experiment was established in 1986 with a strip-plot experimental design that crosses fire and fertilisation treatments (Seastedt et al., 1991). There are eight main blocks: Four blocks are annually burned by drip torch ignition in early spring, when fuel moisture content is lowest across the region (Bragg, 1982), and the other four blocks are not burned. Each fertilisation treatment combination has one replicate plot per fire treatment block ($n = 4$). Within blocks, we sampled 12.5 × 12.5-m plots that were fertilised for 31 years (1986–2016) with 10 g N m^{-2} year^{-1} as NH_4NO_3 (recovering treatment) and never-fertilised plots (control treatment). In 2017, we ceased the N-fertilisation treatment at the whole plot scale and established four 1 × 1-m subplots within each recovering plot, continuing to fertilise subplots at the same rate (fertilised treatment). Due to spatial constraints, we used the recovering and control treatments to measure above-ground plant recovery, and used the control, recovering and continually fertilised treatments to measure soil and root recovery (Nieland et al., 2021).

2.3 | Sample collection and processing

Soil samples were collected at the peak (June–July) and late (August–September) portions of the growing season for five consecutive years (Figure S1). Four 2-cm diameter, 15-cm deep mineral soil cores were taken from each experimental plot and subplot and combined into one composite sample for each treatment replicate. Soils were sieved through a 4 mm mesh to remove rocks and organic debris while preserving soil aggregate structure, and immediately placed on ice until arriving in the laboratory. Soils were stored at 4°C for no more than 1 week while N-cycling assays were completed.

2.4 | Soil available N and microbial N-cycling

To estimate total soil available inorganic N, four resin bags were buried 10 cm below the soil surface in each replicate plot during the growing season in 2017 and 2019–2021 (Baer & Blair, 2008). At the end of the growing season, surviving bags were removed, and resin-sorbed N was extracted in 2 M KCl. NH_4^+-N and NO_3^--N in the extracted solution were quantified using modified indophenol and VCl_3/Griess reagent methods, respectively (Hood-Nowotny et al., 2010), and measured spectrophotometrically using a FilterMax F5 Multimode Microplate Reader (Molecular Devices, San Jose, CA, USA). Quantities of resin-sorbed N were averaged by plot for subsequent analysis.

Nitrification potential was measured by recording the change in NO_3^--N 24 h after spiking a slurry of 2.5 g of soil in a 1-mM phosphate buffer solution with a saturating amount of ammonium (Petersen et al., 2012; Taylor et al., 2010). Denitrification enzyme activity under non-limiting C and N conditions was measured by adding glucose and NO_3^- to an anoxic vessel containing a slurry of 5 g of soil in deionised water with chloramphenicol, and recording the change in N_2O-N over a 1 h incubation period, with acetylene added to prevent N_2O reducing to N_2 (Groffman et al., 1993). In parallel, denitrification potential was assayed using the same approach, but with no added C or N and a 3 h incubation, which better approximates rates measured in ambient soil conditions (Vega Anguiano et al., 2024). N_2O-N was quantified using a Shimadzu GC 2014 fitted with an electron capture detector (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA). An estimate of potential seasonal cumulative N loss in the top 15 cm of soil through denitrification was calculated by summing the average denitrification potential flux (in g N m^{-2}) for each treatment, using the study site bulk density of 1.097 g cm^{-3} (Carson, 2013) and assuming a constant flux over each 183-day (April–September) growing season.

2.5 | Above-ground plant responses

Above-ground net primary productivity (ANPP) was estimated at the end of each growing season in control and recovering plots. Plant biomass was clipped inside two 0.1 m^2 quadrants per plot, dried at 60°C, sorted by plant functional group after drying and weighed (Blair & Zeglin, 2023). Both herbaceous and woody ANPP are reported, but because woody ANPP is underestimated with the quadrat approach, we centred our inferences on herbaceous ANPP. Plant community composition was measured using two 5 m^2 circular subplots within each plot, with canopy cover of each plant species estimated using a modified Daubenmire scale (Blair, 2023).

2.6 | Ecosystem C and N stocks

Above-ground and below-ground ecosystem C and N stocks were estimated at the end of the growing season in 2021. To estimate above-ground ecosystem C and N stock, after whole-plant biomass in the experimental plots was sorted, clipped, dried and weighed for ANPP, it was then ground using a Wiley mill, and %C and %N were quantified using a LECO TruSpec CN Combustion Analyser (LECO, St. Joseph, MI, USA) at the Kansas State Soil Testing Lab. To estimate the amount of N vulnerable to pyrovolatilisation, above-ground plant litter was collected 4 weeks before the prescribed fire in spring 2021 in the burned treatment plots, using the same quadrat clipping and sorting approach as for live biomass. After drying, sorting and weighing, composite subsamples of entire plants were ground using an 8000D mixer/mill (SPEX, Metuchen, NJ, USA) and %C and %N were quantified using a FlashEA 1112 NC Analyser (Thermo Fisher Scientific, Waltham, MA, USA). To estimate below-ground (0–15 cm depth) C and N stocks, four 5-cm diameter, 15-cm depth soil cores from each experimental plot or subplot were collected and stored at 4°C in the lab during processing. Roots were extracted, dried at 60°C and weighed as a plot composite sample, and the sieved soil was also composited by plot. Both roots and soils were processed as noted above to quantify C and N with a FlashEA 1112 NC Analyser.

Above- and below-ground plant C and N stocks were extrapolated from the areal footprints of the clipping quadrat and soil core, respectively. Soil %C and %N were converted to soil C and N stocks using a bulk density of 1.097 g cm^{-3} (Carson, 2013). Fertiliser-N retention in the soil of each fire treatment was expressed as a percent of the cumulative amount added over the 30-year experiment (totalling 300 g N m^{-2}) and was calculated using the approach defined by Clark et al. (2009). The annual N loss due to fire in 2021 was estimated as the spring 2021 litter N stock, and this loss was extrapolated to the other study years by applying the proportion of ANPP in 2020 that remained as litter in spring 2021 to the ANPP in other years. This approach assumes similar levels of litter decomposition each fall and winter, and complete combustion of the remaining plant litter N each spring.

2.7 | Statistical analysis

Linear mixed models were used to evaluate the fixed effects of fire, fertilisation and year; with block, subplot within whole plot and day of year (scaled to zero) as random effects; on measured ecosystem properties using the *lmerTest* package for R (Kuznetsova et al., 2017). For C:N ratio of the dead plant biomass, we included random intercepts for litter functional group (i.e. forb or grass) and position within litter type (i.e. standing or laying on soil surface) in the mixed models to account for possible differences in N content from immobilisation. Linear models were used for C and N stocks. Indicator species analysis (SIMPER) was used to identify changes in dominant plant species for burned prairies (Oksanen et al., 2019). Certain response variables were naturally log-transformed (resin-sorbed N, N-cycling potentials, shoot C:N and total plant N stock) or square root-transformed (total and herbaceous ANPP) to meet model assumptions of normal distribution. In the case that a linear mixed model resulted in a singular fit, the subplot within whole plot random effect was removed to reduce model complexity (Barr et al., 2013). Analysis of variance (ANOVA) was used to calculate *F*- and *p*-values for each linear model, and pairwise comparisons for significant effects or their interactions were evaluated using Tukey's post hoc analyses (Lenth, 2016). Following the post hoc analyses, we defined an ecosystem property as 'recovered' if it was statistically similar in both control and recovering treatments, but different in the fertilised treatment relative to both control and recovering treatments. Data were handled and visualised using *tidyverse* (Wickham et al., 2019) in R v4.3.3 (R Core Team, 2024) within the Rstudio environment v2023.12.1 + 402 (Posit Team, 2024).

3 | RESULTS

3.1 | Delayed recovery of the nitrogen cycle

Resin-sorbed N—an index of soil inorganic N availability—partially recovered, decreasing 84% in the first year of fertiliser cessation relative to the continuously fertilised treatment, but it did not recover further in subsequent years, remaining an order of magnitude higher in recovering soils than in never-fertilised soils (Figure 1a,b). Unburned prairie soils had higher resin-sorbed N than burned prairie soils across all N-fertilisation treatments (Figure 1a,b; $F_{1,6} = 22.2$, $p = 0.003$), but, contrary to our predictions, prescribed fire did not affect the proportional recovery of resin-sorbed N (Table S1). However, the level of resin-sorbed NO_3^--N relative to NH_4^+-N did recover more strongly in the annually burned than the unburned treatment: The resin-sorbed NO_3^--N:NH_4^+-N ratio was similar in

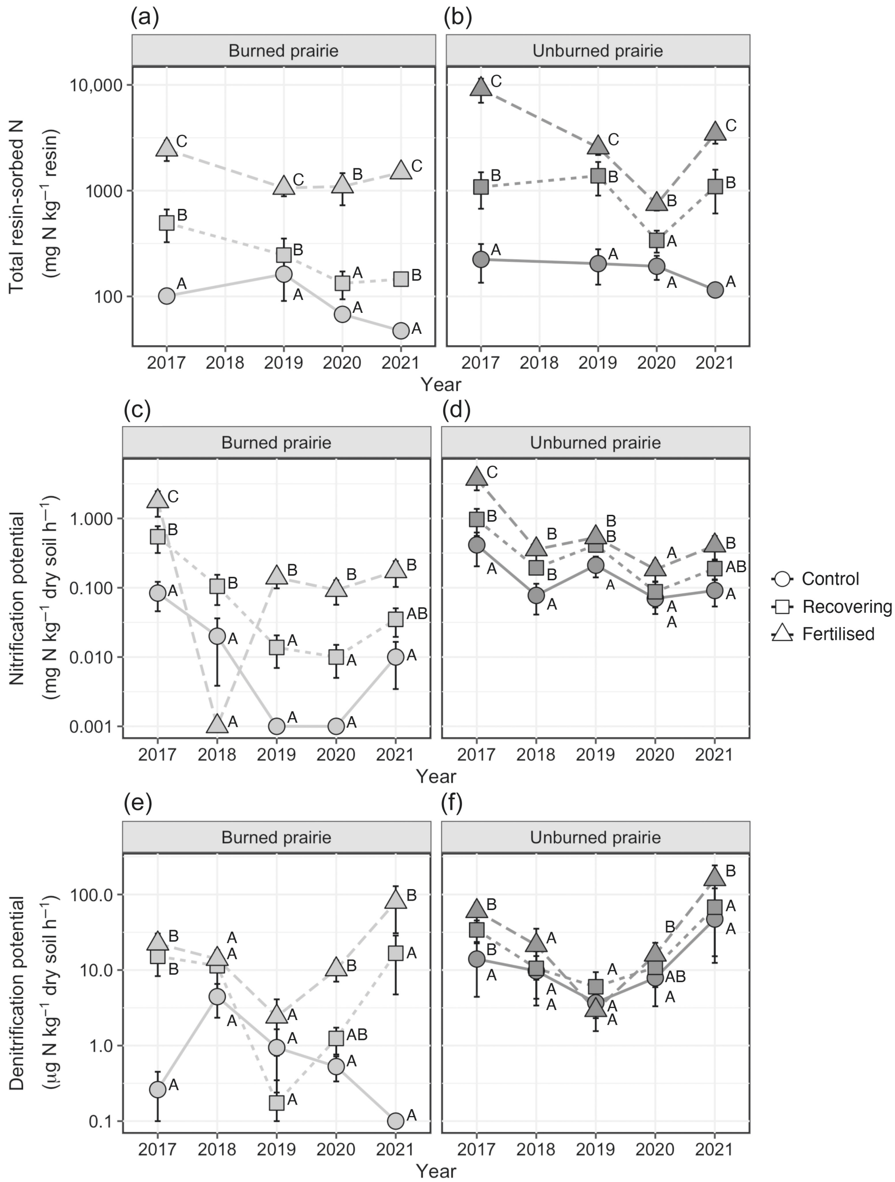

FIGURE 1 Total resin-sorbed N extracted from surviving resin bags during the growing season in 2017 and 2019–2021 (a, b), and nitrification potential (c, d) and denitrification potential (e, f) rates estimated twice during the growing season in 2017–2021, for both burned and unburned prairie treatments. Each point is the average annual value for the fertilisation treatment, and the error bars are ±1 standard error (SE) ($n=8$). Letters denote post hoc differences between fertilisation treatments within the year and fire treatment at $p \leq 0.05$.

recovering and never-fertilised burned treatment soils, with both treatments lower than the continuously fertilised burned treatment (fire × fertilisation interaction: $F_{2,56}=5.2$, $p=0.008$; Figure S2).

Soil microbial N-cycling potentials dropped after ceasing N-fertilisation, but their recovery trajectories varied among the measured processes (Table S1). Nitrification potential recovered in the third-year post-cessation in burned prairies, 1 year earlier than unburned prairies based on statistical results (fire × fertilisation × year interaction: $F_{8,197}=4.0$, $p<0.001$); though rates remained lowest, sometimes below detectable levels, in never-fertilised soils (Figure 1c,d). In contrast, denitrification potential recovered in both burned and unburned prairies 5 years after fertiliser cessation (fertilisation × year interaction: $F_{8,198}=4.1$, $p<0.001$), yet rates were similar across all fertilisation treatments in the second- and third-year post-fertiliser cessation for both fire treatments (Figure 1e,f), with rates lowest in 2019. Maximum denitrification enzyme activities, assayed with glucose and nitrate added, did not respond to fertilisation or fire treatment, or vary interannually (Figure S3; Table S1).

3.2 | Plant responses to nitrogen fertiliser cessation

Above-ground net primary productivity (ANPP) of herbaceous plants did not recover after ceasing N-fertilisation for 5 years (Figure 2c,d; Table S1), and, contrary to our predictions, this positive legacy of fertilisation across the 5-year period was stronger in burned prairies ($ANPP_{Recovering} - ANPP_{Control} = 446 \pm 247\ g\,m^{-2}$) than unburned prairies ($80 \pm 34\ g\,m^{-2}$). In turn, higher herbaceous growth in the recovering burned prairie produced more litter ($332\ g\,m^{-2}$) from the 2020 growing season that could be consumed by fire in 2021 ($t=2.53$, $p=0.045$), but only an additional $1.1\ g\,N\,m^{-2}$ was vulnerable to loss

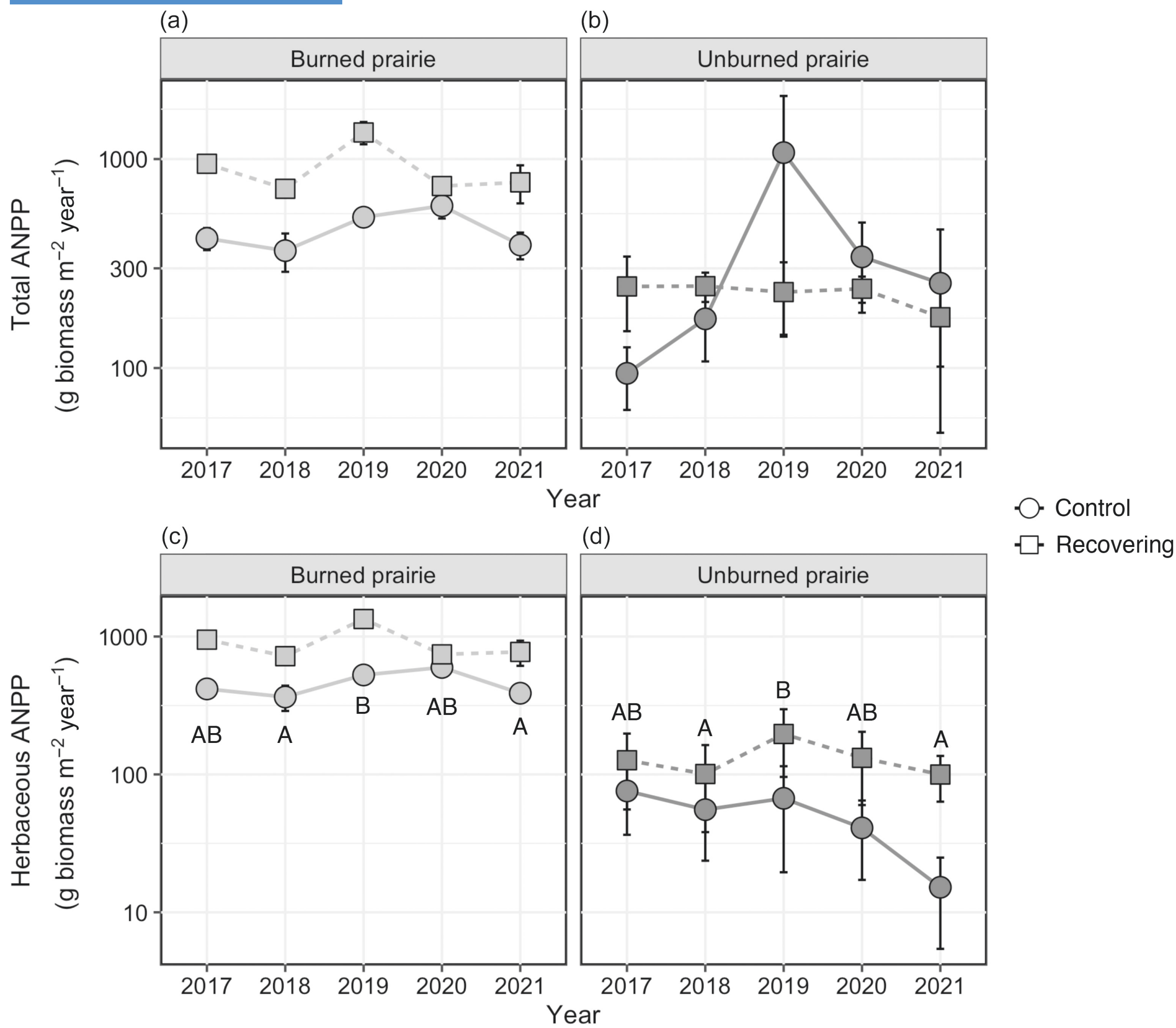

FIGURE 2 Total (a, b) and herbaceous (c, d) ANPP in 2017–2021 for both burned and unburned prairie treatments, excluding the still-fertilised subplots. Each point is the average ANPP for the fertilisation treatment, and the error bars are ±1 SE ($n=4$). Letters denote post hoc differences among years at $p \leq 0.05$.

through pyrovolatilisation ($p=0.052$; Figure S4). Helping to explain this small difference in litter N stock, the live plant shoot C:N ratio during the 2021 growing season was surprisingly higher (less N relative to C) in the recovering treatment than the control treatment (Figure 3a), indicative of a negative legacy (Sala et al., 2012). The root tissue C:N ratio in the recovering treatment was similar to that in control prairies, and in burned prairies, the mean root C:N in control and recovering treatments was higher than in the continuously fertilised treatment (Figure 3b). Because of the recovery in plant tissue N levels post-fertilisation, there was no difference in total plant N stock between control and recovering prairies in either fire treatment (Figure 4a,b; Table S2).

While plant tissue chemistry rebounded after fertiliser cessation, the plant community composition did not. Switchgrass (*Panicum virgatum*) replaced big bluestem (*Andropogon gerardii*) as the dominant plant species in chronically fertilised burned prairies (Carson et al., 2019), and remained dominant following fertiliser cessation (SIMPER, $p=0.001$): *P. virgatum* was 90±3% of total cover in the recovering treatment, relative to 16±8% in the never-fertilised treatment (Figure S5).

3.3 | Partial recovery of soil nitrogen

Despite no recovery of ANPP and delayed recovery of N-cycling microbial processes, surface soil N stocks partially recovered (Figure 4c,d). Furthermore, the difference in soil N stock between the still-fertilised and recovering treatments, that is, the estimated N loss following treatment cessation, was similar in burned and unburned prairies: 20 and 28 g N m^{-2}, respectively. In burned prairie soils, the estimated cumulative N loss from pyrovolatilisation was 20±2 g N m^{-2} (Figure S6), based on the respective measurement of 0.43±0.10 %N and 0.62±0.18 %N in grass and forb litter before spring burning in 2021. Using grass tissue %N reported

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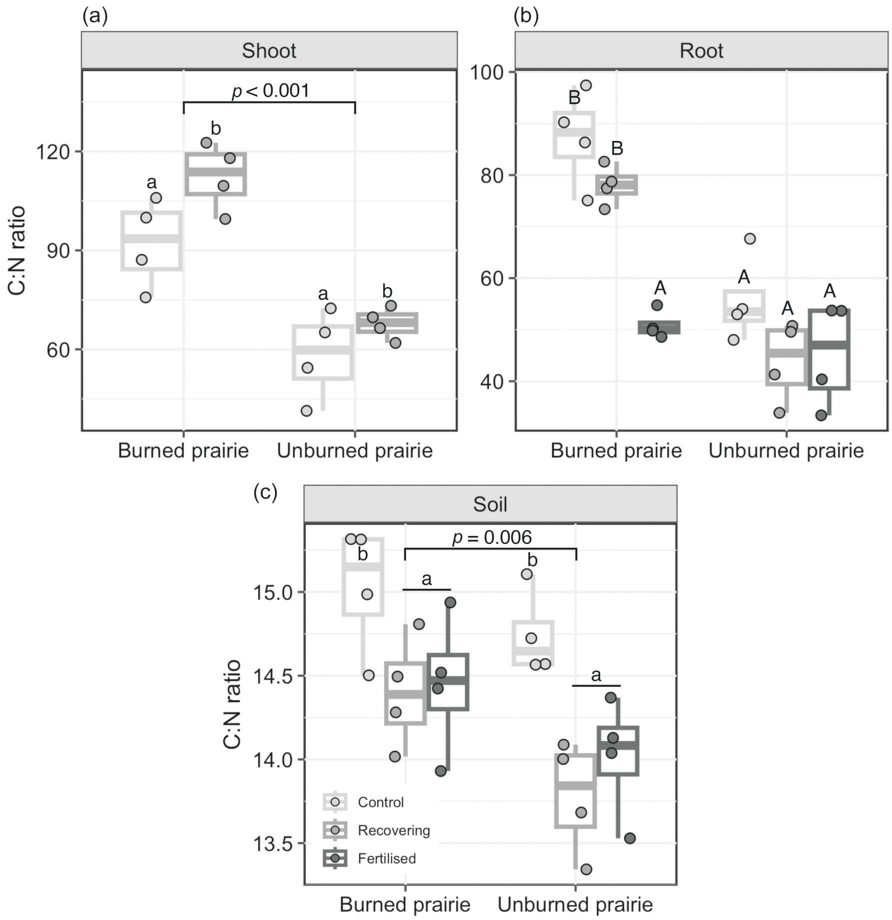

FIGURE 3 C:N ratio of shoots (a), roots (b) and soils (c) in burned and unburned prairies at the end of the 2021 growing season. Each point is the average C:N ratio for each individual plot corresponding to the fertilisation treatment, with the different fertilisation treatments denoted by colour ($n=4$). Shoot C:N ratios were not collected for the still-fertilised subplots. Capital letters for root C:N denote post hoc differences between combined field treatments, while lowercase letters in the shoot and soil C:N indicate post hoc differences between fertilisation treatments, at $p \leq 0.05$.

elsewhere (0.4–0.7 %N; Norris et al., 2001; Reed et al., 2005; Roley et al., 2018), this loss could range from 19 to 32 g N m^{-2}. In contrast, unburned prairie soils could have lost up to 94 ± 109 g N m^{-2} from denitrification during the recovery period, nearly three times greater than the 32 ± 18 g N m^{-2} estimated from burned prairie soils (Figure S6). After considering diurnal patterns and correcting for potential overestimation from single time-point denitrification measurements (maximum mean bias value = 58.8%; Wu et al., 2021), the estimated maximum N loss to denitrification in the unburned prairies is 54.7 g N m^{-2} and is 18.8 g N m^{-2} in the burned prairies. After 30 years, up to 20%–40% of the total 300 g N m^{-2} added experimentally was retained in surface mineral soil, based on data collected 1 year after fertiliser cessation (Nieland et al., 2021); 5 years after cessation, however, fertiliser-N retention in soil was only 3% and 12% in the recovering burned and unburned treatments, respectively.

Soil C stock was also lower in the recovering treatments than in the continuously fertilised soils, though fertilisation treatments did not differ statistically (Figure S7). Notably, the soil total C:N ratio was significantly lower in continuously fertilised treatments relative to control conditions, but did not recover following fertiliser cessation in either fire treatment, and was also lower in unburned than burned prairies independent of fertiliser treatment (Figure 3c).

4 | DISCUSSION

We monitored soil microbial N-cycling and plant recovery for 5 years after ceasing long-term N-fertilisation at a field experiment with contrasting fire treatments, to test the hypothesis that fire-mediated removal of exogenous ecosystem N results in faster and greater recovery of ecosystem properties in burned prairies. Contrary to this hypothesis, the material legacy of total soil N was similar between burned and unburned prairies, and there were minimal differences in soil microbial N-cycling recovery between fire treatments. Moreover, high herbaceous ANPP persisted in the recovering burned prairie, although plant tissue chemistry recovered in burned prairies, meaning less combustible N in litter. Therefore, our results suggest that (1) fertilisation-induced shifts in functional traits of soil microbial and plant populations can persist after ceasing N-fertilisation, and can in turn (2) support different legacies of ecosystem function during ecosystem recovery that drive comparable levels of N loss.

4.1 | Recovery of nitrogen availability incomplete, unaffected by fire

Plant available resin-sorbed N fell immediately after ceasing N fertiliser application, but did not return to control levels, nor did

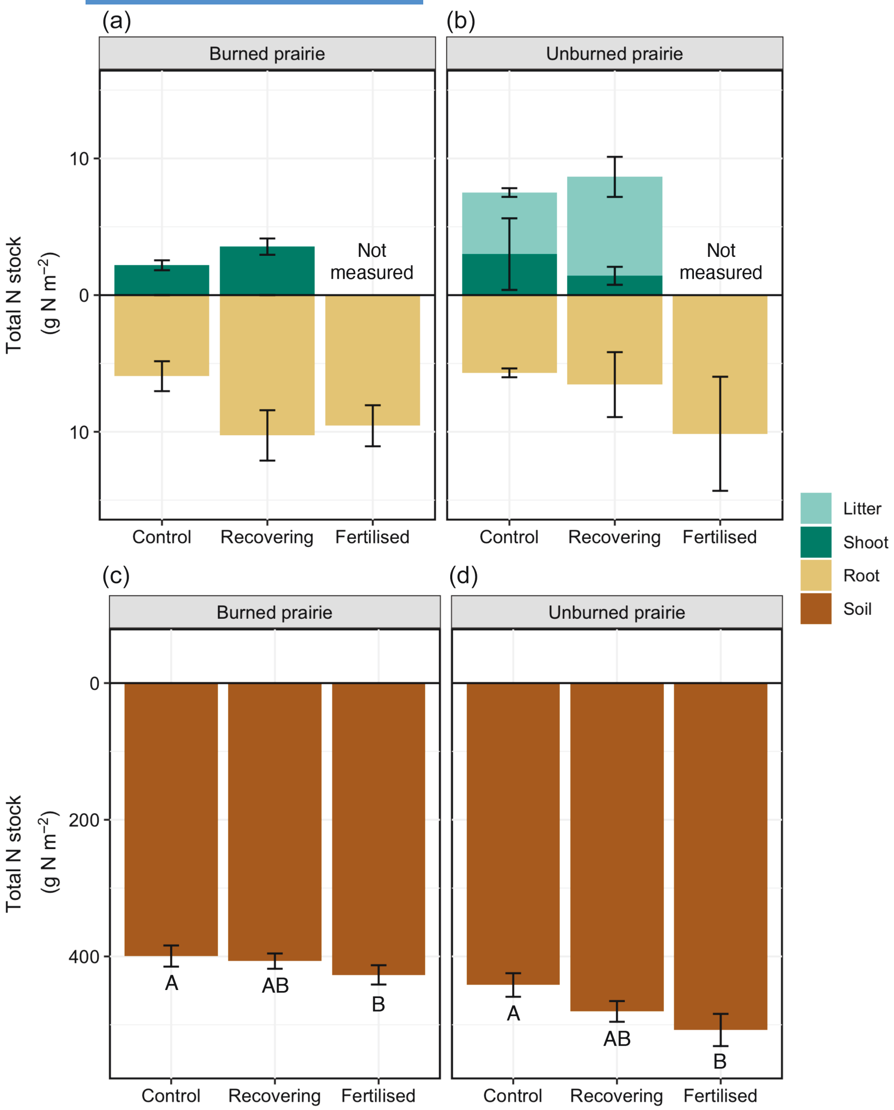

FIGURE 4 Plant (a, b) and soil (c, d) N stock in 2021 for both burned and unburned prairie treatments. Roots and soil were collected from the top 15 cm soil depth. Each bar is the average stock for the fertilisation treatment, and the error bars are ±1 SE ($n = 4$). Capital letters denote post hoc differences among fertilisation treatments at $p \le 0.05$.

burning enhance its recovery (Figure 1a,b). This positive legacy in N mineralisation, the process that increases resin-sorbed N, has also been observed at other N-cessation field experiments (Clark et al., 2009; O'Sullivan et al., 2011) and is often attributed to the higher N litter and SOM pools accumulated during fertilisation (Clark et al., 2009; Frankenberger & Abdelmagid, 1985; Liu et al., 2020; Manzoni et al., 2008). Net N mineralisation is linked to low C:N of decomposing organic matter, which originates from plant litter and accumulates in the soil (Hobbie, 2015; Parton et al., 2007). In the burned treatment of this study, the consistently lower C:N ratio of SOM post-fertilisation more likely explains the positive legacy of N mineralisation, since plant tissue C:N either fully recovered or exhibited a negative legacy of fertilisation (Figure 3). While fire in grassy ecosystems drives soil N availability at multiple time scales (Blair, 1997; Johnson & Matchett, 2001; Romanyà et al., 2001), in this experiment, annual prescribed fire did not affect recovery of soil N availability for the first 5 years following N-fertiliser cessation.

4.2 | Delayed recovery of microbial N-cycling minimally affected by fire

As we predicted, nitrification potential recovered faster in the burned treatment, but only by 1 year relative to the unburned treatment (Figure 1c,d). Microbial nitrifiers grow slowly using ammonium for cellular energy via chemolithoautotrophy and can be outcompeted by plants and microbial heterotrophs (Gill et al., 2023; Schimel & Bennett, 2004), so stronger competition for ammonium under more N-limited conditions in burned prairies could explain the faster recovery of nitrification potential. Indeed, the NO_3^--N:NH_4^+-N ratio recovered in burned prairies (Table S1), meaning lower nitrate production and providing further evidence of greater recovery of nitrification in the burned treatment. However, since soil N availability remained elevated in the recovering treatment (Figure 1a,b), plant–microbe competition for ammonium was still weaker than in unfertilised conditions and cannot fully explain the results. At this field experiment, fertilisation increased total ammonia oxidiser abundance, but bacterial, not archaeal, nitrifying populations recovered (Nieland et al., 2021). Archaeal ammonia oxidisers are more competitive than bacterial ammonia oxidisers at lower substrate levels typical of unfertilised conditions (Prosser & Nicol, 2012; Verhamme et al., 2011), so the lack of recovery of this microbial functional group is a putative information legacy supporting higher nitrification rates in the previously fertilised soils.

In contrast, denitrification potential recovered within the same time frame in both fire treatments (Figure 1e,f). Heterotrophic denitrifiers require many conditions to be met for high process rates (Wallenstein et al., 2006); thus, interannual variability in denitrification activities was expected (Groffman et al., 1993). Precipitation in the second and third years of fertiliser cessation was substantially below and above average, respectively (Figure S1), which likely affected denitrification potentials. Denitrification response could have been limited by nitrate or C substrate availability, or less anoxic conditions, in the dry year. In the wet year, low denitrification was more surprising, given limited oxygen availability in wetter soils, and higher NO_3^--N:NH_4^+-N ratio levels (Figure S2); however, plants could assimilate more N with water limitation alleviated (Ren et al., 2017), and because nitrate is highly mobile, more leaching was possible during this wet year. Instead of denitrifier abundance, some aspects of substrate availability more likely control denitrification recovery, in a fire-independent manner.

4.3 | Legacies in plant productivity and species dominance

Grassland primary production is generally limited by N availability, and our results show that high ANPP can persist for at least 5 years after ceasing N-fertilisation (Figure 2). This positive legacy of N-fertilisation on ANPP was clearly supported by herbaceous growth (Figure 2c,d) which is not surprising since herbaceous species are usually responsive to N-addition (Hall et al., 2011; Yahdjian et al., 2014). However, in the unburned treatment, the absolute N-fertilisation effect on ANPP ($g\,m^{-2}$) was smaller, probably because herbaceous growth was lower overall in unburned prairies due to woody encroachment (Ratajczak et al., 2011; Tooley et al., 2022); because of lower herbaceous growth, the N-fertilisation effect size was stronger in unburned prairies (Unburned: 224%; Burned: 101%). Therefore, N availability alone does not explain the strong positive legacy of fertilisation on ANPP, especially given the loss of exogenous N via pyrovolatilisation in burned prairies. Although perennial grasses conserve N overwinter by retranslocating up to 70% of their N in leaves and stems to below-ground organs (Hayes, 1985), the lower quality of growing plant tissues post-fertilisation than in the never fertilised treatment does not mechanistically support this positive legacy.

Instead, a shift in the dominant plant species more likely explains the positive legacy in ANPP (Figure S5). *Panicum virgatum* is a highly productive species that is a good competitor under N-enriched conditions, because it intercepts a large proportion of light and has flexible N demand physiology (Dybzinski & Tilman, 2007; Harpole & Tilman, 2006; Tilman, 1982), and in this experiment, *P. virgatum* remained productive even though N availability and plant tissue quality decreased. Thus, plant species turnover due to chronic N-addition prevented herbaceous ANPP recovery from long-term fertilisation, constituting another information legacy. In other long-term fertilisation experiments, plant turnover constrained post-fertilisation recovery (Isbell et al., 2013), but litter removal through fire was postulated to accelerate recovery (Meyer et al., 2023; Storkey et al., 2015). Contrary to predictions from other studies, in this experiment, fire did not promote the recovery of plant composition or ANPP within 5 years of fertiliser cessation because species-specific stoichiometric flexibility constrained recovery, which is also contrary to some predictions based on plant stoichiometric homeostasis (e.g. Yu et al., 2015).

4.4 | Soil nitrogen legacies maintained by plant and microbial feedbacks

Unexpectedly, the recovery of total soil N was similar in magnitude between fire treatments (Figure 4c,d). A previous ^{15}N-ammonium tracer study at this experiment showed high levels of N immobilisation in plants and soils in unfertilised, burned prairies (Dell et al., 2005; Dell & Rice, 2005), with 78% of the ^{15}N-ammonium remaining 5 years after labelling. While our results seem to contradict this observation, the same study also found that N retention decreased substantially after 8 years of fire suppression with only 52% of the ^{15}N remaining, showing that the N retention capacity of this ecosystem can change over time. Based on our data, after 30 years, chronically N-fertilised prairies may have further lost capacity to retain N (Aber et al., 1998; Lovett & Goodale, 2011), but more work would be needed to confirm this directly. Most notably, despite the similar levels of recovery, different pathways contributed most to soil N loss and ecosystem recovery. Fire losses alone could account for the N recovery in burned prairies, while soil microbially mediated N losses were substantially higher in unburned prairies.

The most likely legacy soil N pools vulnerable to loss during recovery are SOM in macroaggregates or particulate organic matter (POM). In the local ^{15}N-ammonium tracer study, 93%–94% of N retained in both burned and unburned prairies was in the soil microbial and SOM pools (Dell et al., 2005; Dell & Rice, 2005). In our study, the C:N ratio of SOM was reduced by both long-term fire suppression and N-fertilisation, but did not rebound after ceasing fertilisation (Figure 3c). The lower total soil C:N from fertilisation suggests an enrichment of microbial necromass, because soil microbial biomass, particularly of bacteria, has lower C:N than plant biomass (Cleveland & Liptzin, 2007; Cotrufo et al., 2015; Liang et al., 2017; Strickland & Rousk, 2010); thus, the lack of recovery in soil C:N represents another material legacy. Microbial necromass can persist as mineral-associated organic matter (MAOM) for decades to centuries because of chemical bonding to clay mineral surfaces (Kögel-Knabner et al., 2008; Lavallee et al., 2020; Von Lützow et al., 2007), so it is unlikely that MAOM pools changed over 5 years. Instead, the partial recovery in total soil organic matter N and C stocks is more likely related to changes in other SOM pools. For example, upon cessation of fertilisation, physically protected SOM in aggregates may have become vulnerable to microbial breakdown through modified plant–fungal relationships. In the same field experiment, 17 years of N-fertilisation induced stronger P-limitation (Wilson et al., 2009); thus, more plant root arbuscular mycorrhizal fungal colonisation increased the proportion of soil macroaggregates (Johnson et al., 2015; Wilson et al., 2009), while fungicide application decreased the macroaggregate proportion (Wilson et al., 2009). With the relief of P-limitation following N-fertiliser cessation (Nieland et al., 2024), the mycorrhizal relationship becomes less necessary for the host plant, so macroaggregate loss was likely during the recovery phase. Furthermore, POM is the most vulnerable N substrate for microbial decomposition (Lavallee et al., 2020; Mooshammer et al., 2014). Although root biomass and N stock were similar among fertilisation treatments (Figure 4a,b; Table S2), the potentially large POM pool in fine roots is difficult to measure directly and may have been better accounted for in our soil total N measurements than our root measurements.

4.5 | Legacies of nitrogen eutrophication

Evidence from this field experiment and other studies to date support the idea that historical N eutrophication can create legacies that constrain contemporary ecosystem function. The positive legacy in net N mineralisation at this field experiment, quantified from resin-sorbed N, is consistent with other N-cessation experiments (Clark et al., 2009; Olff et al., 1994; O'Sullivan et al., 2011), suggesting a widespread legacy that can persist for at least a decade. In this experiment, persistently higher N availability could be related to the material legacy of lower C:N in the SOM, despite substantial recovery of total soil N pools. Also, a positive legacy in nitrification has emerged in other experiments (Olff et al., 1994; Stienstra et al., 1994), and previous research suggests that this legacy may be connected to an information legacy of shifts in ammonia-oxidising populations (Nieland et al., 2021), in addition to higher ammonium availability.

Not all findings at this field experiment were consistent with other studies. We found no effect of N removal from fire on the recovery of plant community composition, contrasting with other grassland experiments where mowing supported recovery (Hu et al., 2021; Storkey et al., 2015), illustrating how different management practices that remove exogenous N may not result in similar ecosystem recovery outcomes. Additionally, we found no evidence that N-enriched litter promotes higher net N mineralisation (Clark et al., 2009); rather, if necromass is responsible for the lower soil C:N, then faster growth and turnover of soil microbial biomass (Blaško et al., 2013; Hart et al., 1994) in fertilised conditions may support higher N availability. Long-term N-fertilisation increased the abundance of soil microbial copiotrophs (i.e. populations with putatively faster growth rates) in this field experiment (Carson & Zeglin, 2018; Nieland et al., 2021, 2024), supporting this mechanism. Also, in contrast to many field experiments where soil pH decreases from N-fertilisation (Riggs & Hobbie, 2016; Treseder, 2008) and constrained recovery from eutrophication (Bowman et al., 2018), soil pH minimally decreased at this site (Nieland et al., 2024). In more arid grasslands, while both fire and gaseous loss pathways would likely be weaker due to lower plant production and unfavourable conditions for denitrification, N pool recovery could be similar due to decreased plant and microbial N demand (Broderick et al., 2022) which reduces the potential for N retention in less mesic systems (McCulley et al., 2009). Overall, despite some generality in N-cycling legacy mechanisms, it is likely that site-specific characteristics (including soil factors, climate and precipitation variability and management strategies and capacities) will determine local trajectories in ecosystem recovery from N eutrophication.

5 | CONCLUSIONS

In summary, we found that the ecological memory of N eutrophication via plant and microbial feedbacks shapes contemporary ecosystem structure and function even after cessation of chronic N-fertilisation. Although land management affects ecosystems, our results demonstrate that contrasting fire regimes can elicit similar recovery outcomes (e.g. soil total N loss) because different mechanisms predominate under different practices (pyrovolatilisation with fire management vs. soil microbially mediated loss in the absence of fire). Because of site-specific characteristics, however, it is important to monitor the recovery of individual ecosystem properties from N eutrophication. Therefore, we encourage others to quantify the prevalence and persistence of different legacies from N-fertilisation experiments to better understand ecosystem responses to the continued decreases in N availability.

AUTHOR CONTRIBUTIONS

Matthew A. Nieland and Lydia H. Zeglin conceived the ideas and methodologies; Matthew A. Nieland collected and analysed the data; Matthew A. Nieland led the writing of the manuscript with Lydia H. Zeglin providing feedback. Both authors gave final approval for publication.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

All data are available through the Konza Prairie Long-Term Ecological Research website, at https://doi.org/10.6073/pasta/cb24fd55a0102723882ad33ed3f787b9 and https://doi.org/10.6073/pasta/41f0e5aa1781e722e7922aaaa6b57143, hosted by the Environmental Data Initiative.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Cumulative precipitation during the growing season (April–September) collected near the BGP experiment at KBPS in 1986–2021, shown yearly during the study period (2017–2021).

Figure S2. Ratio of NO_3^--N:NH_4^+-N sorbed from surviving resin bags during the growing season in 2017 and 2019-2021.

Figure S3. Denitrification enzyme activity, under non-limiting C and N conditions, estimated twice during the growing season in 2017–2021.

Figure S4. Combustible plant litter (a), total combustible N in litter (b), and grass and forb litter C:N ratio (c) quantified before the 2021 prescribed fire in burned prairies, summarized by boxplots.

Figure S5. Percent cover of big bluestem (*A. gerardii*) and switchgrass (*P. virgatum*) in burned prairies in 2021.

Figure S6. Estimated cumulative nitrogen loss in burned and unburned recovering prairies.

Figure S7. Plant (a, b) and soil (c, d) C stock in 2021 for both burned and unburned prairie treatments.

Table S1. Statistical results for soil available N, microbial N-cycling, and above-ground net primary productivity.

Table S2. Statistical results for ecosystem C and N stocks.

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