

GRASSLAND ESTABLISHMENT UNDER VARYING RESOURCE AVAILABILITY: A TEST OF POSITIVE AND NEGATIVE FEEDBACK

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Abstract. The traditional logic of carbon (C) and nitrogen (N) interactions in ecosystems predicts further increases or decreases in productivity (positive feedback) in response to high and low fertility in the soil, respectively; but the potential for development of feedback in ecosystems recovering from disturbance is less well understood. Furthermore, this logic has been challenged in grassland ecosystems where frequent fires or grazing may reduce the contribution of aboveground litter inputs to soil organic matter pools and nutrient supply for plant growth, relative to forest ecosystems. Further, if increases in plant productivity increase soil C content more than soil N content, negative feedback may result from increased microbial demand for N making less available for plant growth. We used a field experiment to test for feedback in an establishing grassland by comparing aboveground net primary productivity (ANPP) and belowground pools and fluxes of C and N in soil with enriched, ambient, and reduced N availability. For eight years annual N enrichment increased ANPP, root N, and root tissue quality, but root C:N ratios remained well above the threshold for net mineralization of N. There was no evidence that N enrichment increased root biomass, soil C or N accrual rates, or storage of C in total, microbial, or mineralizable pools within this time frame. However, the net nitrogen mineralization potential (NMP) rate was greater following eight years of N enrichment, and we attributed this to N saturation of the microbial biomass. Grassland developing under experimentally imposed N limitation through C addition to the soil exhibited ANPP, root biomass and quality, and net NMP rate similar to the ambient soil. Similarity in productivity and roots in the reduced and ambient N treatments was attributed to the potentially high nitrogen-use efficiency (NUE) of the dominant *C₄* grasses, and increasing cover of legumes over time in the C-amended soil. Thus, in a developing ecosystem, positive feedback between soil N supply and plant productivity may promote enhanced long-term N availability and override progressive N limitation as C accrues in plant and soil pools. However, experimentally imposed reduction in N availability did not feed back to reduce ANPP, possibly due to shifts in NUE and functional group composition.

Key words: carbon amendment; grassland ecosystems; nitrogen enrichment and saturation; plant productivity; progressive nitrogen limitation; tallgrass prairie restoration.

INTRODUCTION

Feedback between plants and soil has important implications for long-term productivity and the cycling of nutrients in ecosystems (Vitousek 1982, Johnston et al. 2004, Ehrenfeld et al. 2005). Decomposition rates and patterns of soil N supply are influenced by litter quality (i.e., C:N or lignin:N ratios), as determined by plant species composition and/or N content (Melillo et al. 1982, Blair et al. 1990, Prescott 2002). In general, positive feedback is expected in higher fertility systems when organic matter inputs with low C:N ratio increase N supply to plants by promoting N mineralization during decomposition (Vitousek 1982, Pastor et al. 1984, Hobbie 1992, Aerts 1995). This positive feedback mechanism has also been invoked to explain ecosystem responses to elevated N inputs, either through atmo-

spheric deposition or fertilization (Aber et al. 1993, Tateno and Chapin 1997). Likewise, positive feedback is predicted in low fertility systems as a result of production of plant litter with low N content (high C:N ratio), which enhances N immobilization and reduces N supply to plants, further decreasing plant productivity (Chapin 1991). Clark et al. (2005) also concluded that feedback can occur through litter quality, but propose alternative mechanisms including variation in the conversion rate of litter to different forms of N (i.e., complex organic N substrates vs. simple inorganic N ions) or the fraction of N recycled.

The relationship between plant and soil C and N interactions has been intensively modeled for forest ecosystems, under assumptions of steady-state conditions and a closed N cycle, with and without inorganic N inputs (Tateno and Chapin 1997). Simulated changes in ecosystem C and N pools and fluxes during 10 years of N enrichment led to predicted increases in plant C and N, net primary productivity, litter N content, and net N

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mineralization rates (Tateno and Chapin 1997). This positive feedback mechanism has been challenged in grassland ecosystems (Knops et al. 2002), where less N is returned to the soil system via aboveground litter inputs, since much of the plant N is translocated to roots during senescence, and surface litter can be removed by fire (Knapp and Seastedt 1986, Blair 1997). Knops et al. (2002) proposed that plant tissue C:N ratio may be slow to influence N cycling and availability because plant N inputs must first pass through the soil organic matter (SOM) pool, where microbial demand for N regulates transformations of N. Furthermore, if N cycling is regulated by the effects of plant-derived C on the microbial N loop (Hart et al. 1994), then N released from the SOM may invoke negative feedback if mineralized N increases belowground accrual of C, and subsequent microbial demand for N. Logically, there is potential for C and N cycling in ecosystems to be linked through either positive or negative feedback. However, little is known about the potential for feedback to develop in ecosystems recovering from disturbance, where accumulations of soil C and N are below steady-state conditions and accruing over time.

Soil C storage results from the balance between inputs of C from primary producers and losses of C during decomposition, and pools and fluxes of C and N vary with a system's condition relative to steady state. In the Central Plains of North America, native grassland soils contain high levels of SOM due to comparatively high belowground productivity of the dominant grasses and slow decomposition rates under relatively dry conditions (Risser and Parton 1982). Consequently, SOM-rich grasslands are characterized by high gross, but low net, N mineralization rates; cultivated grassland soil shows the opposite patterns (Schimel 1986). The conversion of grasslands to row-crop agriculture can result in 25–60% declines in soil C storage (Haas et al. 1957, Coleman et al. 1984, Parton et al. 1988, McLauchlan 2006). Reestablishment of the dominant perennial grasses catalyzes a transition from lower to higher steady-state soil accumulations of SOM and associated nutrients, and increases in soil C during grassland recovery from cultivation have been well documented (Gebhart et al. 1994, Knops and Tilman 2000, Kucharik et al. 2001, Baer et al. 2002, McLauchlan 2006, McLauchlan et al. 2006). Carbon and nitrogen interact during ecosystem recovery from disturbance to promote progressive N limitation (Luo et al. 2004). During grassland reestablishment following cultivation, available N pools and net N mineralization rates decline rapidly with increases in root biomass, root C:N ratio, microbial biomass, and soil C (Baer et al. 2002).

Dynamic changes in plant community composition and belowground C pools, particularly labile and active pools (Staben et al. 1997, Baer et al. 2002, McLauchlan et al. 2006), over time during grassland development may modulate feedback between plants and soil. We quantified aboveground net primary productivity

(ANPP), root biomass and quality, soil C and N pools and transformations, and plant functional group composition periodically during eight years of grassland establishment under enriched-N, ambient, and reduced-N conditions. This experimental framework allowed us to test whether positive or negative feedback occurs during conditions that are not steady state and that characterize ecosystem recovery. We hypothesized that annual N enrichment would promote positive feedback through increased ANPP and production of roots with higher N content, which would increase labile soil N and N supply to plants. Alternatively, if N enrichment increased plant C assimilation disproportionately, resulting in greater belowground inputs of lower quality roots, then increases in soil C pools would promote microbial demand for N and reduce the supply of N to plants from the soil system (i.e., negative feedback between initial N supply and subsequent plant productivity). We also tested for positive feedback in grassland establishing under reduced N availability created by manipulation of the soil C pool. We predicted that low N availability resulting from enhanced microbial demand for N in C-amended soils (i.e., alleviated C limitation) would increase microbial demand for N, reduce N supply and availability to plants, which would result in depressed ANPP, less root biomass, and higher root C:N ratio that would further reduce N availability. Ideally, tests for feedback between plants and soil under varying nutrient regimes would hold plant composition constant. All of our experimental treatments were seeded with the same species and density, but species and functional group sorting over time are inherent to ecosystem recovery following disturbance. Thus, we examined changes of grasses and legumes over time to elucidate whether sorting of these key functional groups might modulate positive or negative feedback under varying resource availability.

MATERIALS AND METHODS

Study site

Research plots were located in a lowland agricultural field at Konza Prairie Biological Station (see Plate 1), 9 km south of Manhattan, Kansas, USA (39°05' N, 96°35' W). The soil was a gently sloping (0–1%) Reading silt loam (mesic Typic Arguidoll) formed by colluvial and alluvial deposits. Prior to cultivation, the vegetation would have been representative of lowland native tallgrass prairie, dominated by the warm-season (C₄) grasses *Andropogon gerardii* Vitman, *Andropogon scoparius* Michx., *Sorghastrum nutans* (L.), and *Panicum virgatum* L., interspersed with a variety of less common grass and forb species (Abrams and Hulbert 1987).

Experimental design and grassland establishment

As part of a larger grassland restoration experiment, sixteen 6 × 8 m plots separated by 6-m buffers were delineated in the agricultural field in 1997 (Baer et al. 2003). Results reported here are from a subset of plots (*n*

= 4) that included both soil depth and nutrient manipulations. A factorial combination of two levels of soil depth (deep and shallow) and three levels of soil N availability (reduced, ambient, and enriched) randomly assigned to horizontal and vertical strips produced two 2×2 m replicate subplots of six different soil treatment combinations in each of four 6×8 m plots. The two levels of soil depth were achieved by excavating all plots to a depth of ~ 25 cm and placing native limestone slabs in 2×6 m alternating strips in each plot. The soil was then replaced, leveled, and disked (2–3 cm deep). Three levels of N availability were randomly assigned to 2×8 m strips. To reduce soil N availability, 5.5 kg dry sawdust/m² (49% C; C:N ratio = 122) was incorporated into the soil prior to planting (February, 1998) to approximate C levels in native prairie soil ($\sim 3\%$ C). This application rate was based on a mean initial C content of 1.5% and bulk density of 1.2 g/cm³ in the 0–15 cm depth in the agricultural soil. Following the C amendment, all plots were tilled to promote similar conditions prior to sowing. This one-time addition of recalcitrant C significantly reduced N availability and net N mineralization rates for at least the initial three years of grassland establishment (Baer et al. 2003). To maintain N limitation over time, sugar was applied at a rate of 84.2 g C/m² (~ 200 g sucrose/m²) three times during the growing season, starting in 2005. Strips assigned to the enriched-N treatment were fertilized with ammonium nitrate at a rate of 5 g N/m² annually, following emergence of plants each year. All sampling was conducted within the 12 subplots (2×2 m) in each of the four blocks ($n = 48$).

In April 1998, all plots were sown with 42 native prairie species according to a log-normal distribution with species assigned to dominant, common, frequent, or uncommon grass categories. Baer et al. (2003) provide details on seed sources, storage treatment, and seeding procedures. Deer were excluded from the experimental area with a fence. Prescribed fire is a common management practice in tallgrass prairie, and the entire field site was burned in early spring prior to the second, fourth, sixth, seventh, and eighth growing seasons.

Plant productivity, cover, and root allocation

Aboveground NPP was sampled in 1998, 1999, 2000, 2004, and 2005 (Table 1). At the end of each growing season, all vegetation in a 0.10-m² area within each subplot was clipped at the soil surface, separated into current and previous year's biomass, dried at 60°C, and weighed. Current year's biomass was used to estimate ANPP (Briggs and Knapp 1991).

Percent cover of each plant species was determined in spring and summer for all plants rooted within two permanently located 50×50 cm quadrats in every subplot in 1998, 1999, 2000, 2003, and 2005 (Table 1). The maximum cover value of each species from these seasonal surveys was used to calculate total cover and mean cover of grass and legume functional groups.

Root biomass and tissue C and N were quantified in 2004 (Table 1). In each subplot a 5 cm diameter \times 20 cm deep core was collected and stored at 4°C. In the laboratory, samples were crumbled by hand over a 4 mm diameter sieve to separate large root segments from the soil. Fine roots were hand picked from the soil. No attempt was made to distinguish between live and dead roots. Roots were rinsed with deionized water, dried at 55°C, weighed, ground, and percentage of C and N were determined by dry combustion coupled with gas chromatography on a Carlo Erba CN Analyzer (Carlo Erba, Milan, Italy).

Availability and transformations of N

The surface 10 cm of soil in each plot was sampled for availability and transformations of N in 2003 and 2005. Two 2 cm diameter cores were removed and composited per subplot, and passed through a 4-mm sieve. Inorganic N concentrations were determined by extracting 10–12 g of field-moist soil with 50 mL of 2 mol/L KCl by agitating solutions on an orbital shaker at 200 rpm for 1 h before filtering solutions through 0.4- μ m polycarbonate membranes. To assess net nitrogen mineralization potential (NMP), a second 10–20 g subsample of soil was incubated in the laboratory at $\sim 25^\circ\text{C}$ for 30 days and 12 days in 2003 and 2005, respectively, and then extracted for inorganic N using the same methods. Difference in NH₄-N, NO₃-N, and NH₄-N + NO₃-N between the incubated and initial soil cores was used to calculate daily net ammonification, nitrification, and mineralization rates (Robertson et al. 1999). In 2005, in situ relative inorganic N availability was estimated in all subplots using ion exchange resins (Binkley 1984). Resin bags were constructed of nylon, and contained 20 g of a 1:1 mixture of cation (Dowex 50 WX2) and anion (Dowex 1X8-50) resins (Dow Chemical, Midland, Michigan, USA), preloaded with H⁺ and Cl⁻, respectively. One resin bag was buried in the surface 10 cm of each subplot in June and retrieved in September. Following retrieval, bags were stored at 4°C, rinsed with deionized water, and extracted with 75 mL of 2 mol/L KCl using the methods as for net NMPs. Extracts were analyzed for inorganic N using segmented flow analysis on an Alpkem autoanalyzer (Alpkem, Clackamas, Oregon, USA) from 1998 to 2003 and on an OI Analytical Flow IV autoanalyzer (formerly Alpkem; College Station, Texas, USA) in 2005. The indophenol blue method was used for colorimetric determination of NH₄-N, and diazotization with sulfanilamide after reduction of nitrate to nitrite through a cadmium coil was used for NO₃-N determinations (Keeney and Nelson 1982).

Soil C and N pools

Total carbon and total nitrogen in the surface 10 cm of soil was measured in each subplot prior to planting in 1998 and during each growing season in 1998, 1999, 2000, 2004, and 2005 (Table 1). Total C was also

TABLE 1. Summary of plant and soil sampling times (years and months), 1998–2005.

Variable	Year (years since establishment)							
	1998 (0–1)	1999 (2)	2000 (3)	2001 (4)	2002 (5)	2003 (6)	2004 (7)	2005 (8)
ANPP	Sep	Sep	Sep	–	–	–	Sep	Sep
Plant cover	Jun, Sep	Jun, Sep	Jun, Sep	–	–	Jun, Sep	–	Jun, Sep
Roots	–	–	–	–	–	–	Sep	–
Extractable N	+	+	+	–	–	Jul	–	Sep
Net NMP	+	+	+	–	–	Jul	–	Sep
Resin N	+	+	+	–	–	–	–	Jun–Sep
Total C and N	Feb, Jul	Jul	Jul	–	–	Sep	–	Sep
PMC	Jun	–	Mar	–	–	–	–	–
MB C and N	+	+	+	–	–	–	–	Sep

Notes: Variables measured before April of 1998 (prior to planting) were considered time-0 measurements. A plus sign indicates data from sampling in a prior study (see Baer et al. 2003); a minus sign indicates that the parameter was not sampled in the specified year. Abbreviations: ANPP, aboveground net primary productivity; net NMP, net nitrogen mineralization potential; PMC, potentially mineralizable carbon; MB C and N, microbial biomass carbon and nitrogen.

measured in the surface 5 cm of soil at the onset of the first growing season and prior to the third growing season (Table 1) to evaluate recovery of the mineralizable C pool as a proportion of the total C pool. Prior to planting and any soil manipulations, 10 soil cores were removed from each whole plot and composited to estimate initial C and N levels. For all other sampling events, two soil cores were removed from directly beneath and between rows of planted grasses, composited by subplot, and stored at 4°C. In the laboratory, soil samples were homogenized through a 4 mm diameter sieve. A 50-g subsample of soil was weighed, dried at 105°C, and reweighed to determine gravimetric soil water content. A 20-g subsample of soil was dried at 50°C, finely ground, and used to determine the percentage of C and N by dry combustion on a Carlo Erba CN Analyzer from 1997 to 2003 and then on a Thermo CN Analyzer (formerly Carlo Erba; CE Elantech, Lakewood, New Jersey, USA) in 2004 and 2005. Bulk density was quantified in four subplots of each treatment combination in each block to convert concentrations to g/m².

The size of the potentially mineralizable carbon (PMC) pool was determined by quantifying cumulative CO₂ efflux from long-term incubations of soil according to Rice and Garcia (1994) and Omay et al. (1997). Samples for PMC were taken shortly following the emergence of sown native plants in June 1998 and in March 2000. Two 5 cm diameter × 5 cm deep soil cores adjacent to and between plants were composited by subplot. Soils were sieved (4-mm) and repacked to dry mass-based bulk density in 10 cm long (5 cm diameter) PVC tubes lined with fine polyester material to contain the soil. Each core was placed in an airtight container with 5 mL of water to maintain humidity and sealed with a lid fitted with an airtight rubber septum. Soil cores were preincubated for one week at 25°C then leached in 50-mL increments with 300 mL of 0.01 mol/L CaCl₂. Following leaching, each soil core received 50 mL of a N-free nutrient solution (KH₂PO₄ [100 ppm P], K₂SO₄ [24 ppm K], MgSO₄ [113 ppm Mg], and CaSO₄ [4

ppm Ca]) and was equilibrated at 0.033 MPa for 6 h. Cores were incubated in the laboratory at 25°C and CO₂-C was sampled every three days during the first month, weekly during the second month, and every two weeks for the duration of the incubation period. Following sampling, jars were opened to allow CO₂ to equilibrate with the atmosphere. Headspace gas samples were analyzed using a gas chromatograph equipped with a thermal conductivity detector and a 2-m Poropak column (Shimadzu, Kyoto, Japan). Carrier gas (He) flow rate and column temperature were 14 mL/min and 70°C, respectively. Samples were incubated for 211 days and 212 days in 1998 and 2000, respectively, at which time all data could be modeled to quantify the mineralizable pool of C. Soil cores were leached every six weeks to remove accumulated inorganic N.

Soil microbial biomass carbon (MBC) and nitrogen (MBN) in the surface 10 cm of soil were determined in 2005 using the fumigation-incubation procedure (Jenkinson and Powlson 1976). At the end of the 10-day incubation, a 0.5-mL sample of headspace gas was removed from each serum bottle containing 8–10 g of soil and analyzed for CO₂ as for PMC. Following CO₂ measurements, fumigated and unfumigated samples were extracted, filtered, and analyzed for inorganic N concentrations (NH₄-N + NO₃-N) using the same methods as for net nitrogen mineralization potentials. Biomass C and N were calculated from the difference between fumigated and unfumigated CO₂-C and inorganic N, divided by a correction factor of 0.4 for MBC and 0.6 for MBN, respectively (Voroney and Paul 1984).

Statistical analyses

Plant and soil responses to the experimental treatments were analyzed according to a split-block design using the mixed-model procedure in SAS, Version 9.1 (SAS Institute 2004). Repeated measures were included in the analyses of aboveground net primary productivity, plant cover, and soil C pools in the surface 5 cm of soil. For repeated-measures analyses, we chose covariance structures that minimized Akaike's Information

TABLE 2. KCl-extractable inorganic nitrogen and potential net nitrogen transformation rates in the surface 10 cm of soil in 2003 and 2005 (years 6 and 8, respectively) of an establishing grassland.

Inorganic N pools and transformation rates	Year	Enriched N	Ambient N	Reduced N
Extractable NO ₃ -N (mg/m ²)	6	412.3 ^a (116.5)	158.5 ^b (63.8)	102.7 ^b (78.5)
	8	375.1 ^a (147.5)	156.6 ^b (110.0)	<0.01 ^c (<0.01)
Extractable NH ₄ -N (mg/m ²)	6	54.3 (11.7)	73.2 (31.9)	72.1 (34.9)
	8	54.0 (16.5)	59.5 (25.9)	20.4 (3.8)
Net ammonification rate (mg·m ⁻² ·d ⁻¹)	6	-29.8 (11.4)	-50.9 (31.3)	-42.2 (27.6)
	8	22.6 × 10 ⁻² (0.97)	3.3 × 10 ⁻³ (1.39)	3.6 (1.2)
Net nitrification rate (mg·m ⁻² ·d ⁻¹)	6	48.4 ^a (3.4)	37.9 ^{ab} (8.5)	24.4 ^b (11.2)
	8	24.8 ^a (7.8)	6.5 ^b (6.6)	4.9 ^b (2.9)
Net N mineralization (mg·m ⁻² ·d ⁻¹)	6	47.4 ^a (3.3)	36.3 (7.8) ^{ab}	23.0 ^b (10.7)
	8	25.0 ^a (8.0)	6.5 ^b (7.0)	8.6 ^b (3.8)
Resin-collected NO ₃ -N (µg/bag)	8	11 152.4 ^a (3327.1)	390.4 ^b (125.9)	67.1 ^c (24.3)
Resin-collected NH ₄ -N (µg/bag)	8	997.2 ^a (75.0)	705.7 ^b (220.0)	400.9 ^b (57.0)
Resin-collected total inorganic N (µg/bag)	8	12 129.6 ^a (3281.8)	1096.1 ^b (256.7)	467.7 ^c (64.8)

Notes: Inorganic nitrogen collected passively on ion exchange resin bags buried in the surface 10 cm of soil was used as an index of relative inorganic N availability in 2005. Values are given as means (with SE in parentheses). Means accompanied by the same lowercase letter within a row were not significantly different ($\alpha = 0.05$).

Criterion and Schwartz's Bayesian Criterion (Littell et al. 1996). When significant interactions were detected, contrast and estimate statements were constructed to test a priori comparisons (Milliken and Johnson 1992), specifically whether treatments varied within a year and whether changes occurred within a treatment over time ($\alpha = 0.05$; $P < 0.10$ reported). Although inorganic N was measured in 2003 and 2005, repeated-measures analyses were not performed due to different sampling times each year. The soil depth treatment was included in all statistical analyses, but did not significantly affect any of the belowground parameters. Thus, results focus on the consistently strong main effects of the nutrient treatment on ecosystem structure and processes. We report means and standard errors calculated from the mean of each treatment among the independent whole plots ($n = 4$) where above- and belowground parameters were measured in four subplots of each nutrient treatment.

Cumulative mineralized C (CO₂-C, g/m²) from each soil core was fit to one-pool, two-pool, and mixed-order kinetic models (Stanford and Smith 1972, Brunner and Focht 1984) using the nonlinear model-fitting procedure in SAS 9.1 (SAS Institute 2004). The most appropriate model was selected based on convergence to a unique solution, error estimates for the model parameters, and sums of squares for the model residual error. This modeling approach has been used successfully to estimate the mineralizable pool of C and decay coefficients of mineralization for grassland (Rice and Garcia 1994, Williams et al. 2004) and agricultural soils in the same region (Omay et al. 1997). Once the potentially mineralizable carbon pool and mineralization rate constants of were determined, they were analyzed using the same univariate methods.

Changes in total carbon and total nitrogen over time were analyzed using simple linear regressions (SAS Institute 2004). If regressions were significant for more than one treatment, slopes and elevations of regressions were compared using analysis of covariance in the general linear models procedure in SAS 9.1 (SAS Institute 2004).

RESULTS

Inorganic nitrogen dynamics

In the first three years of grassland establishment (1998–2000) all indices of soil nitrogen (N) availability (soil extractable inorganic N, potential net N mineralization rates, and ion exchange resin-collected inorganic N) demonstrated that the initial carbon (C) addition (sawdust) significantly reduced N availability and net nitrogen mineralization potential (NMP) relative to ambient soil; whereas annual fertilization increased N availability and NMP relative to the ambient soil (Baer et al. 2003). Although soil was not sampled in 2001 and 2002, the C amendment likely maintained a degree of N limitation during this interval, as NMP rate in the C-amended soil increased from a mean net nitrogen immobilization rate of $-11.7 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ in 1998 to a mean net nitrogen mineralization rate of $1.7 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ (Baer et al. 2003). Over this 3-yr interval, NMP rate increased $6.67 \text{ mg}/\text{yr}$ ($n = 12$, $r^2 = 0.51$, $P = 0.001$), which would maintain lower N supply from the C-amended soil relative to the ambient soil ($20.3 \text{ mg}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ in 2000 [Baer et al. 2003]) for approximately two additional years (2003).

In 2003 (Table 2), extractable NO₃-N was significantly different between the enriched and reduced N treatments, with intermediate extractable NO₃-N in the ambient soil ($F = 4.60$, $df = 2, 76$, $P = 0.043$; Table 2). The same pattern of inorganic N availability among treatments was observed in 2005 at the end of year 8 ($F = 4.82$, $df = 2, 6$, $P = 0.056$). In year 8, total extractable inorganic N and extractable NO₃-N in the C-amended soil were not significantly different from zero ($P > 0.999$) following the sucrose additions. Resin-collected N further substantiated that the sucrose additions successfully reestablished a strong gradient of N availability over the growing season, as indexed by total inorganic N ion capture on exchange resins ($F = 73.8$, $df = 2, 38.1$, $P < 0.001$). Differences among treatments were primarily due to differences in resin-collected

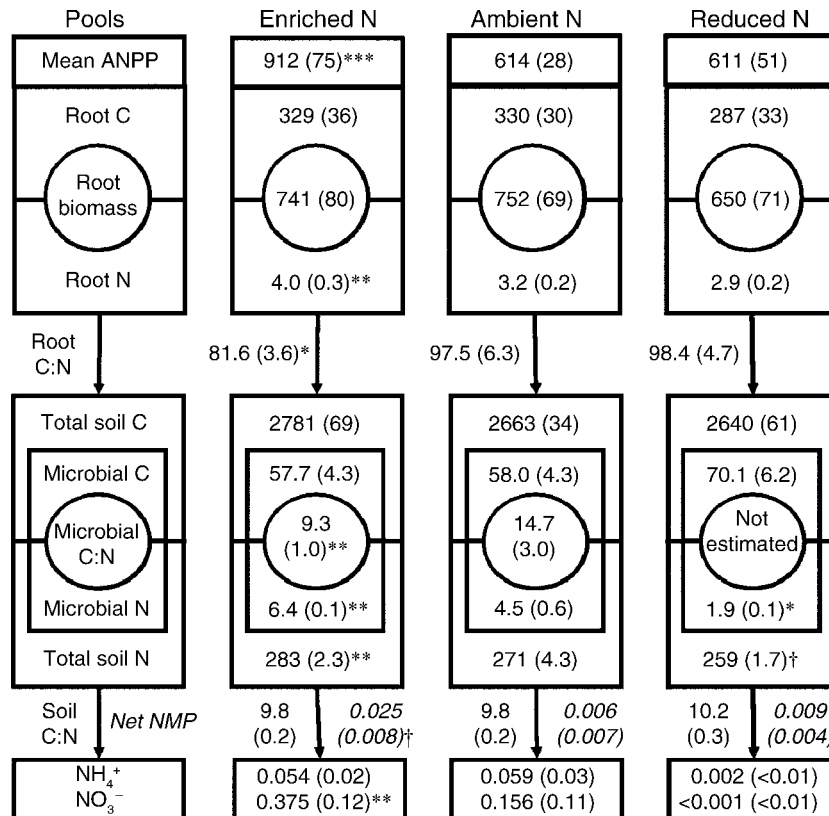


FIG. 1. Ecosystem aboveground net primary productivity (ANPP) and belowground C and N pools and C:N ratios of pools following 7–8 growing seasons of grassland establishment in soil with varying resource availability (enriched, ambient, and reduced N treatments). The left-hand column is the key identifying the components of the diagram for each treatment. ANPP represents the mean for each nutrient treatment averaged over all collection years (1998–2005). Carbon and nitrogen storage and C:N ratio of roots were measured following seven growing seasons of grassland establishment (2004). Soil total and microbial C and N pools, microbial C:N ratios, net NMP rates, and extractable inorganic N pools were measured following eight growing seasons of grassland establishment (2005). Fluxes are italicized. All pools and fluxes are presented in g m^{-2} and $\text{g m}^{-2} \text{yr}^{-1}$, respectively. Each value represents the mean (with SE in parentheses) among the four independent blocks containing each of the three nutrient treatments. The significance level of comparisons between the enriched-N and reduced-N treatments relative to ambient soil conditions is indicated.

† $P < 0.10$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

$\text{NO}_3\text{-N}$ ($F = 85.8$, $df = 2$, 38.1 , $P < 0.001$), although $\text{NH}_4\text{-N}$ exhibited similar trends (Table 2).

Soil nutrient treatments maintained a gradient of net nitrogen mineralization potential (NMP) rates into 2003 ($F = 8.08$, $df = 2$, 6 , $P = 0.020$), which were highest in N-amended soil and lowest in C-amended soil (Table 2). There was a similar pattern of net nitrification rates among the soil treatments in 2003 ($F = 6.74$, $df = 2$, 6 , $P = 0.029$; Table 2). In 2005, NMP ($F = 3.22$, $df = 2$, 12 , $P = 0.075$) and net nitrification ($F = 4.23$, $df = 2$, 12 , $P = 0.041$) rates were greater in the N-amended treatment than in either the non-amended or C-amended treatments, which were not different than one another.

Aboveground net primary productivity and belowground storage of C and N in roots

Over all years, aboveground net primary productivity (ANPP) was greater in the N enrichment treatment

relative to the ambient and reduced-N soil treatments ($F = 17.85$, $df = 2$, 28 , $P < 0.001$), which were similar to one another (Fig. 1). Root biomass and C storage in roots were similar among the soil nutrient treatments ($P > 0.10$; Fig. 1). Percentage of N in roots, however, was higher in the enriched-N soil ($F = 3.49$, $df = 2$, 15 , $P = 0.056$), resulting in greater N storage in roots ($F = 3.92$, $df = 2$, 12 , $P = 0.049$) and a reduction in root C:N ratio relative to the ambient and reduced-N soil treatments ($F = 3.34$, $df = 2$, 9 , $P = 0.082$; Fig. 1).

Soil C and N pools

Total soil carbon increased linearly over the 8-yr period in the ambient-N and enriched-N soil, but did not change after the initial increase in the C-amended soil (Fig. 2). Rates of total carbon accrual in the ambient-N and enriched-N treatments were similar ($P > 0.10$), so a pooled slope model was used to test for differences in the

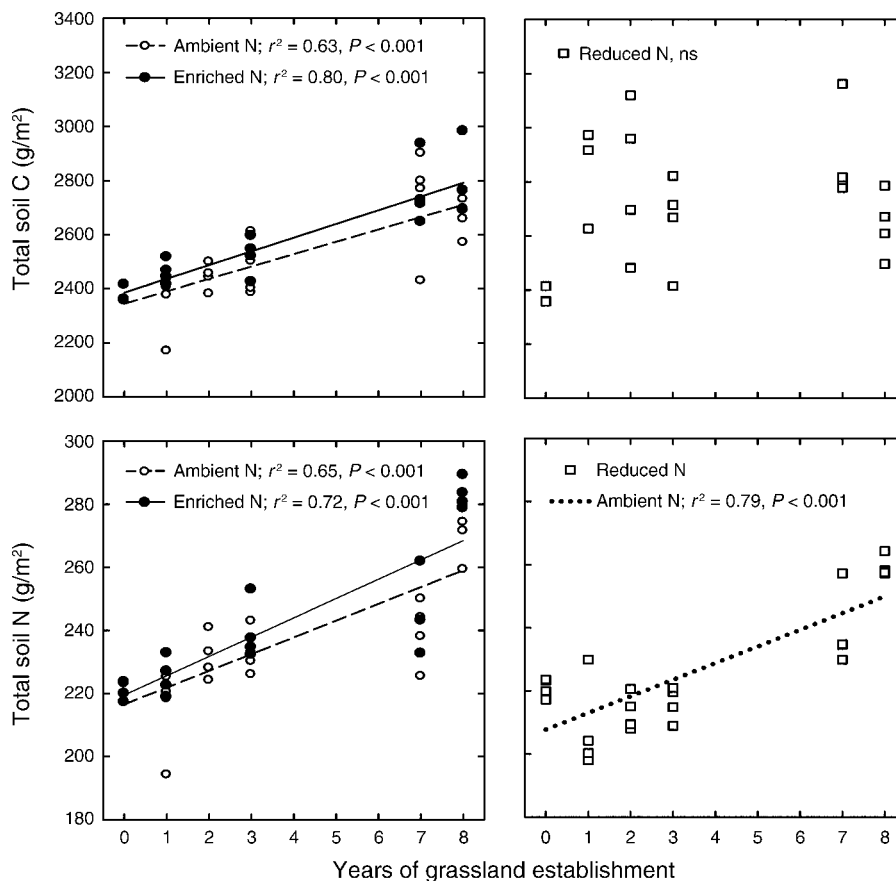


FIG. 2. Simple linear regressions (SLR) of changes in total carbon and nitrogen among the soil nutrient treatments during grassland establishment. Measurements were made at a depth of 0–10 cm. Regression equations for changes in total carbon in the ambient and enriched-N soil were $y = 45.74x + 2344.21$ and $y = 50.81x + 2385.87$, respectively; the relationship for soil C in the reduced-N (C-amended) treatment was nonsignificant (ns). Regression equations for changes in total nitrogen in the ambient, enriched-N, and reduced-N (C-amended) soil were $y = 5.31x + 216.5$, $y = 6.13x + 219.5$, and $y = 5.27x + 207.7$, respectively.

elevation of the regressions. The elevation of the total carbon accrual regression for the enriched-N soil was higher than the ambient soil by 59.7 g/m^2 ($F = 4.02$, $df = 2, 1$, $P = 0.052$). Total N increased over time in all soil nutrient treatments and the slopes of all regressions were similar ($P > 0.10$). The N accrual regression in the C-amended soil, however, had an elevation that was 9 g N/m^2 and 15 g N/m^2 lower than the ambient and enriched-N treatments, respectively ($F = 8.66$, $df = 2, 2$, $P < 0.001$).

The potentially mineralizable carbon (PMC) pool was measured in the surface 0–5 cm of soil prior to the first and third growing seasons (Tables 1 and 3). The cumulative amount of C mineralized conformed to a first-order model described by the following equation (Brunner and Focht 1984): $C_{\text{cum}} = \text{PMC} [1 - \exp(-kt)]$, where C_{cum} is the cumulative amount of C mineralized after time t (days), PMC is the mineralizable pool of C (g/m^2), and k represents the rate constant of mineralization (d^{-1}). The PMC pool was affected by a significant interaction between the soil nutrient treatments and time, resulting from differential changes in PMC

between years in the C-amended and non-C-amended soils ($F = 49.0$, $df = 2, 12.7$, $P < 0.001$; Table 3). In 1998, the initial C amendment elevated PMC by an order of magnitude relative to the non-amended soils (Table 3). Over time, PMC increased in both the ambient and enriched-N soil, and two years of N enrichment did not affect the size of the PMC pool. In contrast, the C-amended soil lost 45% of the PMC pool between 1998 and 2000. Prior to the third growing season (2000), the PMC pool in the C-amended soil remained more than two times that of the non-C-amended soils (Table 3). The ratio of PMC:TC also showed a significant interaction between the soil nutrient treatments and time ($F = 38.76$, $df = 2, 11.6$, $P < 0.001$; Table 3). In 1998, PMC was only 7.5% of total carbon (TC) in the non-C-amended soils, whereas >60% of the total carbon pool was mineralizable following the C addition (Table 3). Changes in PMC:TC over time reflected changes in PMC pool (Table 3). Rate constants of mineralization (k) also exhibited an interaction between the nutrient treatments and time ($F = 24.0$, $df = 2, 18.2$, $P < 0.001$). The rate constant in the C-amended soil was lower than

TABLE 3. Changes in total soil carbon (TC), potentially mineralizable carbon (PMC), mineralizable percentage of the total carbon pool (PMC/TC), and rate constants (k) of mineralization over time under three N treatments.

Soil N treatment	Years	TC (g/m ²)	PMC (g/m ²)	PMC/TC (%)	k (d ⁻¹)
Reduced	0	1431 ^c (101.4)	873.0 ^d (137.9)	61.13 ^d (8.22)	0.0043 ^b (0.0006)
	2	1541 ^d (87.6)	483.9 ^c (72.4)	30.75 ^c (2.96)	0.0068 ^c (0.0006)
Ambient	0	1132 ^a (11.2)	85.3 ^a (6.7)	7.55 ^a (0.59)	0.0096 ^a (0.0004)
	2	1292 ^b (24.7)	224.6 ^b (25.7)	17.45 ^b (2.44)	0.0094 ^a (0.0004)
Enriched	0	1159 ^a (22.6)	87.2 ^a (10.0)	7.54 ^a (0.82)	0.0104 ^a (0.0004)
	2	1324 ^b (44.4)	203.7 ^b (12.0)	15.34 ^b (0.45)	0.0092 ^a (0.0003)

Notes: Carbon was measured at 0–5 cm depth under reduced-N (C-amended), ambient, and enriched-N soil treatments at the onset (1998, time 0), and after two years (prior to the third growing season in 2000) of grassland establishment. Means (with SE in parentheses) accompanied by the same lowercase letter within a column were not significantly different ($\alpha = 0.05$).

the ambient and enriched-N treatments in both years; k increased over time in the C-amended soil due to an increase in the total carbon pool and reduction in the PMC pool (Table 3).

As with total and mineralizable C pools, annual N additions did not increase the microbial C pool relative to the other treatments despite eight years of N enrichment. In year 8, microbial biomass carbon (MBC) was similar in the ambient and enriched-N soil treatments, and was marginally higher in the C-amended soil relative to controls ($F = 2.55$, $df = 2, 28$, $P = 0.096$). Nitrogen content of the microbial biomass differed among all soil nutrient treatments ($F = 36.3$, $df = 2, 27.2$, $P < 0.001$), with lowest microbial biomass nitrogen (MBN) in the reduced-N soil and highest biomass N in the enriched-N soil. Similar C and greater N in microbial biomass of the enriched-N soil resulted in a lower C:N ratio of the microbial biomass relative to the ambient soil treatment (Fig. 1). Estimates of MBC and MBN in the C-amended soil resulted in C:N ratios well above that which occurs for soil microflora (Stark and Hart 2003), likely due to limitations of the fumigation-incubation procedure in soils amended with C. It appears that N released from the fumigation was rapidly immobilized during incubation, which resulted in an underestimate of MBN. For this reason, C:N of the C-amended soil was not compared to the other soil treatments.

Plant cover

The experimental grassland was dominated by native plant species by year 2 (Baer et al. 2003). Grasses dominated total plant cover in all treatments over time, and in every year total grass cover was highest in the enriched-N soil and lowest in the reduced-N soil, resulting in a strong main effect of the nutrient treatment on grass cover across all years ($F = 13.4$, $df = 2, 6$, $P = 0.006$; Fig. 3). Cover of legumes exhibited an interaction between the nutrient treatments and time ($F = 3.93$, $df = 8, 165$, $P < 0.001$), resulting from a linear increase in legume cover in the reduced-N soil ($r^2 = 0.73$, $P < 0.001$) and no change in the enriched-N soil (Fig. 3). Legume cover in the ambient soil treatment was intermediate, relative to other treatments, by year 8.

DISCUSSION

Steady-state transition

Understanding the extent to which resource availability and plant composition influence productivity and C and N dynamics is needed to predict the trajectory of recovery in ecosystems following long-term disturbance. In the case of grasslands restored in former agricultural fields, this includes initial conditions of low total soil C and N and relatively high initial N availability, followed by increasing N limitation as C accrues in perennial plant biomass and soil pools over time (Baer et al. 2002). During ecosystem reassembly, net primary productivity is the primary source of C for accumulating soil organic matter (SOM) pools (Parton et al. 1988), and ANPP recovers rapidly during grassland reestablishment on formerly cultivated soil (Baer et al. 2002, 2003). Within three years of grassland establishment, mean ANPP in the ambient unmanipulated soils of our experimental grassland was similar to or higher than ANPP in frequently burned lowland native prairie at Konza Prairie Biological Station (22-yr mean ANPP = 527.5 ± 26.9 g·m⁻²·yr⁻¹, mean \pm SE; Knapp et al. 1998). Furthermore, belowground biomass following seven growing seasons of grassland establishment exceeded that of frequently burned native prairie (~ 600 g/m²) at the same depth (Blair 1997).

Repeated long-term soil disturbance, particularly cultivation, results in loss of total and active soil C and N pools and can lead to new lower equilibrium levels of soil C and N (Mann 1986, Burke et al. 1995, Knops and Tilman 2000). As a result, these pools have the potential to accrue significant C and N once disturbance is removed and native vegetation is restored (Lal 2003). Total soil C and N accrual rates in this study (45.7 g C·m⁻²·yr⁻¹ and 5.7 g N·m⁻²·yr⁻¹ in the surface 10 cm, respectively) were similar to those in other former agroecosystems seeded with C₄ grasses (Potter et al. 1999, Baer et al. 2002, McLauchlan 2006, McLauchlan et al. 2006). If these rates remained linear over time, recovery of total soil C and N to levels representative of steady-state conditions in local native tallgrass prairie (total carbon, 3000–4000 g/m² [Smith and Johnson 2003]; total nitrogen, 300–400 g N/m² [Lett et al. 2004]) would take an estimated 14–36 years.

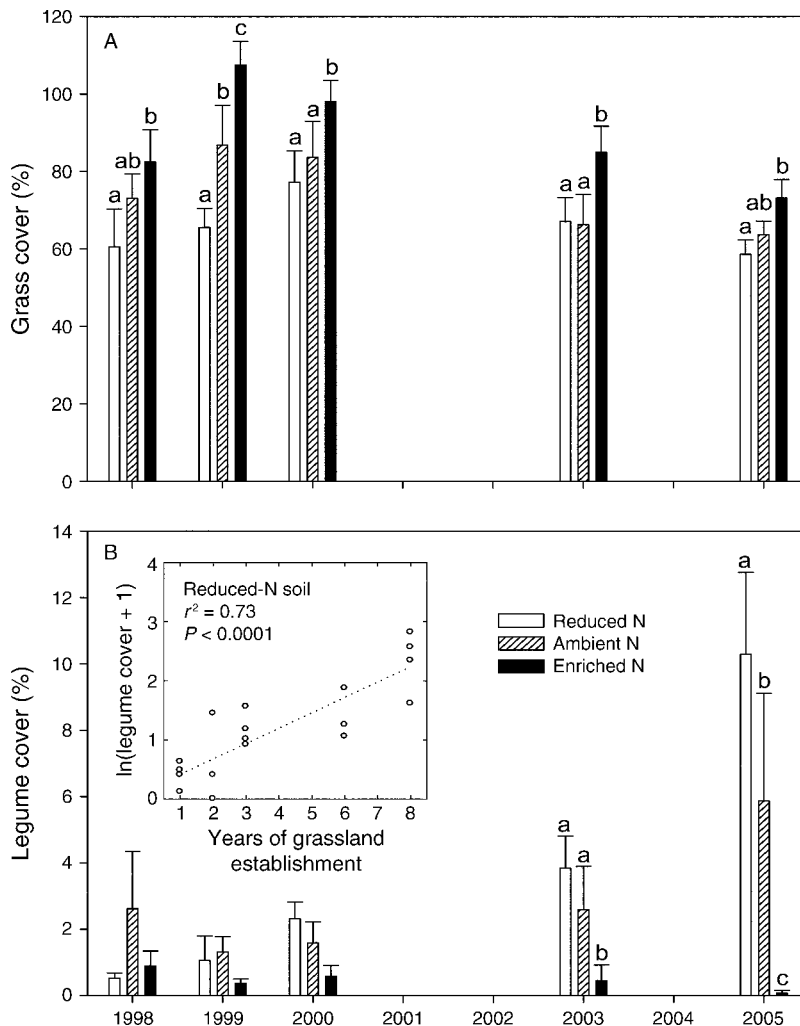


FIG. 3. Percent cover (mean + SE) of (A) grasses and (B) legumes each year during grassland establishment, 1998–2005. A linear increase in cover of legumes over time occurred only in the reduced-N soil treatment (panel B inset graph). Means within a year accompanied by the same lowercase letter were not significantly different ($\alpha = 0.05$). For the years 1998–2000 there were no significant differences in legume cover among treatments.

The potentially mineralizable carbon (PMC) pool is sensitive to changes in recent C inputs (Rice and Garcia 1994, Williams et al. 2004), and in an ecosystem transitioning from depleted C pools, PMC accumulation may represent the primary source of energy for C-limited microbial populations, and a key process underlying the development of N limitation over time. Although changes in the active or mineralizable C pools have been estimated using indices such as short-term rates of CO₂ efflux and/or organic matter fractionations (Robles and Burke 1997, Staben et al. 1997, Baer et al. 2002, Camill et al. 2004, McLauchlan et al. 2006), this is, to our knowledge, the first study to quantify changes in the PMC using long-term incubations that provide reasonable estimates of the C content in the “active + slow” SOM pools in recovering grasslands (Paul et al. 1999). The increase in total carbon in the surface 5 cm of soil prior to and following two growing seasons of

grassland establishment was nearly entirely attributed to accrual of C in the PMC pool. The distribution of C (PMC:TC) in the two-year restored grassland was more similar to native prairie, where the PMC pool (ranging from 360 g to 516 g C/m²) represents 26.8% of the total carbon pool at this depth (Rice and Garcia 1994, Williams et al. 2004). The microbial C pool represents ~3% of the total C pool in nearby native grassland soil (Rice and Garcia 1994) and comprised 2.17% ± 0.15% of the total C pool following seven growing seasons of grassland establishment.

Increases in belowground C pools during ecosystem transition from low equilibrium levels toward new steady-state conditions may feed back to limit the availability and supply of N for plant growth (i.e., progressive N limitation; Luo et al. 2004). In the ambient soil treatment, inorganic N availability, as indexed by extractable and resin-collected nitrate and



PLATE 1. Restored experimental grassland established in 1998 at the Konza Prairie Biological Station, a Long-Term Ecological Research site in Manhattan, Kansas (USA). Treatments include manipulations of soil nitrogen availability and soil depth in combinations designed to alter plot-level heterogeneity. Photo credit: S. G. Baer.

net nitrogen mineralization potentials, declined significantly after one year of grassland establishment (Baer et al. 2003), and has been maintained at low levels, representative of native prairie soil (Blair 1997), over time. The ability of the grassland restored under ambient soil conditions to maintain ANPP comparable to native prairie ecosystems, despite reduction in the available N pool over time, is likely due to the dominance of C_4 grasses with high nutrient-use efficiency and an increase in legume cover over time.

Feedback between plant reassembly and soil processes under varying resource limitations

Positive feedback between productivity, plant tissue quality, and soil nutrient supply is predicted for high-fertility systems (Melillo et al. 1982, Vitousek 1982, Hobbie 1992, Aerts 1995, Tatenno and Chapin 1997). Annual N enrichment increased percentage of N in root tissue but did not increase root biomass, resulting in greater N storage and lower C:N ratio of root tissue relative to ambient conditions. The N additions may have resulted in an absolute decline in root C:N ratio or fertilization may have reduced the rate of increase in root C:N ratio over time relative to the ambient treatment, where the establishment of C_4 grasses would be expected to increase root C:N ratios over time (Baer et al. 2002). Positive feedback through faster decomposition of higher quality (lower C:N) roots, relative to the ambient soil, seems unlikely in our system as root

biomass C:N ratio was well above the theoretical threshold for mineralization of N, due to persistent dominance of C_4 grasses with high nutrient-use efficiency at this low level of fertilization (Wedin and Tilman 1996). Similarity in root biomass between the enriched and ambient soil did not support the alternative feedback mechanism of a greater fraction of N recycled despite the high C:N (low quality) of root tissue (Clark et al. 2005).

Root biomass, however, does not provide information on productivity or turnover of fine roots that represent the primary supply of plant C and N to the SOM. Less production or allocation of C to fine roots may occur in high-fertility sites with less competition for N and greater competition for light (Craine et al. 2002), but turnover of fine roots may be higher in response to nutrient enrichment (Olf et al. 1994, van der Krift and Berendse 2002). Furthermore, divergence in functional composition of the plant community between the ambient and fertilized treatments, and lower diversity in the fertilized treatment (Baer et al. 2003, 2004) limits our interpretation of the direct effects of N enrichment on plant C and N inputs through root systems in the developing grassland, since differences in species composition can impose larger variation in tissue chemistry (hence inputs) than N alone (Dijkstra et al. 2006). The enriched soil was nearly exclusively dominated by C_4 grasses, which also could dampen the strength of positive feedback as litter from these grasses promotes

lower N mineralization rates and N availability relative to other functional groups in grassland soil (Wedin and Tilman 1990, Tilman and Wedin 1991, Wedin and Pastor 1993, Craine et al. 2002, Dijkstra et al. 2006).

Negative feedback in response to N enrichment would be expected if enhanced plant productivity promoted N immobilization and subsequently reduced the supply of N for plant growth (Knops et al. 2002). In our experiment, despite chronically higher ANPP in the fertilized soil, N enrichment did not increase root biomass, C storage in roots, or any soil C pool relative to ambient conditions to promote negative feedback through this mechanism. Furthermore, the importance of higher ANPP and aboveground litter inputs in N and C cycling were considered negligible under the frequent burning regime. The establishing grassland represented a C accruing system, and it is possible that negative feedback may take longer than eight years to detect, as changes in soil C pools have taken four to eight years to respond to fertilization in native prairie at steady state (Rice and Garcia 1994).

Our results suggest positive feedback in the developing grassland was occurring through the influence of N enrichment on the soil microbial biomass. The fertilized soil contained a similar microbial biomass (C) to the ambient soil, but with a higher N content and lower C:N ratio. Microbial biomass response to N enrichment ranges from stimulation (Fisk and Schmidt 1996, Johnson et al. 1998, Li et al. 2006) to suppression (Smolander et al. 1994, Fahey et al. 1998, Bowden et al. 2004, Burton et al. 2004, Wallenstein et al. 2006). The physiological activity of the microbial biomass, rather than size, likely modulates positive or negative feedback between N enrichment and N mineralization. For example, a small but physiologically active microbial community can produce negative feedback (Fisk and Fahey 2001) if N immobilization is proportional to microbial growth (Schimel 1988). Our results were more consistent with patterns of N saturation (Aber et al. 1989), at least in the microbial biomass, with higher biomass N content and net nitrification rates in the fertilized soil relative to ambient soil. Thus, N enrichment during ecosystem transition can override the progressive N limitation, expected to develop while C and N are accruing in plant and SOM pools (Luo et al. 2004).

A unique aspect of this study was that we reestablished species in soil with a gradient of N availability. We predicted that positive feedback would occur in soil supplemented with C to promote microbial immobilization of N and reduce N supply for plant growth. The reduced-N treatment differed from the ambient soil in total soil N content, which was diluted from the addition of the recalcitrant C substrate with a wide C:N ratio, which also limited N availability for incorporation into the microbial biomass. Despite greater N limitation in the C-amended soil, ANPP, root biomass, and root C:N ratio was similar to the ambient soil. Two mechanisms may be invoked to explain the lack of positive feedback

in the reduced-N treatment. First, C₄ grasses dominated species composition and they exhibit high productivity under low N availability (Epstein et al. 1997). Second, increasing cover of a plant functional group associated with symbiotic N-fixing bacteria, and consequently less dependence on N supply from soil for growth, may have partly compensated for N limitation to plant growth imposed by the C amendment to the soil.

Implications for community and ecosystem reassembly

Chronic soil disturbance, widespread application of fertilizers, and increasing atmospheric deposition have collectively increased N availability and cycling in many ecosystems (Vitousek et al. 1997). Alteration of N storage and fluxes has important implications for plant diversity, as species loss is a common occurrence under fertilization (Suding et al. 2005), and inverse relationships between N availability and diversity have been reported in many herbaceous systems (Gough et al. 2000), including our restored grassland (Baer et al. 2003). In addition to the short-term (less than three years) negative effect of N enrichment on community structure, this longer term investigation of C and N interaction in the developing grassland indicates that recovery of ecosystem function is not enhanced beyond an increase in ANPP following eight years of N enrichment. Detailed studies are needed to elucidate the effects of chronic nutrient enrichment on fine-root production and turnover on microbial activity and composition that are instrumental in the longer term protection of soil C and N in stable pools (Jastrow et al. 1998, Six et al. 2000, 2006) needed to sustain productivity over the long term.

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