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Woody Plant Encroachment by *Juniperus virginiana* in a Mesic Native Grassland Promotes Rapid Carbon and Nitrogen Accrual

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Abstract

The cover and abundance of *Juniperus virginiana* L. in the U.S. Central Plains are rapidly increasing, largely as a result of changing land-use practices that alter fire regimes in native grassland communities. Little is known about how conversion of native grasslands to *Juniperus*-dominated forests alters soil nutrient availability and ecosystem storage of carbon (C) and nitrogen (N), although such land-cover changes have important implications for local ecosystem dynamics, as well as regional C and N budgets. Four replicate native grasslands and adjacent areas of recent *J. virginiana* encroachment were selected to assess potential changes in soil N availability, leaf-level photosynthesis, and major ecosystem C and N pools. Net N mineralization rates were assessed in situ over two years, and changes in labile soil organic pools (potential C and N mineralization rates and microbial biomass C and N) were determined. Photosynthetic nitrogen use efficiencies (PNUE) were used to examine differences in instantaneous leaf-level N use in C uptake. Comparisons of ecosystem C and N stocks revealed significant C and N accrual in both plant biomass and soils in these newly established forests, without changes in labile soil N pools. There were few differences in monthly in situ net N mineralization rates, although cumulative annual net N mineralization was greater in forest soils compared to grasslands. Conversely, potential C mineralization was significantly reduced in forest soils. Encroachment by *J. virginiana* into grasslands results in rapid accretion of ecosystem C and N in plant and soil pools with little apparent change in N availability. Widespread increases in the cover of woody plants, like *J. virginiana*, in areas formerly dominated by graminoid species suggest an increasing role of expanding woodlands and forests as regional C sinks in the central U.S.

Key words: *Juniperus virginiana*; woody plant encroachment; grassland conversion; invasion; nitrogen cycling; nitrogen use efficiency; mineralization; carbon storage; land cover change.

Introduction

Grasslands and savanna ecosystems comprise a large portion of the terrestrial biosphere, and are important in regional and global biogeochemical cycles (Field and others 1998). Changes in these ecosystems caused by woody plant encroachment can significantly alter rates of ecosystem carbon (C) gain or loss, with potential global consequences for soil and atmospheric chemistry (Archer and

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others 2001; Pacala and others 2001). In North America, the abundance and cover of several genera of woody plants, including *Juniperus*, have increased dramatically beyond their historical range and distribution in grasslands and savannas during the last 150 years, converting millions of hectares of grassland or savannas to woodlands or forests (Van Auken 2000; Archer and others 2001). These newly established woodlands and forests may serve as significant, if transient, sinks for atmospheric CO_2 (Archer and others 2001; Norris and others 2001a; Albani and others 2006). For example, aboveground net primary productivity was much greater in *J. virginiana* forests (~10 Mt ha^{-1} y^{-1}) compared to grassland sites in similar locations (~3.7 Mt ha^{-1} y^{-1}), leading to a substantial increase in aboveground C storage, as well as potential changes in belowground C storage and nutrient cycling (Norris and others 2001a; Smith and Johnson 2003; Norris and others 2007). Changes in the rates and magnitude of C accrual, as well as shifts in C allocation from below- to aboveground, in *J. virginiana* forests relative to the grasslands they replace could have significant consequences for both soil chemistry and regional ecosystem exchange processes (Moiser 2001). Concurrent changes in litter quantity or quality and microclimate may also alter key ecosystem processes or properties that affect decomposition and soil nutrient availability (Austin and Vivanco 2006; Throop and Archer 2007), and ultimately, alter feedbacks with plants that may, in turn, affect ecosystem function, particularly C uptake.

Juniperus virginiana occurs in every state east of the 100th meridian and parts of southern Canada (Fowells 1965). Historically, *J. virginiana* in the eastern Central Plains was restricted to areas that were protected from intense grassland fires such as rocky outcrops, canyons or areas with shallow soils. In the absence of fire, *J. virginiana* can increase in density and size at rates that allow canopy closure in less than 40 years, and it has encroached into grasslands in the eastern Central Plains at an unprecedented rapid rate affecting millions of hectares as a result of changing land-use practices (Schmidt and Leatherberry 1995; Briggs and others 2002, 2005). In the Central Plains, *J. virginiana* often forms dense, nearly monospecific stands that alter forest floor microclimate with respect to light, temperature, and moisture, and substantially reduces plant richness in the understory (Briggs and others 2002, 2005; McKinley and others 2008).

In grasslands of the eastern Central Plains, plant productivity is potentially limited by multiple factors, including water, light, and nitrogen (N) (Blair 1997; Blair and others 1998; Knapp and others 1998). The relative importance of these factors is highly influenced by fire regime and ungulate grazing intensity (Johnson and Matchett 2001). However, in areas of *J. virginiana* encroachment, both fire and grazing are typically absent. The removal of these disturbances, coupled with changes in plant traits (that is, tissue chemistry and resource allocation), may alter ecosystem N cycling and subsequent availability of soil N for plant uptake. Ultimately, differences in plant available N and utilization of N in forest and grassland soils may influence plant productivity, as well as potential rates of ecosystem C accretion and storage.

Norris and others (2007) reported that mean aboveground plant productivity of recently established *J. virginiana* forests was about 2.5× greater than that of comparable grasslands (similar soils and topographic position). Greater primary productivity of *J. virginiana* forests, relative to the grasslands they replace, may increase N demand and elicit immobilization of significant quantities of N in plant biomass, litter, and soil organic matter (SOM). In addition, the establishment of *J. virginiana* forests alters several of the factors that control soil N mineralization and N availability. Specifically, conversion of grasslands to *J. virginiana* forests alters litter quality (Norris and others 2001b), soil temperature (Smith and Johnson 2004; McKinley and others 2008), and possibly other factors (for example, soil biota) that influence soil N mineralization. Immobilization of N in plant and soil pools, coupled with changes in litter chemistry that reduce decay rates (Norris and others 2001b), may reduce the size of labile N pools and ultimately N availability. High productivity of *J. virginiana* forests may be facilitated by high plant or ecosystem nitrogen use efficiency (NUE). Norris and others (2007) found that annual ecosystem NUE in *J. virginiana* forests, measured as ANPP:litterfall N, was more than double that of the grasslands they replaced. Leaf-level NUE was not measured in that study. Species with high NUE could maintain high productivity under conditions of reduced N availability, but this would be expected to reinforce plant–soil feed back loops and maintain low soil N availability through production of relatively low quality substrates (for example, wider C:N ratios). Thus, there is reason to expect that conversion of native grasslands to *J. virginiana* woodlands will alter soil N dynamics.

Our general hypothesis was that replacement of native grassland species with a non-N-fixing tree species would result in accrual of plant biomass and litter (Tilman and others 2000; Archer and others

2001; Norris and others 2001a) that, in the absence of additional N inputs, would lead to reduced soil N availability through greater N sequestration in plant biomass, changes in substrate quantity and quality that promote microbial immobilization of N, and altered abiotic conditions that slow decomposition and mineralization. These predicted changes in soil N cycling and reductions in N availability might be offset by intrinsic characteristics of *J. virginiana* (for example, high NUE), to allow the observed increases in productivity following forest encroachment into grasslands, despite expected lower N availability. However, few studies have simultaneously investigated changes in plant and soil C and N pools, N availability, and leaf-level nitrogen use efficiencies in the context of whole ecosystem function in grasslands and newly established *J. virginiana* forests growing on similar soils and under similar climates. Our objective was to assess soil N availability and a suite of ecosystem attributes at multiple paired grassland-forest sites, using a combination of concurrent complementary field and laboratory assays to understand how *J. virginiana* forests sustain high productivity and transform ecosystem properties after invading native grasslands. Specifically, we (1) examined rates and seasonal patterns of in situ net N mineralization; (2) compared major ecosystem pools of C and N, labile soil N pools, and photosynthetic N use efficiencies; and (3) conducted parallel laboratory assays of potential C and N mineralization to assess changes in the storage, cycling, and use of these elements.

Materials and Methods

General Site Description

This study was conducted in the Flint Hills of NE Kansas, USA (39°05′ N, 96°35′ W), where prevalent native vegetation is tallgrass prairie, dominated by perennial, warm-season (C_4) grasses including big bluestem (*Andropogon gerardii* Vit.), little bluestem (*Schizachyrium scoparium* Michx.), indiangrass (*Sorghastrum nutans* Nash), and switchgrass (*Panicum virgatum* L.). These C_4 grasses contribute the majority of aboveground net primary productivity (ANPP) (Knapp and others 1998). However, a highly diverse mixture of less abundant species, including C_3 grasses, sedges, and a diverse array of forbs, contribute to the high floristic diversity of these grasslands. The native tallgrass prairie flora also includes a number of native woody plants, such as buckbrush (*Symphoricarpos orbiculatus* Moench.), redcedar (*Juniperus virginiana* L.), smooth sumac (*Rhus glabra* L.), and rough-leaved dogwood (*Cornus drummondii* CA May), which can be locally abundant, especially in prairie that is burned infrequently (Briggs and others 2005). Average annual precipitation is 835 mm with 75% falling during the growing season (Bark 1987). Topographic relief divides the landscape into upland plateaus with shallow soils, slopes with outcrops of limestone, and lowlands with deeper alluvial and colluvial soils.

Experimental Design

Four replicate sites, each comprising contiguous *J. virginiana* forest adjacent to native grassland, were chosen in the northern Flint Hills, in close proximity (<1 km) to the Konza Prairie Biological Station (KPBS), the primary location of the Konza Prairie Long-Term Ecological Research (LTER) program. This allowed the use of a variety of baseline data on ecological processes in native grassland. All study sites were located in uplands, with shallow mineral soils (~10 cm deep) overlying fragmented limestone layers, the most common topoedaphic condition for *J. virginiana* forest encroachment in this region. Three of the sites had silty clay loam soil (fine, mixed, active, mesic Udothentic Haplustols) and the fourth site had a silt loam soil (fine, mixed, superactive, mesic Udertic Argiustolls). Each *J. virginiana* forest (≥0.5 ha) had dense (680–1,360 trees ha^{-1}), complete canopy cover. Tree diameter at breast height (dbh) ranged from 15.2 to 22.1 cm (mean = 18.1 cm). Historical aerial photographs since 1950 and analysis of soil organic carbon (SOC) isotopic composition were used to verify the recent replacement of native grasslands with *J. virginiana* forests and estimate the age of each forest stand. Estimated ages of individual stands ranged from 35–75 years, with a mean around 45–50 years. Each forest site was located adjacent to a native grassland site in the same topographic position and soil type. Grassland sites were not grazed in the recent past (>15 years, personal communication) and had a contemporary average fire return interval of 1–2 years as a result of prescribed fires conducted in early spring. The forest sites remained unburned. In each vegetation type (*Juniperus* forest or grassland) at each replicate study site, one 50 m transect (n = 4/vegetation type) was established and used to randomly locate plots where soil and other ecosystem measurements were made. Assays of in situ soil net N mineralization, and soil sampling for microbial biomass and potentially mineralizable C and N, were done in randomly assigned 2-m^2 plots (n = 6/site).

Collections of mineral and organic soil, foliar litter, and root biomass to quantify C and N pools were taken from larger 25-m^2 plots (n = 6/site) along these same transects.

C and N Stores: Aboveground Biomass

Aboveground biomass was measured at the seasonal peak (August 2005) in grassland sites by clipping all vegetation within 0.1-m^2 quadrats and weighing the dried (60°C) samples. Sub-samples of plant material were analyzed for C and N content with a Carlo Erba model NA1500 C/N analyzer (Milano, Italy). Aboveground biomass, biomass C and biomass N for *J. virginiana* sites was estimated using allometric equations developed for this region (Norris and others 2001a) applied to all trees in twenty 25-m^2 quadrats along each 50-m transect.

C and N Stores: Soil

Total soil C concentrations were sampled in 25-m^2 plots (5 composite soil cores (2-cm diam. × 10-cm depth) per plot, six replicate plot samples per site). This procedure was later repeated with 2-cm diam. soil cores divided into 2-cm depth increments to examine changes in the amount and isotopic composition of SOC with depth. Soils were ground, dried at 105°C for 48 h, weighed (~25 mg) into silver capsules, and treated with 2 M HCl to remove inorganic carbon ($CaCO_3$) prior to analysis. Percent C and N was determined with a Carlo Erba model NA1500 C/N analyzer (Milano, Italy). Total soil organic C and N were expressed on a per square meter basis after accounting for soil bulk density.

Decarbonated sub-samples of soil were used to determine $\delta^{13}C$ values at each depth increment to assess replacement of C_4-derived SOC with new C_3 forest inputs. A Thermo Finnnigan Delta Plus mass spectrometer (samples combusted with a CE Elemental Analyzer with ConfloII) was used to determine isotopic values of C ($\delta^{13}C$). A mixing model (Balesdent and others 1988; Arrouays and others 1995) was used to calculate percent soil C converted from C_4-C to C_3-C: $f = \delta - \delta_0/\delta_1 - \delta_0$, where $\delta = \delta^{13}C$ of current soil, f = percent C from C_3 juniper, $\delta_0 = \delta^{13}C$ value of original C_4-derived soil (soil $\delta^{13}C$ from the paired grassland and corresponding depth) and $\delta_1 = \delta^{13}C$ value of new C_3 inputs (C_3 *J. virginiana* litter and roots).

Surface litter in the forested sites was collected by removing the entire O-horizon in 20 × 50 cm quadrat. Litter samples were dried, weighed, and ground for total C and N analysis.

Microbial Biomass

Soil microbial biomass C and N was determined in mid-growing season (July 2004) and at the beginning of the non-growing season (October 2004) using a chloroform fumigation-incubation technique (Jenkinson and Powlson 1976). Microbial biomass C was determined by difference in the amount of CO_2-C respired from fumigated and non-fumigated samples, measured with a Shimadzu GC-8A gas chromatograph. Microbial biomass N was determined by difference in the amount extractable inorganic N from fumigated and non-fumigated samples. Inorganic nitrogen (NH_4^+-N and NO_3^- + NO_2^--N) concentrations were determined colorimetrically (described later). Conversion factors (K_C and K_N) of 0.41 and 0.54 were used for C and N, respectively (Horwarth and Paul 1994).

Net Nitrogen Mineraliztion

In situ net N mineralization rates were measured 15 times from June 2003 to June 2005, using an intact soil core method. The O-horizon (litter layer) was removed in *J. virginiana* sites, and polyvinyl chloride (PVC) tubes (5 cm diam × 12.5-cm length) were driven 10 cm into the mineral soil. A cap prevented precipitation from entering the core. To prevent anaerobic conditions, two 0.5-cm i.d. holes were drilled in the exposed portion of the pipe. Cores were incubated in the field for 30–35 d (T_1). A second core (5-cm i.d. × 10 cm) was taken 5–10 cm from the in situ PVC core to measure initial (T_0) inorganic nitrogen concentrations.

Soils were prepared for extraction by passing through a 4-mm soil sieve and mixing thoroughly by hand. Ammonium and nitrate were extracted from 10 to 12 g of field moist soil with 50 ml of 2 M KCl, agitated for 1 h on an orbital shaker (200 rpm), and filtered through a 0.4-μm polycarbonate membrane. Inorganic nitrogen (NH_4^+-N and NO_3^- + NO_2^--N) concentrations were determined colormetrically with an Alpkem® Flow Solution Autoanalyzer (Wilsonville, Oregon).

Net N mineralization was calculated as the final concentration (μg NH_4^+ + NO_3-N g^{-1} dry soil) of inorganic nitrogen (T_1) minus the concentration at the start of the field incubation (T_0). Analogous calculations were done separately with ammonium and nitrate concentrations to determine net ammonification and nitrification rates, respectively. Mean cumulative annual net N mineralization, ammonification, and nitrification rates were calculated for each site by summing daily rates

measured during the 30 d periods and extrapolating daily rates for intervals between incubation periods. Monthly mean temperatures and precipitation during the study period were obtained from a weather station at the Konza LTER site.

Mineralizable C and N

Aliquots of soil were taken from the initial cores (T_0) used for in situ net N mineralization assays in July and October 2004 to determine potentially mineralizable C and N in soils collected in the growing and non-growing seasons, respectively. Ten-gram aliquots (dry wt. equivalent) of soil were placed in 150 ml serum bottles and brought to 30% soil moisture (or 60% water filled pore space). Each serum bottle was sealed with a rubber septum and aluminum closure, and incubated in the dark at 25°C for 31 days. Carbon dioxide concentrations in the headspace were measured every 2–3 days with a Shimadzu GC-8A gas chromatograph. After each measurement, the serum bottles were opened for 2 h to allow equilibration with ambient O_2 and CO_2 levels. The CO_2-C flux per gram of soil was summed over the incubation period to calculate cumulative carbon mineralization. At the end of the incubation, soils were extracted with 2 M KCl to determine inorganic N concentrations (described previously). Potentially mineralizable N was determined using a formula similar to that used to calculate field-based net mineralization rates, but expressed as μg N g^{-1} dry-soil 30 d^{-1}. Total soil carbon and nitrogen concentrations were measured in separate aliquots of T_0 soils, and used to determine yield of mineral C and N per gram organic C and N in the bulk soil, respectively.

Photosynthetic Nitrogen Use Efficiency (PNUE)

Leaf-level photosynthetic rates (A_{max}) were measured seven times in growing and non-growing seasons with a LiCor 6400 portable infrared gas analyzer system. Terminal portions of exposed shoots (relatively young leaf tissue) of *J. virginiana* or a single leaf of *A. gerardii* were sealed in a chamber equipped with a red-blue diode light source for 3–4 min while CO_2 assimilation was measured. Measurements were made under a photosynthetic photon flux density (PPFD) of 1,500 μmol m^{-2} s^{-1} (high light conditions), constant CO_2 (375 μl l^{-1} CO_2), and ambient air temperate and humidity.

Leaf area for *A. gerardii* was estimated by measuring the mid point width of the leaf in the cuvette and multiplying by the cuvette length (2 cm). Projected leaf area of *J. virginiana* was measured with a LI-3100 leaf area meter (Li-Cor, Lincoln, Nebraska, USA). No attempt was made to account for the three-dimensional shape of the leaves. Specific leaf mass for *J. virginiana* was determined by dividing the dry weight of the sampled leaves by the leaf area. Published values of specific leaf mass of *A. gerardii* in burned sites were used for comparisons (Knapp and others 1998). Leaf tissues were dried at 60°C, ground, and analyzed for N content. Photosynthetic nitrogen use efficiency (PNUE) was calculated by dividing A_{max} (μmol CO_2 m^{-2} s^{-1}) by the nitrogen content (mol N m^{-2}).

Data Analyses

A randomized complete block was used as the experimental design. Measurements obtained from the six plots along a given transect (forest or grassland) were averaged to obtain a single mean value for each discrete site for statistical analysis (n = 4/vegetation type). Significant differences in treatment means for field and laboratory assays of C and N mineralization, were determined using repeated-measures analysis of variance (ANOVA) (alpha = 0.05) (SAS, version 8.02). Differences in soil, root, and litter C and N, and microbial biomass in forests and grasslands were determined using analysis of variance (ANOVA) (alpha = 0.05) (SAS, version 8.02). A simple linear regression model was used to determine the relationship between the percentages of SOC of forest origin in forest soils by soil depth and to assess the relationship between leaf N content and net photosynthetic rates in *A. gerardii* and *J. virginiana*.

Results

Ecosystem C and N

Encroachment of *J. virginiana* led to large accumulations of C and N in newly established *J. virginiana* forests relative to the grasslands they replaced (Table 1). Carbon accumulation in aboveground plant biomass increased by a factor of 37, from a mean seasonal maximum of 163 g C m^{-2} in grasslands to 6,065 g C m^{-2} in *J. virginiana* stands. Similar changes in N storage in aboveground biomass occurred, although the magnitude of the difference between N accumulation in grassland and forest sites was not as large. Total N accumulation in aboveground biomass increased by a factor of 16, from a mean seasonal maximum of 3.0 g N m^{-2} in grasslands to 48 g N m^{-2} in forest sites. Differences in the rate of change in aboveground biomass C and

Table 1. Comparisons of Carbon and Nitrogen Standing Stocks in Selected Ecosystem Compartments in Native Grasslands and Adjacent Areas of Recent *J. virginiana* Forest Encroachment

Ecosystem compartment	Grassland	Forest
Carbon Stocks (g m^{-2})		
Aboveground biomass	163 ± 35	6,065 ± 74
Soil		
O-horizon	0	1,540 ± 51
A-horizon (upper 10 cm)	3,443 ± 188	3,871 ± 119
Microbial biomass C	118 ± 8	129 ± 11
Total	3,606 ± 787	11,476 ± 634
Nitrogen stocks (g m^{-2})		
Aboveground biomass	3.0 ± 0.6	48 ± 7
Soil		
O-horizon	0	56 ± 4
A-horizon (upper 10 cm)	298 ± 13	329 ± 9
Microbial biomass N	17 ± 2	16 ± 1
Extractable N	0.10 ± 0.02	0.08 ± 0.03
Total	301 ± 32	433 ± 14

Means (±SE) are based on n = 4 sites per vegetation type.

N in *J. virginiana* forests, relative to grasslands, resulted in a shift in mean C:N ratios in aboveground biomass from approximately 54 in grasslands to approximately 126 in *J. virginiana* forests.

Changes in C and N in the organic and mineral soil also occurred with conversion to *J. virginiana* forests. The largest difference was in the surface O-horizon, which was absent in the grasslands due to frequent fires, but which accrued significant amounts of C and N following *J. virginiana* encroachment. Carbon and N in the O-horizon of *J. virginiana* forests were 1,540 ± 51 g C m^{-2} and 56 ± 3.8 g N m^{-2}, respectively. Organic C and N were also significantly greater in the mineral soil (A-horizon, 0–10 cm) of *J. virginiana* forests compared to grasslands. Soil organic carbon (SOC) increased about 12% in *J. virginiana* forests, and was significantly greater (3,871 ± 119 g C m^{-2}) compared to grassland soils with 3,443 ± 188 g C m^{-2} (ANOVA, $F_{1,6} = 7.42$, $P = 0.01$, Figure 1A). Soil organic nitrogen (SON) increased similarly (10%) from 298 ± 12.8 g N m^{-2} in grassland to 329 ± 9.4 g N m^{-2} in *J. virginiana* forest (ANOVA, $F_{1,6} = 8.27$, $P = 0.01$, Figure 1B). Concurrent increases in soil organic C and N did not significantly alter soil C:N ratios in *J. virginiana* (11.77 ± 0.17) compared to grassland soils (11.41 ± 0.20) (ANOVA, $F_{1,6} = 1.87$, $P = 0.18$, Figure 1C).

The distribution of SOC with depth in the A-horizon was similar in *J. virginiana* and grassland soils (Figure 2A), as evidenced by no significant interaction between soil depth and vegetation type (ANOVA, $F_{4,30} = 0.25$, $P = 0.91$). However, there was significantly greater SOC across all depths in the *J. virginiana* soils (ANOVA, $F_{4,30} = 6.48$, $P = 0.02$) and SOC decreased significantly with depth in both vegetation types (ANOVA, $F_{1,30} = 12.61$, $P < 0.01$). The contribution of new forest-derived C to total SOC pools following *J. virginiana* encroachment was not uniform (Figure 2B). Although about 34% of the total SOC in the upper 10 cm of *J. virginiana* soils was of C_3 (forest) origin, the greatest relative contribution (48%) occurred in the 0–2 cm soil increment, and the amount of accumulated SOC of forest origin decreased predictably and linearly with depth ($R^2 = 0.97$, $y = 49.33 + 3.623x$, $P \leq 0.01$, using single mean site values for each depth increment) to about 17% in the 8–10 cm soil increment.

Microbial Biomass C and N

Soil microbial biomass is an important short-term source and sink of plant available nutrients, and is crucial for conserving N in tallgrass prairie (Garcia and Rice 1994). Microbial biomass C and N comprised a small portion (3% C and 5–6% N) of the total soil organic pools of the A-horizon, and did not differ between *J. virginiana* and grassland soils (ANOVA, $F_{1,6}$, $P \geq 0.05$) in either the growing or non-growing seasons (Figure 3).

Potentially Mineralizable C and N

There was a general tendency for lower C mineralization in laboratory incubations of forest soils on a bulk soil basis (Figure 4A, repeated-measures ANOVA, $F_{2,5} = 4.46$, $P = 0.077$), as well as lower C yield (μg C g^{-1} soil-C) compared to grassland soils

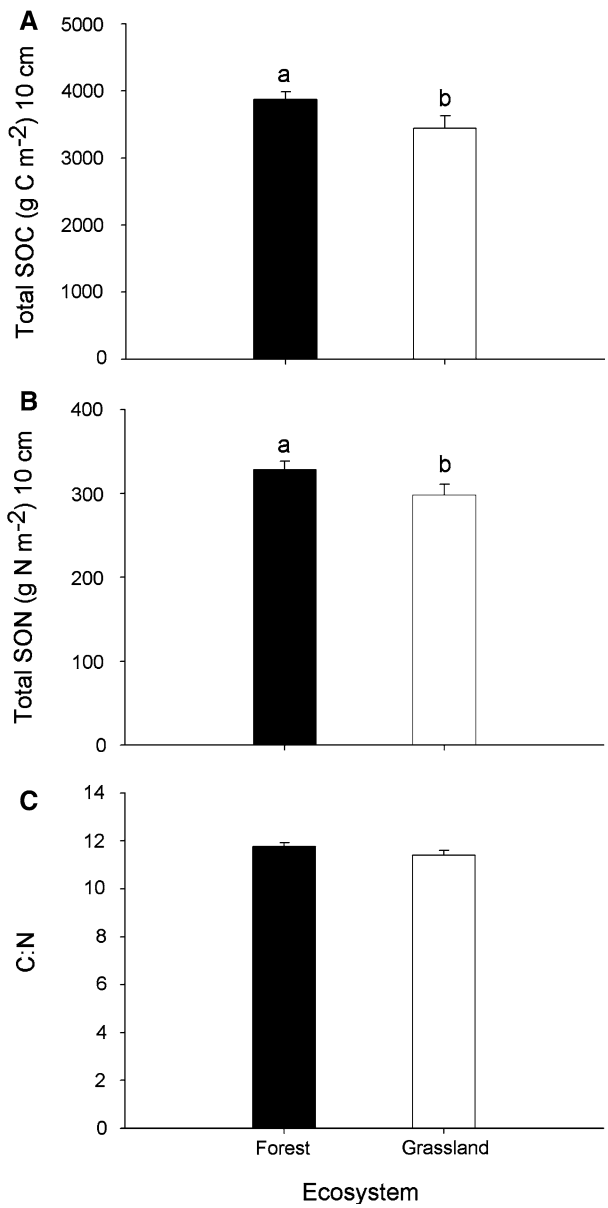

Figure 1. Total soil organic carbon (SOC) mean ± SE (**A**), total soil organic nitrogen (SON) (**B**), and the carbon to nitrogen (C:N) ratio (**C**) of the mineral soil in *J. virginiana* forest and grassland soil. Different letters indicated significant differences ($P < 0.05$).

(Figure 4B, repeated-measures ANOVA, $F_{2,5} = 6.68$, $P = 0.039$). Potential N mineralization rates were not statistically different in bulk soils of grasslands and forests, although there was a trend for forest soils to have greater rates than grassland soils in both growing (61%) and non-growing seasons (240%) (Figure 4A, repeated-measures ANOVA, $F_{2,5} = 1.54$, $P = 0.301$). Similarly, potential yield of N (μg N g^{-1} soil-N) was not significantly different in forest soils relative to grassland

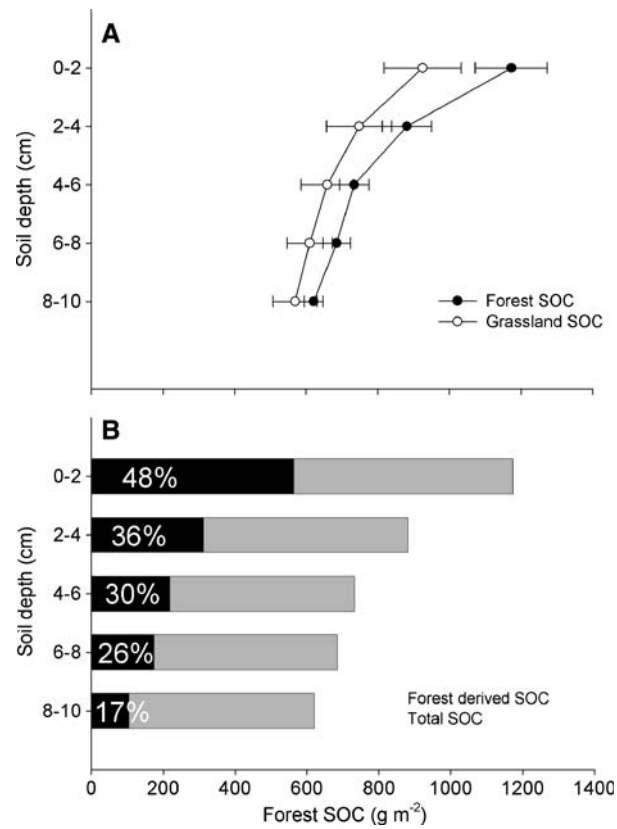

Figure 2. Total SOC (mean ± SE) by depth in forest and grassland soils (**A**); closed circles are total forest SOC and open circles are grassland SOC. The percent contribution of forest-derived (C_3) organic C by depth (**B**); the solid portion of each bar is new C_3-SOC, and the shaded portion is grassland carbon composing the remainder of SOC.

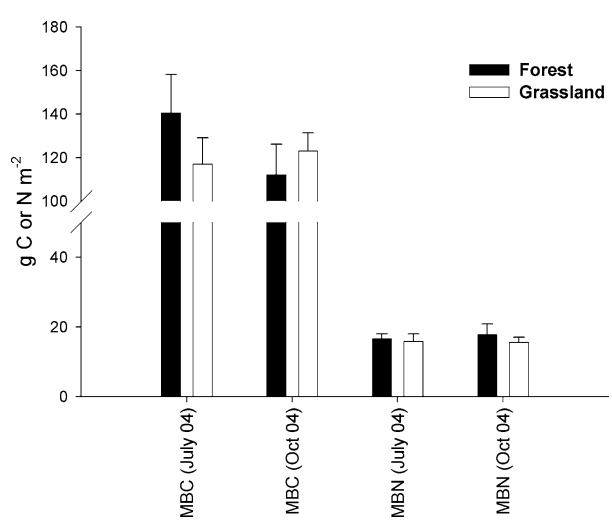

Figure 3. Microbial biomass carbon (MBC) and nitrogen (MBN) (mean ± SE) measured in the growing (July) and non-growing season (October) (note: break between 50 and 100 on *Y*-axis).

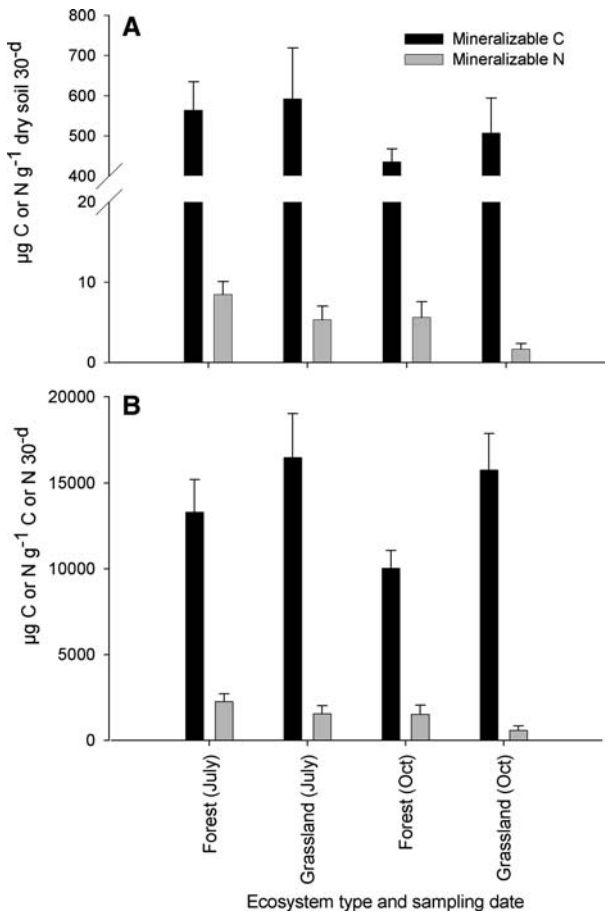

Figure 4. Potentially mineralizable carbon and nitrogen (mean ± SE) measured with 30-day laboratory incubations in forest and grassland soils collected in July (growing season) and October (non-growing season) 2004 (note: break between 20 and 400 on *Y*-axis) (**A**). Potentially mineralizable carbon and nitrogen are also expressed on a total soil C or N basis to provide an index of potential yield (**B**).

soils (Figure 4B, repeated-measures ANOVA, $F_{2,5}$ = 1.01, P = 0.428).

Net Soil N Fluxes

There were no significant differences in monthly in situ net N mineralization rates of forest and grassland soils (repeated-measures ANOVA, $F_{6,1}$ = 23.08, P = 0.158) (Figure 5A). There were cyclical seasonal variations, with peak rates (~0.10 μg N g^{-1} soil d^{-1}) of net N mineralization for both forest and grassland soils occurring in spring and summer, and the lowest rates (<0.03 μg N g^{-1} soil d^{-1}) occurring in winter. There was one period (Dec 03–Jan 04) where large negative net N mineralization (net immobilization) rates occurred in both forest (−0.08 μg N g^{-1} soil d^{-1}) and grassland soils (−0.05 μg N g^{-1} soil d^{-1}).

Net nitrification rates were also similar in forest and grassland soils for most sampling periods (Figure 5C) (repeated-measures ANOVA, $F_{6,1}$ = 24.96, P = 0.152). Net nitrification rates were relatively high (≥0.05 μg NO_3-N g^{-1} soil d^{-1}) in both forest and grassland soils in non-winter months. Lowest nitrification rates (≤ 0 μg NO_3-N g^{-1} soil d^{-1}) generally occurred in winter (Dec–Feb), with one exception being a negative rate in grassland soils only in June 2004. Net nitrification most closely tracked monthly and seasonal trends in temperature and precipitation (Figure 5D).

Mean cumulative annual net N mineralization rates (see inset Figure 5A) for June 2003–June 2004 and June 2004–June 2005 were significantly greater (by ~46%) in forest soils (11.52 ± 0.38 μg N g^{-1} soil y^{-1}) compared to grassland soils (7.90 ± 0.26 μg N g^{-1} soil y^{-1}) ($F_{1,2}$ = 60.67, P = 0.016). However, cumulative annual net nitrification rates were not significantly different for forest (12.67 ± 0.01 μg NO_3-N g^{-1} soil y^{-1}) and grassland soils (11.54 ± 2.91 μg NO_3-N g^{-1} soil y^{-1}) ($F_{1,2}$ = 0.157, P = 0.731).

Photosynthetic Nitrogen Use Effeciency

Leaf-level photosynthetic rates were greater in *A. gerardii* compared to *J. virginiana* for much of the growing season (Figure 6A). However, there was no C uptake by *A. gerardii* in the non-growing season (Nov–April) when grasses were senescent. *Juniperus virginiana* trees had maximum rates of photosynthesis during the late and early growing season for grasses. Leaf tissue N concentrations were maintained at approximately 1.5% in *J. virginiana* shoots over the entire year (Figure 6B). However, leaf tissue N in *A. gerardii* reached a peak of 2.5% at the beginning of the growing season, and then decreased steadily, leveling off at approximately 1.5% from July through early October when there was a precipitous drop to less than 0.5% N as leaf N was resorbed prior to senescing. Photosynthetic nitrogen use efficiency (PNUE) was always more than an order of magnitude greater in *A. gerardii* (>300 μmol CO_2 [mol N] $^{-1}$ s^{-1}) than *J. virginiana*, which had a maximum PNUE of 26 μmol CO_2 [mol N] $^{-1}$ s^{-1} in May 2006 (Figure 6C). The maximum PNUE in *A. gerardii* (413 μmol CO_2 [mol N] $^{-1}$ s^{-1}) occurred in August 2005, and was caused by very high photosynthetic rates (Figure 6A) relative to other months, while leaf N remained at moderate levels (Figure 6B). The great disparity in PNUE estimates was attributable, in large part, to much greater specific leaf mass (and total N content) of *J. virginiana*, rather than large differences in leaf N concentration or photosynthetic rates.

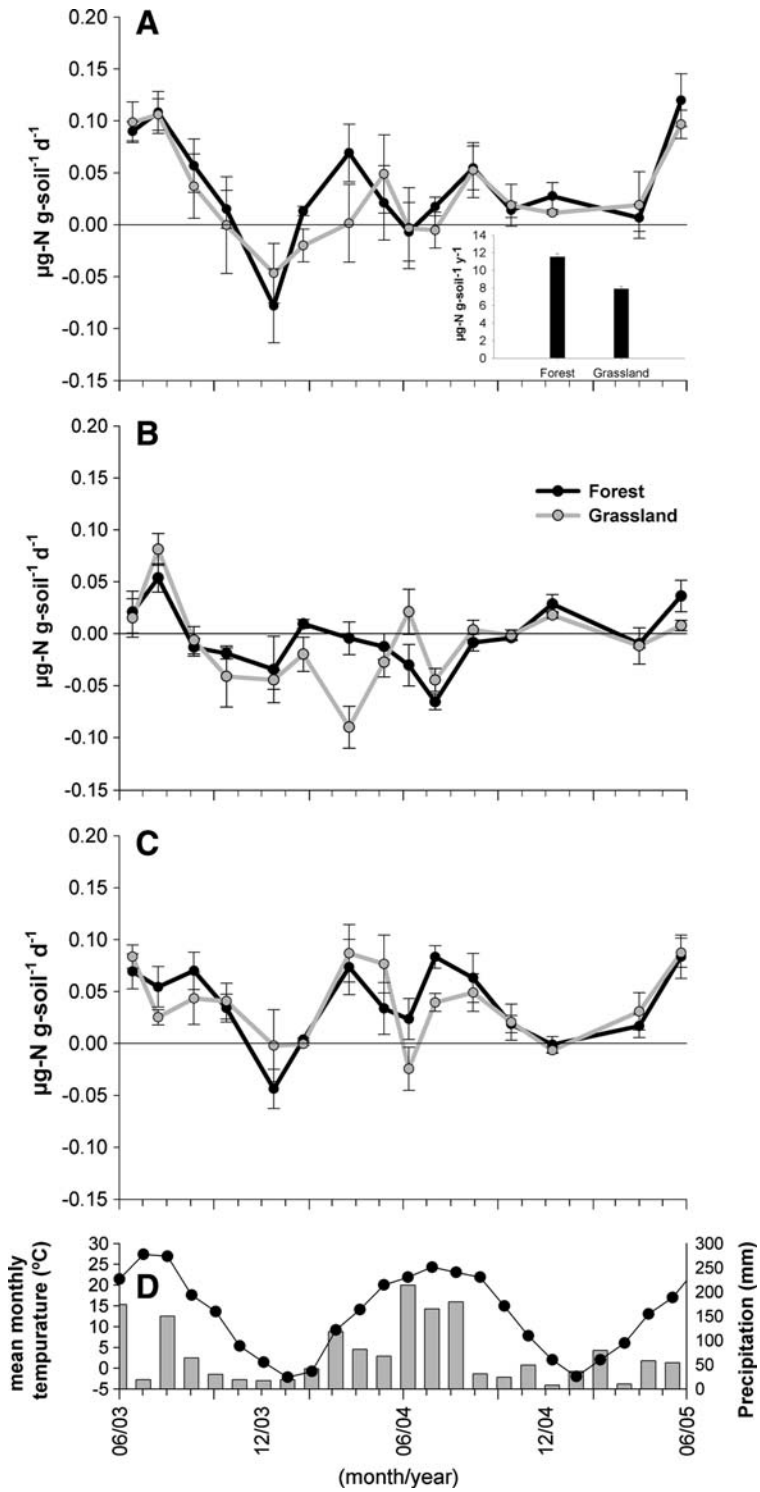

Figure 5. Net nitrogen mineralization rates (mean ± SE) (**A**), net ammonification rates (**B**), and net nitrification rates (**C**) based on in situ assays on 15 sampling periods from June 2003 through June 2005. Mean monthly temperature (*dotted line*, left *Y*-axis) and precipitation (*bars*, right *Y*-axis) during the study period are also presented (**D**).

Discussion

Substantial changes in aboveground primary productivity and the storage of C and N in plant and soil pools occurred following replacement of native grasslands by *J. virginiana* forests. However, the hypothesized changes in plant–soil feedback loops

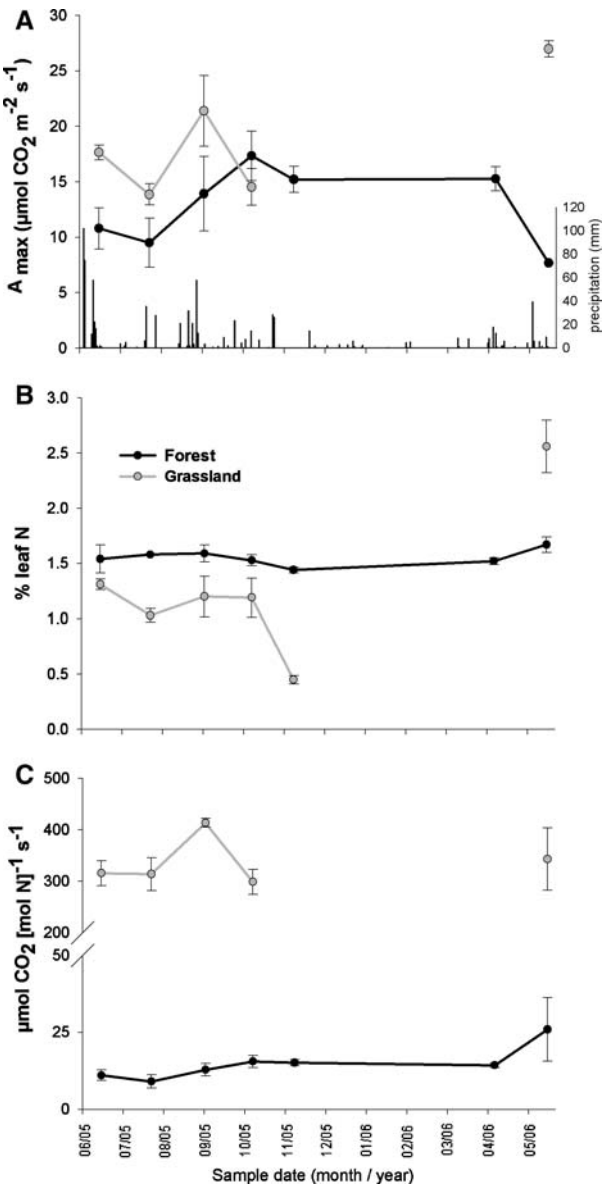

Figure 6. Net photosynthetic rates (A_{max}) (*solid line*, left *Y*-axis) and precipitation (*bars*, right *Y*-axis) (**A**), percent leaf N (**B**), and leaf-level photosynthetic nitrogen use efficiency (**C**) (mean ± SE) of *J. virginiana* and *A. gerardii*, a dominant C_4 grass in tallgrass prairie.

that were predicted to decrease soil N availability and exacerbate potential N limitation following conversion of grassland to *J. virginiana* forests did not occur. There was no evidence of changes in soil labile N pools (for example, microbial biomass), or reductions in substrate quality (potentially mineralizable N) or field N mineralization rates measured over a two-year period. This phenomenon raises two questions: (1) are there specific mechanisms that compensate for the large amounts of N immobilized in *J. virginiana* plant and soil pools, which might otherwise lead to decreased N availability? and (2) are there plant–soil interactions that allow these forests to maintain similar labile N pools and a trend for greater N availability?

Ecosystem C and N Accrual

Large differences in the productivity of *J. virginiana* forests compared to the grasslands they replace (Norris and others 2001a) contribute to large accumulations of C and N in a relatively short period (~45 years). Ecosystem C accrual in *J. virginiana* forests in the current study was significant; an additional 7,870 g C m^{-2} in *J. virginiana* forests compared to paired grassland sites (Table 1). If our estimates of C storage included root biomass of *J. virginiana*, total ecosystem C accrual could be much greater. Root:shoot ratios of *J. virginiana* may range from about 25% (Cairns and others 1997) to 40% as seen in *J. occidentalis* (Miller and others 1990). Considering the aboveground biomass of these forests (15,641 g m^{-2}) there could be as much as 6,257 g m^{-2} of belowground biomass. Assuming a root tissue C concentration of about 50%, these forests could sequester 1,955–3,128 g C m^{-2} in belowground biomass, compared to 430–543 g C m^{-2} in local grasslands (Seastedt and Ramundo 1990).

Although *J. virginiana* forests store the bulk of newly accrued C aboveground, there were also significant increases in soil organic C and N (Figures 1A, B, 2). The accrual of total SOC was relatively uniform with depth (Figure 2A); however, replacement of old SOC with new forest inputs was greatest at the mineral soil surface (48%) and decreased rapidly with depth (Figure 2B). Carbon accrual in *J. virginiana* forest mineral soils is important in reducing potential losses, such as during fire, thus allowing long-term C sequestration. Our results contrast with Jackson and others (2002), who found an apparent 57% reduction in SOC (59% in SON) in the top meter after 40 years of *Juniperus* encroachment in a Texas grassland, but are consistent with other studies that have found SOC accretion in shallow soils (≤ 35 cm) associated with encroachment of *Juniperus* and other coniferous trees (Miller and others 1990; Klemmedson and Tiedemann 2000; Bates and others 2002; Smith and Johnson 2003; Grünzweig and others 2007). Jackson and others (2002) reported that the greatest differences in *Juniperus*-derived SOC (assessed with $\delta^{13}C$) and SOC were found between approximately 20–100 cm, which suggests that SOC dynamics and storage potential could be different at sites with deep soils. The lack of differences

in soil C:N ratios in this study (Figure 1C), despite significant gains in total SOC and N, may help maintain site fertility and contribute to high productivity in forested sites. Increased SOC and N without changes in soil C:N ratios have also been observed with encroachment of *Prosopis glandulosa*, a N-fixing shrub, in arid grasslands (Hughes and other 2006; Wheeler and others 2007).

Juniperus occidentalis, endemic to the northwestern U.S., has also promoted large accumulations of C after encroaching into grasslands. For example, total ecosystem C (that is, aboveground biomass, litter and upper 10 cm of soil), of a mature (108- to 231-year-old) *J. occidentalis* forest was 13,622 g C m^{-2} (Tiedemann and Klemmedson 2000) compared to 11,476 g C m^{-2} for the younger (35- to 75-year-old) *J. virginiana* forests in this study. However, ecosystem C allocation differed between these two species, with more C allocated to aboveground biomass and less to mineral soil in *J. occidentalis*, whereas litter C totals were nearly identical for both *Juniperus* species. In comparison, encroachment in the southern Great Plains by *P. glandulosa* shrubs resulted in an accumulation of 1,900–4,300 g C m^{-2} in comparable pools within 21–68 years (Hughes and others 2006).

Significant ecosystem N accumulation of approximately 132 g N m^{-2} also occurred following *J. virginiana* encroachment, amounting to a 44% increase compared to grasslands. *Juniperus occidentalis* caused similar increases in ecosystem N accrual (51%) after encroaching into adjacent grassland (Tiedemann and Klemmedson 2000). Significant N accrual has occurred in other cases of woody plant encroachment into grasslands (Hibbard and others 2001; Hughes and others 2006; Liao and others 2006; Wheeler and others 2007), but the dominant woody invaders in these studies typically have been species with the potential to fix N. However, the potential role of N-fixation in promoting ecosystem N accrual in newly established woodland ecosystems is unclear (Wheeler and others 2007). Nitrogen accrual may be a ubiquitous and necessary change in ecosystem properties with woody plant encroachment into North American grasslands.

Accrual of ecosystem N ($\sim$3 g N m^{-2} y^{-1}) in aboveground plant biomass and shallow soil pools, coupled with sustained higher aboveground productivity, provides strong evidence that *J. virginiana* communities can compensate for greater N immobilization in plant and soil organic pools. Maintenance of labile N pools (for example, microbial biomass N and potentially mineralizable N) (Figures 3, 4) and greater N availability (cumulative annual net N mineralization) in shallow mineral soils of *J. virginiana* forests provide evidence that plant–soil feedback loops do not decrease N availability and increase potential soil N limitations.

Differences in plant or ecosystem level NUE of *J. virginiana* may contribute to the high productivity of these forests. Higher ecosystem-level NUE in *J. virginiana* forests relative to grasslands was noted in a previous study (Norris and others 2007). However, photosynthetic nitrogen use efficiency (a leaf-level metric of N use) of *J. virginiana* as measured in this study was over an order of magnitude less than that of the dominant C_4 grass, *A. gerardii*, in adjacent grasslands (Figure 6C). Higher PNUE in *A. gerardii* is attributable to greater photosynthetic rates (Figure 6A) and less N on a specific leaf mass basis. The leaf N concentration in living *A. gerardii* tissue varied considerably over the growing season, with a high in May of 2.56% ± 0.24 to a July low of 1.03% ± 0.06, and N concentration was significantly related to photosynthetic rates (df = 6, R^2 = 0.646, $P \leq 0.01$). Conversely, leaf N concentration varied little in *J. virginiana* tissue over the entire year, and there was no relationship between leaf tissue N concentration and photosynthetic rates (df = 6, $R^2 \leq 0.01$). These instantaneous measurements of NUE at the leaf level demonstrate that *J. virginiana* cannot assimilate C at nearly the same rate as a dominant C_4 grass per unit N over short-time periods, but *J. virginiana* can retain adequate N content in their leaves to maintain photosynthesis year round (Knapp and others 2008). Greater canopy leaf area, longer leaf life-span, and most notably the ability to photosynthesize year round when grasses are senescent contribute to greater overall annual ecosystem NUE (Norris and others 2007) and primary productivity (Field and Mooney 1983; Miller and others 1987; Escudero and Mediavilla 2003) despite very low instantaneous N-use efficiencies of these *Juniperus* forests.

The long-term suppression of fire in *J. virginiana* forests eliminates ecosystem N losses by combustion, which is a major pathway of N loss in regional grasslands (Blair and others 1998), and likely contributes to increased total ecosystem N storage in *J. virginiana* forests. Depending on aboveground standing stocks, grasslands lose substantial amounts of N (1–4 g N m^{-2}) when burned (Ojima and others 1994; Blair 1997), and elimination of fire in *J. virginiana* forests allows conservation of N that would otherwise be volatilized. The average standing stock of N in aboveground grassland biomass at our sites was about 3 g N m^{-2} during the mid-growing season, but decreased to 1.8 g N m^{-2} following senescence. Assuming that these forests developed

in the absence of fire for an average of approximately 45–50 years and that the adjacent grasslands burned annually, the elimination of fire could account for as much as 60–70% of N accrual in *J. virginiana* forests.

It is also possible that additional exogenous sources of N may be important in the N budgets of aggrading *J. virginiana* forests. Estimates of annual above-ground plant N uptake in comparable *J. virginiana* forests obtained from a similar study in the Flint Hills (Norris 2000) indicate that the requirement of *J. virginiana* forests for N (5.9 g N m^{-2} y^{-1}) was about 3.5× greater than that of adjacent grassland communities (1.7 g N m^{-2} y^{-1}) and this demand greatly exceeded estimated annual net N mineralization rates in the top 10 cm of the soil (1.4 g N m^{-2} y^{-1}). Greater plant N demand and sequestration in *J. virginiana* forests suggest that potential N limitations may be alleviated to some extent by inputs from additional exogenous sources (for example, enhanced scavenging of atmospheric inputs), particularly because these forests developed on very shallow soils which might constrain access to deep soil N. However, potential sources of exogenous N, and their importance in *J. virginiana* forest budgets, remains to be investigated (see McKinley and others 2008).

Soil C and N Flux

In total, the results of field and laboratory assays suggest that N availability, as indexed by net N mineralization, was similar or tended to be greater (by ~46% when considering cumulative annual rates) in *J. virginiana* forest soils relative to grassland soils (Figures 4, 5A). This trend for greater N mineralization in forest soils is contrary to our initial prediction of reduced net N mineralization, relative to grasslands. In contrast, and in support of our original hypothesis, potential soil CO_2-C flux (Figure 4A, B) tended to be less in *J. virginiana* forests compared to adjacent grassland soils on a bulk soil basis (repeated measures ANOVA, $F_{2,5} = 4.46$, $P = 0.077$), or relative to the total SOC pool (repeated measures ANOVA, $F_{2,5} = 6.68$, $P = 0.039$). This is consistent with reported slower turnover of C in *J. virginiana* soils, compared to grasslands (Smith and Johnson 2004). Lower soil CO_2-C flux relative to the overall C pool size in *J. virginiana* soils (Figure 4B) may contribute to the significant increase in C concentration in forest mineral soil (40.3 ± 1.2 mg C g $soil^{-1}$) compared to grassland soil (32.5 ± 1.8 mg C g $soil^{-1}$) ($F_{1,6} = 7.42$, $P = 0.01$). Differences in litter quality or abiotic conditions may also facilitate soil C accrual in newly established *J. virginiana* forests. Norris and others (2001b) found that decomposition of fresh *J. virginiana* litter was significantly slower that decomposition of litter from the dominant grass, *A. gerardii*, owing to differences in litter chemistry. *Juniperus virginiana* roots and surface litter have higher percentage of lignin compared to the dominant C_4 grass (*Andropogon gerardii*) (Norris and others 2001b), which is important in controlling decomposition and N mineralization, and forming recalcitrant C compounds (Scott and Binkley 1997). Recalcitrant C sources may become more prevalent in later stages of decomposition, slowing C mineralization in the long term (McClaugherty and Berg 1987), which may contribute to the accretion of soil organic pools and increasing percentage of forest-derived C comprising SOC (38% in the upper 10 cm of mineral soil, Figure 2B).

Greater N mineralization rates in forest soils have important implications for whole ecosystem processes. The ability to maintain, or increase soil N availability, as *J. virginiana* forests encroach into grasslands may contribute, in part, to their greater productivity or C uptake relative to the grasslands they replace, which may affect plant community dynamics and ecosystem C storage. Increases in soil resource availability are commonly observed with shrub/woodland encroachment in arid and semi-arid regions, and with encroachment of N-fixing woody plant species (Schlesinger and others 1990; Scholes and Archer 1997; Hibbard and others 2001; Hughes and others 2006; Liao and others 2006; Wheeler and others 2007). This study suggests that *Juniperus* encroachment may create similar, although smaller magnitude, increases in resource availability in more mesic grasslands.

Juniperus forest soils generally have much lower temperatures (as much as 7°C lower at 5 cm), which may constrain potential C and N mineralization under field conditions (McKinley and others 2008). Smith and Johnson (2004) found that soil temperatures explained most of the variance in field CO_2 flux in *Juniperus* forests and grasslands, and soil CO_2 flux was lower because of lower mean temperatures in *J. virginiana* soils. Comparisons of parallel in situ and laboratory incubations suggest that conditions in the field in both ecosystems are sub-optimal for N mineralization, because in situ net N mineralization rates in the growing season were approximately 6% and 3% of laboratory-based potential net N mineralization rates in *J. virginiana* and grassland soils, respectively. In the non-growing season, in situ net N mineralization rates were a similar proportion (7%) of potential rates in forests, whereas grassland soils seem to be

closer to optimum conditions for N mineralization rates, with in situ rates approximately 34% of potential rates. However, it is also important to note that soils used in the laboratory incubations were disturbed more than the cores incubated in the field, which likely inflated potential N mineralization estimates. Intra-annual variation in temperature and precipitation appear to be important drivers of seasonal differences in field net N mineralization and net nitrification rates in both ecosystems (Figure 5A, C, D), but differences in the microclimate of forests and grasslands may contribute to more subtle differences in C and N cycling between ecosystems.

Differences in substrate composition and microclimate, however, do not create the expected plant–soil feedback loop that would result in lower N availability and stronger N limitation on plant productivity following the encroachment of *Juniperus* forests into grassland. Even though organic C concentrations in forest soils (mineral soil) are greater, and a greater proportion of SOC in the mineral soil is unavailable or slowly utilized by soil microbes, as evidenced by reduced C flux relative to the overall C pool size (Figure 2B), N mineralization tends to be greater than adjacent grasslands. In the long-term, slower C turnover in forest soils may contribute to greater SOC pools in the mineral horizon, an important long-term stable C sink.

Future Directions and Conclusions

For more than a century, an increase in cover and biomass of *Juniperus* spp. and other woody plants has converted millions of hectares of grasslands across a broad spectrum of climatic conditions to woodlands and forests, but the future of these communities and consequences for large-scale biogeochemical processes is uncertain. Ecosystem models that couple biogeochemical processes with plant community changes will be valuable in forecasting the consequences of *Juniperus* forest encroachment in the Great Plains and elsewhere. These models will require detailed field data on C and N pools and fluxes, such as those provided in this and other related studies (Smith and Johnson 2003; Norris and others 2007). Alterations in global or regional climate, increased atmospheric CO_2 concentrations, N-deposition, and human land-use activities that could hasten, halt, or reverse *Juniperus* forest encroachment should be considered in the development of new ecosystem models. In the central Great Plains, most forecasted global changes are expected to favor *Juniperus* forest expansion (Knapp and others 2008). Elsewhere, increases in prolonged drought may lead to large-scale mortality of *Juniperus* spp., as has occurred in southwestern North America (Breshears and others 2005). In parts of the western US, invasions by non-native grasses have altered fire regimes and started to reverse the process of woody plant encroachment (Bradley and others 2006). Although the future of *Juniperus* communities remains obscure, the ability of newly established forests to sustain relatively high productivity for at least several decades has broad implications with respect to rapid stand development, rates of potential ecosystem C accrual, and more broadly, the strength and timing of potential C sinks in ecosystem models developed for the Great Plains, and other grasslands and savannas experiencing similar ecosystem transitions (Van Auken 2000; Briggs and others 2005).

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REFERENCES

Albani M, Medvigy D, Hurtt GC, Moorcroft PR. 2006. The contribution of land-use change, CO_2 fertilization, and climate variability to the Eastern US carbon sink. Glob Change Biol 12:2370–90.

Archer SR, Boutton TW, Hibbard KA. 2001. Trees in grasslands: biogeochemical consequences of woody plant expansion. In: Schulze ED, Harrison S, Heimann M, Holland E, Lloyd J, Prentice I, Schimel D, Eds. Global biogeochemical cycles in the climate system. San Diego: Academic Press. p 115–38.

Arrouay D, Balesdent JB, Mariotti A, Girardin C. 1995. Modeling organic carbon turnover in cleared temperate forest soils converted to maize cropping using ^{13}C natural abundance measurements. Plant Soil 173:191–6.

Austin AT, Vivanco L. 2006. Plant litter decomposition in a semiarid ecosystem controlled by photodegradation. Nature 442:555–8.

Balesdent J, Wagner GH, Mariotti A. 1988. Soil organic matter turnover in long-term field experiments as revealed by carbon-13 natural abundance. Soil Sci Soc Am J 52:118–24.

Bark D. 1987. Konza Prairie Research Natural Area, Kansas. The climates of the long-term ecological research sites. In: Greenland D, Eds. Institute of Artic and Alpine research, occasional paper number 44. Colorado: University of Colorado. p 45–50.

Bates JD, Svejcar TJ, Miller RF. 2002. Effects of juniper cutting on nitrogen mineralization. J Arid Environ 51:221–34.

Blair JM. 1997. Fire, N availability, and plant response in grasslands: a test of the transient maxima hypothesis. Ecology 78:2359–68.

Blair JM, Seastedt TR, Rice CW, Ramundo RA. 1998. Terrestrial nutrient cycling in tallgrass prairie. In: Knapp AK, Briggs JM, Hartnett DC, Collins SL, Eds. Grassland dynamics: long term ecological research in Tallgrass Prairie. New York: Oxford University Press. p 222–43.

Bradley BA, Houghton RA, Mustard JF, Hamburg SP. 2006. Invasive grass reduced aboveground carbon stocks in shrublands of the western US. Glob Change Biol 12:1815–22.

Breshears DD, Cobb NS, Rich PM, Price KP, Allen CD, Balice RG, Romme WH, Kastens JH, Floyd ML, Belnap J, Anderson JJ, Myers OB, Meyer CW. 2005. Regional vegetation die-off in response to global-change-type drought. Proc Natl Acad Sci 102:15144–8.

Briggs JM, Hoch GH, Johnson LC. 2002. Assessing the rate, mechanisms, and consequences of the conversion of tallgrass prairie to *Juniperus virginiana* forest. Ecosystems 5:578–86.

Briggs JM, Knapp AK, Blair JM, Heisler JL, Hoch GA, Lett MS, McCarron JK. 2005. An ecosystem in transition: woody plant expansion into mesic grassland. Bioscience 55:2245–57.

Cairns MA, Brown S, Helmer EH, Baumgarder GA. 1997. Root biomass allocation in the world's upland forests. Oecologia 111:1–11.

Escudero A, Mediavilla S. 2003. Decline in photosynthetic nitrogen use efficiency with leaf age and nitrogen resorption as determinants of leaf life span. J Ecol 91:880–9.

Field F, Mooney HA. 1983. The photosynthesis-nitrogen relationship in wild plants. In: Givnish TJ, Eds. On the economy of plant form and function: proceedings of the sixth Maria Moors Cabot Symposium, evolutionary constraints on primary productivity, adaptive patterns of energy capture in plants. Harvard Forest: Cambridge University Press. p 25–55.

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P. 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281:237–240.

Fowells HA. 1965. Silvics of forest trees of the United States, agriculture handbook No. 271. Washington DC: United States Department of Agriculture.

Garcia FO, Rice CW. 1994. Microbial biomass dynamics in tallgrass prairie. Soil Sci Soc Am J 47:945–51.

Grünzweig JM, Gelgfand I, Fried Y, Yakir D. 2007. Biogeochemical factors contributing to enhanced carbon storage following afforestation of a semi-arid shrubland. Biogeosciences 4:891–904.

Hibbard KA, Archer SR, Schimel DS, Valentine DW. 2001. Biogeochemical changes accompanying woody plant encroachment in a subtropical savanna. Ecology 82:1999–2001.

Horwarth WR, Paul EA. 1994. Microbial biomass. In: Weaver RW, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A, Wollum A, Eds. Methods of soil analysis part 2. Madison: Soil Science Society of America. p 753–73.

Hughes RF, Archer SR, Asner GP, Wessman CA, McMurtry C, Nelson J, Ansley RJ. 2006. Changes in aboveground primary production and carbon and nitrogen pools accompanying woody plant encroachment in a temperate savanna. Glob Change Biol 12:1733–47.

Jackson RB, Banner JL, Jobbágy EG, Pockman WT, Wall DH. 2002. Ecosystem carbon loss with woody plant invasion of grasslands. Nature 418:623–6.

Jenkinson DS, Powlson DS. 1976. The effects of biocide treatments on metabolism in soil – V. A method for measuring soil biomass. Soil Biol Biochem 8:209–13.

Johnson LC, Matchett JR. 2001. Fire and grazing regulate belowground processes in tallgrass prairie. Ecology 82:3377–89.

Klemmedson JO, Tiedemann AR. 2000. Influence of western juniper development on distribution of soil and organic layer nutrients. Northwest Sci 74:1–11.

Knapp AK, Briggs JM, Blair JM, Turner CL. 1998. Patterns and controls of aboveground net primary production in tallgrass prairie. In: Knapp AK, Briggs JM, Hartnett DC, Collins SL, Eds. Grassland dynamics: long term ecological research in tallgrass prairie. New York: Oxford University Press. p 193–221.

Knapp AK, McCarron JK, Silletti AM, Hoch GA, Heisler JL, Lett MS, Blair JM, Briggs JM, Smith MD. 2008. Ecological consequences of the replacement of native grassland by *Juniperus virginiana* and other woody plants. In: Auken OWVan, Eds. Western North American *Juniperus* communities: a dynamic vegetation type. New York: Springer-Verlag New York. p 156–69.

Liao JD, Boutton TW, Jastrow JD. 2006. Storage and dynamics of carbon and nitrogen in soil physical fraction following woody plant invasion of grassland. Soil Biol Biochem 38:3184–96.

McClaugherty C, Berg B. 1987. Cellulose, lignin and nitrogen concentrations as rate regulating factors in late stages of forest litter decomposition. Pedobiologia 30:101–12.

McKinley DC, Norris MD, Blair JM, Johnson LC. 2008. Altered ecosystem processes as a consequence of *Juniperus virginiana* L. encroachment into North America tallgrass prairie. In: Auken OWVan, Eds. Western North American *Juniperus* communities: a dynamic vegetation type. New York: Springer-Verlag New York. p 170–87.

Miller RF, Eddleman LE, Angell RF. 1987. Relationship of western juniper stem conducting tissue and basal circumference to leaf area and biomass. Great Basin Nat 47:349–54.

Miller PM, Eddleman LE, Kramer PJ. 1990. Allocation patterns of carbon and minerals in juvenile and small-adult *Juniperus occidentalis*. Forest Sci 36:734–46.

Moiser AR. 2001. Exchange of gaseous nitrogen compounds between agricultural systems and the atmosphere. Plant Soil 228:17–27.

Norris MD. 2000. Biogeochemical consequences of land cover change in eastern Kansas. Manhattan: Kansas State University, p 112.

Norris MD, Blair JM, Johnson LC. 2007. Altered ecosystem nitrogen dynamics as a consequence of land cover change in tallgrass prairie. Am Midland Nat 158:432–45.

Norris MD, Blair JM, Johnson LC, McKane RB. 2001. Assessing changes in biomass, productivity, and C and N stores following *Juniperus virginiana* forest expansion into tallgrass prairie. Can J Forest Res 31:1940–6.

Norris MD, Blair JM, Johnson LC. 2001. Land cover change in eastern Kansas: litter dynamics of closed-canopy eastern redcedar forests in tallgrass prairie. Can J Bot 79:214–22.

Ojima DS, Schimel DS, Parton WJ, Owensby CE. 1994. Long-term and short-term effects of fire on nitrogen cycling in tallgrass prairie. Biogeochemistry 24:67–84.

Pacala SW, Hurtt GC, Baker D, Peylin P, Houghton RA, Birdsey RA, Heath L, Sundquist ET, Stallard RF, Ciais P, Moorcroft P, Caspersen JP, Shevliakova E, Moore B, Kohlmaier G, Holland E, Gloor M, Harmon ME, Fan S-M, Sarmiento JL, Goodale CL, Schimel D, Field CB. 2001. Consistent land and atmosphere-based US carbon sink estimates. Science 292:2316–20.

Schlesinger WH, Reynolds JF, Cunningham GL, Huenneke LF, Jarrell WM, Virginia RA, Whitford WG. 1990. Biological feedbacks in global desertification. Science 247:1043–8.

Schmidt TL, Leatherberry EC. 1995. Expansion of eastern juniper in the lower Midwest. Natl J Am Forest 12:180–3.

Scholes RJ, Archer SR. 1997. Tree-grass interactions in savannas. Annu Rev Ecol Syst 28:517–44.

Scott NA, Binkley D. 1997. Foliage litter quality and annual net N mineralization: comparison across North American forest sites. Oecologia 111:151–9.

Seastedt TR, Ramundo RA. 1990. The influence of fire on belowground processes of tallgrass prairies. In: Collins SL, Wallace LL, Eds. Fire in North American tallgrass prairies. Norman Oklahoma: University of Oklahoma Press. p 99–117.

Smith DL, Johnson LC. 2003. Expansion of *Juniperus virginiana* L. in the Great Plains: changes in soil organic carbon dynamics. Glob Biogeochem Cycle 17:1–12.

Smith DL, Johnson LC. 2004. Vegetation-mediated changes in microclimate reduce soil respiration as woodlands expand into grasslands. Ecology 85:3348–61.

Throop HL, Archer SR. 2007. Interrelationships among shrub encroachment, land management, and litter decomposition in a semiarid grassland. Ecol Appl 17:1809–1823.

Tiedemann AR, Klemmedson JO. 2000. Biomass and nutrient distribution and system nutrient budget for western juniper in central Oregon. Northwest Sci 74:12–24.

Tilman D, Reich P, Phillips H, Menton M, Patel A, Vos E, Peterson D, Knops J. 2000. Fire suppression and ecosystem carbon storage. Ecology 81:2680–5.

Van Auken OW. 2000. Shrub invasions of North American semiarid grasslands. Annu Rev Ecol Syst 31:197–215.

Wheeler CW, Archer SR, Asner GP, McMurtry CR. 2007. Climatic/edaphic controls on soil carbon/nitrogen response to shrub encroachment in desert grassland. Ecol Appl 17: 1911–28.