CONSEQUENCES OF CONVERSION OF NATIVE MESIC GRASSLAND TO CONIFEROUS FOREST ON SOIL PROCESSES AND ECOSYSTEM C AND N STORAGE

by

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B.S., University of Texas at San Antonio, 1999
M.S., University of Texas at San Antonio, 2002

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Division of Biology
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Abstract

Juniperus virginiana, an important woody plant invader in the U.S. Central Plains, has increased considerably in density and cover in large areas previously dominated by tallgrass prairie. Change in the phenology and nitrogen use efficiency of the dominant plant communities as *J. virginiana* replaces native prairies may lead to increased plant productivity and biomass accumulation, but may also alter the microclimate and litter quality that affect soil microbial communities responsible for key soil processes. I have focused my investigations on changes in key soil processes that could lead to differences in soil N availability, as well as changes in ecosystem C and N pools and fluxes as *J. virginiana* expands into native grasslands. *Juniperus virginiana* forest soils exhibit greater cumulative annual net N mineralization (11.52 ± 0.38 μg N g^{-1} soil y^{-1}) compared to prairie soils (7.90 ± 0.26 μg N g^{-1} soil y^{-1}) ($F = 60.67$, $P = 0.016$), yet slightly reduced potential soil C flux. Examination of internal soil N cycling revealed that both *J. virginiana* and prairie soils minimize potential soil N losses, by rapid microbial immobilization of inorganic N, and constraining nitrification via substrate limitation or environmental constraints. Leaf-level photosynthetic nitrogen use efficiency (NUE) was over a magnitude higher in the dominant grass, *Andropogon gerardii*, but high annual ecosystem-level NUE and greater soil N availability may contribute to the higher productivity and rapid accrual of C in newly established *J. virginiana* forests. Increased plant productivity and elimination of fire in *J. virginiana* forests have allowed at least 80,000 kg ha^{-1} increase in ecosystem C storage in about half a century. Soil organic C, an important long-term sink, has also increased significantly in *J. virginiana* forests, with approximately 34% replacement of C_4 grass-derived soil C with new C from trees in the A-horizon. The observed high productivity of *J. virginiana* and increased N availability necessary to support continued plant biomass accumulation are

possible because of substantial (~ 44%) increase in ecosystem N in measured pools, which is a likely a result of reduced volatilization of N from biomass burning, possible increased exogenous N inputs, and/or N translocation from deeper soil horizons. Reduced fire return intervals in prairie provide an opportunity for *J. virginiana* to establish and facilitate N accrual, which may allow this species to accelerate is own establishment through creating conditions of increased N availability and efficient utilization of N.

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Table of Contents

List of Figures

List of Tables

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CHAPTER 1 - Introduction

Over the last century millions of hectares of grasslands and savannas have been converted to shrublands or forests on every continent except Antarctica (Archer et al. 2001). In North America, woody plant expansion coincided with rapid increases in human populations and subsequent changes in land use and natural disturbance regimes (Archer et al. 2001). Some notable genera of woody plants that are currently invading, and often, replacing grasslands include *Baccharis*, *Juniperus*, *Larrea, Pinus*, *Prosopis, Quercus,* and others (Van Auken 2000). Woody plant encroachment may appreciably alter ecosystem processes, such as rates and patterns of plant carbon (C) assimilation, as well as nutrient cycles. Resulting changes in these ecosystem processes significantly alter the magnitude and allocation ecosystem C and nutrient storage. On a broader scale, expansion of woodland and forest ecosystems may serve as part of a significant C sink in North America (Ciais et al. 1995, Pacala et al. 2001). *Juniperus* is an important woody plant invader in North America, and has received considerable attention because of the large areas in which grassland ecosystems have been converted into woodland or forests by *Juniperus* species invasions over very short-time scales.

Juniperus expansion in North America begun in the late 1800's as European settlement and associated land management practices and other anthropogenic activity became more extensive (Smeins 1983). Historically, *Juniperus* trees grew mainly in areas that were sheltered from intense fire, such as rocky outcrops or areas with shallow soils, because of their sensitivity to fire. Fire suppression and overgrazing by domestic ungulates has been widely implicated in promoting the rapid expansion of these native plants, but other factors associated with global

environmental change (e.g., elevated CO_2 and N deposition) could further facilitate *Juniperus* establishment.

There are 14 species that comprise the genus *Juniperus* in North America. *Juniperus* species have two main growth forms; most are trees that may grow well over 20 m (e.g., *J. virginiana*), and some are prostrate and rarely grow over 2 m in height (e.g., *J. horizontalis*) (Hora 1986). Reports of *Juniperus* expansion in the U.S. have focused on large growth forms (large shrubs or trees) that have encroached into grasslands or other communities. Many of these native *Juniperus* species have increased in cover in many areas of the Intermountain west, Northwest, Southwest, Midwest, and some Eastern communities of the U.S. (Arnold et al. 1964, Buffington and Herbel 1965, Blackburn and Tueller 1970, Dealy et al. 1978, Archer et al. 1988, Idso 1992, Breashears et al. 1997, Laurenroth et al. 1999, Van Auken 2000, Wall et al. 2001, Briggs et al. 2002, Miller et al. 2005). *Juniperus* expansion is not limited to grasslands, but has also been observed in aspen (*Populus temuloides* Michx.) and sagebrush (*Artemisia tridentata*) communities (Roberts and Jones 2000, Wall et al. 2001). *Juniperus virginiana* communities have expanded dramatically in the U.S. Central Plains, as far north as Wisconsin, and south into Texas and Louisiana (Briggs et al. 2002). Also, there has been limited documentation of *J. virginiana* encroachment in Virginia and Kentucky (Rhoades et al. 2004). Fire suppression has been implicated as the primary cause of *Juniperus* expansion into grasslands, but resource competition, plant resource allocation patterns, and differences in plant phenology may contribute to *Juniperus* expansion into other woodland communities (Gholz 1980, Miller et al. 1990, Miller et al. 1992, Scott and Binkley 1997, Van Auken 2000, Wall et al. 2001, Leffler and Caldwell 2005).

Juniperus virginiana L. is the most widely distributed *Juniperus* species in the continental United States, and may be found in every state east of the 100th meridian (Figure 1) (Fowells 1965). In the eastern Great Plains and other areas, *J. virginiana* has encroached into adjacent grasslands at an unprecedented and rapid rate, affecting at least 3 million hectares (Schmidt and Leatherberry 1995, Briggs et al. 2002). *Juniperus virginiana*, like some western *Juniperus* species in North America, forms both dispersed community associations, or often, very dense (130-3500 trees ha^{-1}), nearly monospecific stands (Norris et al. 2001, Briggs et al. 2002, Rhoades et al. 2004) that can modify the microclimate with respect to soil temperature and moisture (see Chapter 5; Smith and Johnson 2003), as well as substantially reduce plant species diversity and richness below the canopy (Lassoie et al. 1983, Gehring and Bragg 1992, Briggs et al. 2002). *Juniperus virginiana* is typically found in more mesic areas (>750 mm) and over a broader range of soils compared to most western *Juniperus* species.

Juniperus virginiana forests that established in areas that were historically native tallgrass prairie in the Central Plains have as much as three times greater aboveground net primary productivity (ANPP) than the grasslands they replaced (Norris 2001). Also, substantial increases and shifts in ecosystem C storage were observed with conversion of tallgrass prairie to *J. virginiana* forests (Norris et al. 2001, Smith and Johnson 2004). The ability of these *J. virginiana* forests to maintain higher productivity than the native grassland communities may be paramount for their establishment and continued expansion by escaping severe damage by fire, and may drive rapid C accrual and changes in other ecosystem properties. High productivity of *J. virginiana* forests may be a consequence of decreased resource limitations and/or greater nutrient use efficiency, though this has not been well studied.

Increased productivity of *J. virginiana* forests should elicit significant soil N limitations compared to prairie that is replaced, due to greater C inputs to the soil and greater N immobilization (Figure 2). However, with increased N limitations there should be ensuing reductions in potential productivity, but the apparent ability of *J. virginiana* forests to sustain higher productivity relative to prairie ecosystems (Norris 2001) contradicts this expectation. Understanding the mechanisms of this apparent contradiction may be critical for furthering our understanding of how grasslands are rapidly converted to *J. virginiana* forests.

Soil nitrogen availability and N utilization by *J. virginiana* may be important to understanding the ability of *Juniperus* species to maintain high productivity. In many North American coniferous and hardwood forests, ANPP has a strong positive correlation with net N mineralization, an index of soil N availability (Reich et al. 1997). Soil nitrogen availability also has a strong, well-documented role in shaping plant community composition, richness and primary productivity in other systems (Tilman 1982, 1987, 1993, Turner et al. 1997, Zavaleta et al. 2003). The increased productivity *J. virginiana* forests may result, at least in part, from increased N availability. However, coniferous species also have greater ecosystem nitrogen use efficiency (NUE) than most other plant life forms, which could also contribute to the observed patterns of greater ANPP and ecosystem C accrual by minimizing the effects of soil nitrogen limitations in *J. virginiana* forest (Chapin 1980, Aerts 1995, Lambers et al. 1998). Thus, both soil N availability and N use efficiency in *J. virginiana* forests may be important in contributing to the high ANPP observed in this ecosystem.

Key factors that control N availability in terrestrial ecosystems may be altered when *J. virginiana* forests encroach into grasslands. For example, soil microbial processes that mediate N cycling are temperature and moisture dependent, and changes in these conditions could

significantly alter soil N availability. Substantial alterations of both soil temperature and moisture relative to the community they replace have been reported in the soils of *Juniperus* forests and other woodlands (Lassoie et al. 1983, Belsky et al. 1989, Harrington 1991, Fulbright et al. 1995, Breshears et al. 1997, Thurow and Hester 1997, Breashears et al. 1998, Wayne 2000, Smith and Johnson 2004). Also, the quantity and quality of organic inputs into the soil through surface and root litter may affect N availability by influencing microbial metabolic activity and rates of N release from the decomposition of these substrates (Zak et al. 1994). Norris (2001) found that both *J. virginiana* litter and the microclimate in *J. virginiana* forests caused slower decomposition compared to the prairie microclimate and *Andropogon gerardii* litter. *Juniperus virginiana* may change factors that influence the complex and dynamic microbial community that mediates soil N cycling and availability (see Figure 3).

The overall objective of this dissertation was to compare the biogeochemistry of recently developed *J. virginiana* forests and the prairie ecosystems they replaced to better understand changes in ecosystem processes and properties, as well as possible mechanisms that promote these changes. I focused my investigations on changes in specific soil processes (i.e., N cycling) that may affect other ecosystem processes (e.g., plant C uptake) and properties (e.g., ecosystem C and N pools), as well as elucidate mechanisms that perpetuate changes in these ecosystem properties. In this dissertation I explored: potential differences in soil N availability under field conditions and proximate causes (Chapter 2), mechanistic and integrated approaches to reveal detailed information on potential differences in specific soil N transformations and their mediation by microbes (Chapter 3), and whole ecosystem accounting of C and N, as well as the role of N in C uptake, to better understand the observed patterns and underlying drivers of N cycling (Chapter 4). In Chapter 5, I discuss my findings in the context of the greater body of

research done on *J. virginiana* encroachment into mesic grasslands in the U.S. Central Plains, and provide a synthesis of results to more fully understand the consequences of this phenomenon.

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Figures

Figure 1-1 Map of the continental U.S. with the distribution of *Juniperus virginiana* in the U.S. and a portion of Canada. *Juniperus virginiana* expansion has been most extensive in the western portion of its range, where it has encroached into in grassland communities of the Central Plains, as far north as Wisconsin and south in Texas and Louisiana. Four study sites were established in Riley County along the perimeter of the Konza Prairie Biological Station (in green).

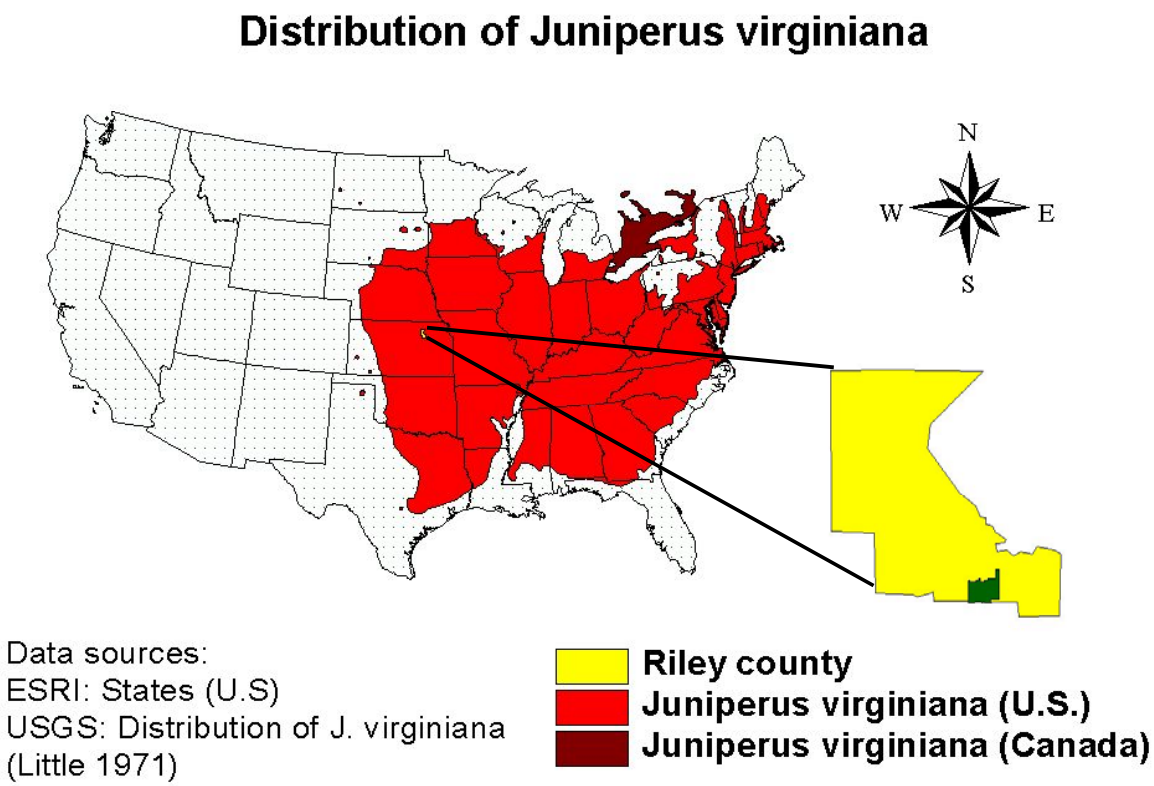


Figure 1-2 A conceptual model illustrating hypothesized mechanisms by which plant-soil feedback loops could increase soil nitrogen limitation in *J. virginiana* forests relative to prairie. *Juniperus virginiana* exhibits greater productivity (NPP) compared to prairie, which causes large amounts of N to accumulate in the plant biomass, as well as soil pools because of greater C inputs and less N input, which could increase N immobilization, reduce potential soil N availability and potential plant productivity. To compensate for limited soil N availability, *J. virginiana* exhibit greater ecosystem nitrogen use efficiency (NUE), which would allow greater productivity with limited soil N availability. As a consequence of strong soil N limitation and high NUE of *J. virginiana*, the resulting litter or C inputs that enter soil organic matter pools would have a greater C:N ratio, which would decrease soil labile N pools and reduce N availability and N uptake by *J. virginiana*. However, productivity may be maintained despite subsequent reductions in soil N availability by increasing NUE, thus creating a self-reinforcing plant-soil feedback loop that exacerbates soil N limitations. (Modified from Lou et al. 2004)

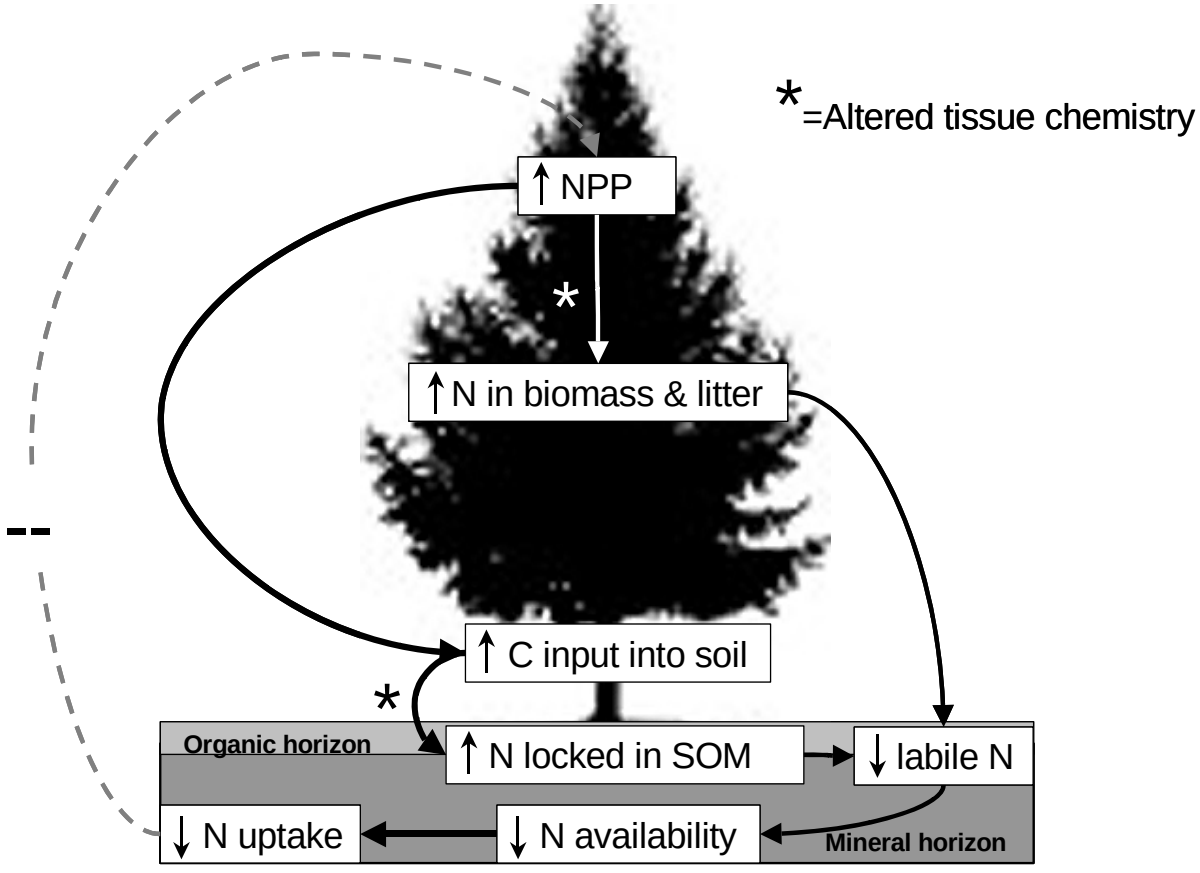
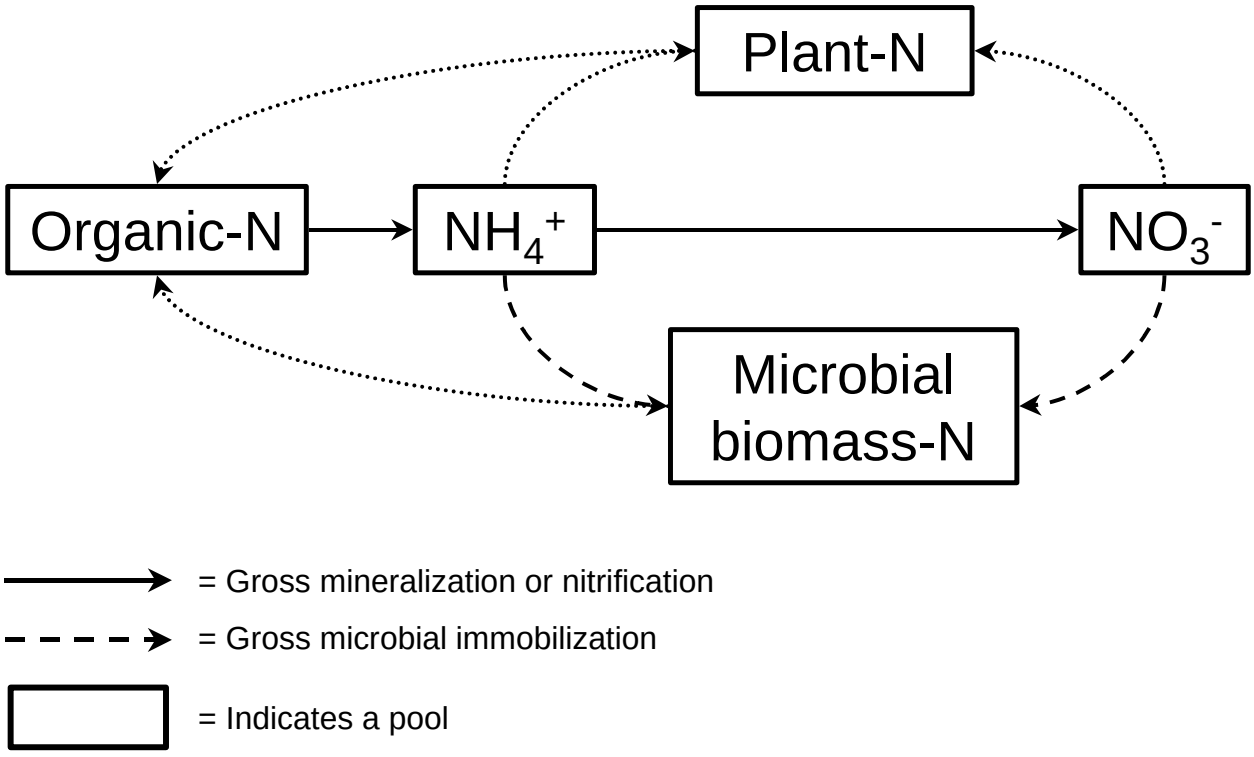


Figure 1-3 A conceptual model illustrating major soil N cycling pathways, including major pools and fluxes. Although soils have large pools if organic N, this form of N is generally unavailable for plant uptake. Plants generally require inorganic N (NH_4^+ and NO_3^-), produced by soil microbes, as their N source in most natural ecosystems. Soil microbes produce ammonium (NH_4^+) during the decomposition of organic nitrogen, a process called nitrogen mineralization. The resulting soil NH_4^+ has three main fates: it can be immobilized in microbial biomass, utilized by plants, or converted by chemoautotrophic bacteria via a two-step process to nitrate (NO_3^-), a process called nitrification. Nitrate that is retained in the ecosystem has two main fates: immobilization by microbes or utilization by plants. In the current paradigm microbes are superior competitors for inorganic N compared to plants, thus plant available N is the amount of inorganic N produced in excess of microbial demands. Net N mineralization is the difference in gross production of inorganic N minus the amount immobilized (consumed) by microbes, and the net difference (net N mineralization) is considered to be the amount of N available for plant uptake.



CHAPTER 2 - *Juniperus virginiana* L. encroachment into tallgrass prairie influences soil nutrient availability

Abstract

Within the last several decades, *Juniperus virginiana* cover and abundance has increased dramatically in areas of the U.S. Central Plains historically dominated by diverse herbaceous grassland communities. Little is known about how conversion of native grasslands to *Juniperus* dominated woodlands or forests affects soil nutrient availability, and such changes have important implications for ecosystem functioning. Four paired sites of mesic grassland (native tallgrass prairie) and adjacent *J. virginiana* forests (converted from prairie in the last 50 yr) were selected to assess differences in soil nitrogen and carbon cycling resulting from this land-cover change. Net N mineralization rates were assessed using intact soil cores incubated *in situ* for 30-day intervals over two years. Potential C and N mineralization rates, indicative of changes in labile C and N pools, were determined by laboratory incubations of soils collected during growing and non-growing seasons. Cumulative annual net N mineralization in the field was significantly greater in forest soils (11.52 ± 0.38 μg N g^{-1} soil y^{-1}) compared to prairie soils (7.90 ± 0.26 μg N g^{-1} soil y^{-1}) ($F = 60.67$, $P = 0.016$), which was qualitatively consistent with laboratory-based estimates of potential N mineralization. Conversely, potential C mineralization tended to be less in forest soils and was significantly lower than in prairie soils in the non-growing season when considered on per gram C basis in the non-growing season ($F = 6.144$, $P = 0.048$). Greater net N mineralization and reduced C mineralization in the soils of forests compared to prairies suggests that forest encroachment into mesic grasslands reduces soil N limitations and supports high plant productivity while simultaneously accumulating soil organic C. Continued change in land-use practices that favor woody plants (e.g., fire suppression)

coupled with the tremendous expansion capabilities of *Juniperus* communities, suggests that these forests may play an increasing role in regional C sinks in the future.

Introduction

Afforestation of grasslands and savannas is at present a worldwide phenomenon, occurring on every major continent except Antarctica. In North America, there are several genera of woody plants that have increased dramatically beyond their historical range and distribution during the last 150 years. Some notable genera include *Baccharis*, *Juniperus*, *Larrea, Pinus*, *Prosopis, Quercus,* and others (Van Auken 2000). Since grassland and savanna ecosystems account for 30–35% of global terrestrial NPP, substantial changes in these ecosystems could have global consequences for soil or atmospheric chemistry (Field et al. 1998). These new woodlands and forests often shift the bulk of the allocation of carbon storage from below- to aboveground and may serve as a significant, if transient, sink for carbon (Archer et al. 2001, Norris et al. 2001a). Concurrent changes in litter quantity or quality and microclimate in these forests may also to change soil nutrient availability, and ultimately, alter feedback patterns that may, in turn, affect ecosystem function, particularly C uptake.

Juniperus virginiana is the most widely distributed *Juniperus* species in the continental United States, occurring in every state east of the 100th meridian (Fowells 1965). Historically, *J. virginiana* in the eastern Central Plains was apparently restricted to areas that were relatively protected from intense grassland fires such as rocky outcrops, canyons or areas with shallow soils. Recently, in the eastern U.S. Central Plains and other parts its range, *J. virginiana* has encroached into adjacent grasslands at an unprecedented rapid rate affecting millions of hectares (Schmidt and Leatherberry 1995, Briggs et al. 2002).

In the Central Plains, *J. virginiana* often forms dense, nearly monospecific stands that change the forest floor microclimate with respect to light availability, soil temperature and moisture, as well as substantially reduce plant species diversity and richness below the canopy (Gehring and Bragg 1992, Briggs et al. 2002, Smith and Johnson 2004). Furthermore, *J. virginiana* encroachment results in substantial changes in ecosystem productivity and shifts in C and N storage patterns (Norris et al. 2001a, Smith and Johnson 2003). For example, annual net plant primary productivity was much higher in *J. virginiana* forests (~10,000 kg C ha^{-1} yr^{-1}) compared to grassland sites in similar locations (~3,700 kg C ha^{-1} yr^{-1}), leading to a substantial increase in aboveground C storage (Norris et al. 2001a). Forest encroachment has not only changed the magnitude, but also the spatial allocation of C and N pools. For example, grasses tend to have ~76% of their biomass belowground at the peak of the growing season (Blair et al. 1998), which is in contrast to forests that have a smaller proportion (assumed to be ~ 25%, (Cairns et al. 1997)) of biomass stored belowground. In spite of lower proportional allocation of plant biomass belowground, estimates of *J. virginiana* root biomass suggest that roots could be significant ecosystem stores of C and N, as well as important pathways for C and N input into soil organic pools. Differences in foliar and root litter quality and quantity, as well as potential changes in rooting depth or structure in *Juniperus* forests could substantially influence nutrient availability compared to the prairies they are replacing.

In systems limited by N availability, N uptake and C storage are often strongly related (Vitousek 1982, Reich et al. 1997, Vitousek 2004). For example, Reich et al. (Reich et al. 1997) found that across many forests that ANPP increased linearly with annual net N mineralization rates, thus productivity of temperature forest ecosystems is driven in part by their N availability.

Nitrogen availability can also shape plant community composition by influencing the success of species with different N requirements (Tilman et al. 1996).

The productivity of mesic grasslands is potentially limited by multiple factors, which include water and light availability, in addition to N availability (Blair 1997, Blair et al. 1998, Knapp et al. 1998). All three of these co-limiting factors are highly influenced by fire regime and ungulate grazing intensity (Risser and Parton 1982). For example, frequent fire in grassland ecosystems tends to lower N availability and grazing generally increases N availability (Risser and Parton 1982, Ojima et al. 1994). However, in *J. virginiana* forests the influences of both fire and grazing have been removed, and the absence these disturbances, coupled with changes in plant characteristics (i.e., tissue chemistry) may alter ecosystem N cycling and subsequent availability of N. Ultimately, differences in plant available N in forest and prairie ecosystems may influence plant productivity, plant community composition, and ecosystem C storage potential.

The establishment of *Juniperus virginana* forests alters many factors that control the processes underlying soil N mineralization and N availability. Net N mineralization (ammonification) rates are defined as the difference between concurrent gross N production and consumption processes over time. Net N mineralization is an index of the rate of supply of N from organic to inorganic N pools, after microbial demands for N have been met. Net N mineralization rates are generally thought to reflect the amount of N potentially available for plant uptake, and thus are considered to be a standard index for measuring plant-available soil N (extractable soil N, net N mineralization rates, and net nitrification rates) (Schimel and Bennett 2004). Both production and consumption processes, and hence net N mineralization, are highly influenced by the chemical composition of organic N being mineralized (substrate quality) and

by a suite of abiotic factors, such as moisture, temperature, pH, and soil organic matter accessibility, which affect the biotic processes of mineralization and consumption (Booth et al. 2005). Conversion of grasslands to *Juniperus virginana* forests influences litter quality, soil temperature and moisture, and possibly other factors that influence soil net N mineralization (Norris et al. 2001; Smith and Johnson 2004).

Further differences in *J. virginiana* forests and prairie ecosystems include the efficiency with which different plant functional types in these communities utilize available resources. Some plant species have evolved high N use efficiency (NUE) in highly N-limited ecosystems. *Juniperus virginiana* forests have much greater (2.5x) annual NUE than prairie communities, meaning these forests fix more carbon per unit N available, which contributes to greater ANPP in these forests (Norris 2000, Norris et al. 2001a). However, species with inherently high productivity and high NUE like *Juniperus* can exhaust soil nutrients, such as N (Grime and Hunt 1975), and produce very poor litter quality with high C:N ratios, which can create feedback loops in the soils that decrease N availability (Seastedt 1991). Therefore, *Juniperus virginiana* encroachment into prairie may be expected to reduce plant available N by producing low quality litter that slows decomposition and net N mineralization. This hypothesis is consistent with a reciprocal litter transplant study (*J. virginiana* forests and prairie) where there was much slower decomposition of *J. virginiana* foliar ~30% and root litter ~65% relative to litter of the dominant grass, and slower decomposition of common substrates in *J. virginiana* forests compared to prairie habitats (Norris et al. 2001b). Lower N availability caused by low tissue quality of *J. virginiana* would likely reinforce a positive feedback loop, which could further reduce available N in *J. virginiana* forests, potentially limiting the long-term productivity of these forests.

In summary, soil net N mineralization rates in *J. virginiana* soils is predicted to be lower relative to prairie soils because of changes in substrate quality concomitant with a less favorable soil microclimate (lower soil temperature and water content) for microbial processes (Smith and Johnson 2004); see Chapter 5). However, nitrogen mineralization in *J. virginiana* soils has not been rigorously investigated with concurrent complementary field and laboratory assays and compared with native grasslands under similar conditions. Changes in soil N cycling and availability may interact with inherent properties of *J. virginiana* forests (e.g., high NUE), which may help explain the observed increase in ANPP and changes in other ecosystem properties. In this study I examined *in situ* net N mineralization patterns coupled with parallel laboratory assays of potential C and N mineralization to determine if there are significant changes in soil C and N cycling with recent encroachment of *J. virginiana* forests into native grasslands. I also examined possible relationships between soil C and N fluxes and some important soil properties to gain an understanding of what soil characteristics may influence these processes, and whether these relationships differ in forest and grassland soils.

Materials and methods

Experimental design

Four paired sites comprised of contiguous or nearly contiguous *Juniperus virginiana* forest adjacent to native prairie were chosen in close proximity (<1 km) to the Konza Prairie Biological Station (KPBS) (39º05'N, 96º35'W). Proximity to the KPBS, the primary location of the Konza Prairie Long-Term Ecological Research (LTER) program, allows the use of a variety of baseline data on ecological processes in native tallgrass prairie. Historical aerial photographs taken since 1950 and analysis of soil organic carbon (SOC) isotopic composition were used to verify recent replacement of native grasslands with *J. virginiana* forests (see Chapter 4). Each

paired site shared similar soil type, slope, position, and aspect. Each *J. virginiana* forest (at least 0.5 ha) was relatively mature (~30–55 yrs) creating dense (680–1,360 trees ha^{-1}), complete or nearly complete canopy cover. Recurring controlled or natural fires in the adjacent grasslands presumably formed the current distinct boundaries between vegetation types. These prairie sites have not been grazed in the recent past (> 15 yrs, personal communication) and have a contemporary average fire return interval of 1–2 yrs, as a result of, controlled burns conducted in early spring. One 50 m transect was established in each vegetation type (*Juniperus* forest or prairie) at each of the four-paired study sites (n = 4), where soil and other ecosystem measurements were made in randomly assigned plots. Assays of *in situ* net N mineralization and collections of foliar litter and root biomass were randomly assigned to 2-m^2 plots (n = 6) along these transects.

Site description

The prevelant native vegetation in the northern Flint Hills is tallgrass prairie, dominated by a matrix of perennial, warm-season C_4 grasses including big bluestem (*Andropogon gerardii* Vit.), little bluestem (*Schizachyrium scoparium* Michx.), indiangrass (*Sorghastrum nutans* Nash) (Anderson et al.) and switchgrass (*Panicum virgatum* L.) (Kuchler 1967; Freeman and Hulbert 1985). These C_4 grasses contribute the majority of aboveground net primary productivity (ANPP) (Knapp et al. 1998). However, a highly diverse mixture of less abundant species, including C_3 grasses and sedges and a diverse array of forbs, contribute to the high floristic diversity of these grasslands (Freeman and Hulbert 1985). The native tallgrass prairie flora also includes a smaller number of native woody plants, such as buckbrush (*Symphoricarpos orbiculatus* Moench.), New Jersey tea (*Caenothus herbaceous* Raf.), smooth sumac (*Rhus glabra* L.) and rough-leaved dogwood (*Cornus drummondii* CA May), which can be locally abundant,

especially in prairie that is infrequently burned (Briggs et al. 2005). Average annual total precipitation is 835 mm with 75% falling during the growing season (Bark 1987). Topographic relief divides the landscape into upland plateaus with mostly shallow soils, slopes with outcrops of limestone, and lowlands with deeper alluvial and colluvial soils (Oviatt 1998). Three of the study sites had silty clay loam soil; fine, mixed, active, mesic Udothentic Haplustols. The fourth site had a silt loam soil; a fine, mixed, superactive, mesic Udertic Argiustolls (United States Department of Agriculture Soil Conservation Service, 1975). The soils at these sites were generally low in nutrients and relatively high in organic carbon.

Net N mineralization and inorganic N

In situ net field N mineralization was measured fifteen times, from June 2003 – June 2005, using an intact soil core method. Soil cores were taken at six plots along each transect (both forest and prairie) at four sites. Once a suitable site in a plot was selected, the O-horizon (litter layer) was removed by hand leaving the A-horizon exposed. After surface litter removal, polyvinyl chloride (PVC) pipes, 12.5 cm long with an internal diameter 5 cm were driven into the ground until only the upper 2.5 cm was visible (effectively including the top 10 cm of the A-horizon). A cap was placed on top of the pipe to prevent 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. Typically, these cores were incubated in the field for 30–35 d (T_1). A second core (5 cm i.d. x 10 cm) was taken 5–10 cm from the *in situ* PVC core with a soil corer to measure initial (T_0) concentrations of inorganic nitrogen. Once either T_0 or T_1 cores were removed from the field, they were placed in sealed Ziploc© bags in an iced cooler and refrigerated (2–4ºC) upon return to the laboratory.

Soils were prepared for extraction by passing through a 4 mm soil sieve and mixing thoroughly by hand. Ammonium and nitrate were extracted from 10–12 g of field moist soil with 50 mL of 2 N KCl, agitated for 1 h on an orbital shaker (200 rpm), and filtered through a 0.4 μm polycarbonate membrane. The extracted samples were stored at 2ºC until analysis. Inorganic nitrogen (NH_4^+-N and NO_3^- + NO_2^- -N) concentrations were determined colormetrically with an Alpkem® Flow Solution autoanalyzer (Wilsonville, Oregon) using the indophenol blue method for NH_4^+-N and cadmium reduction followed by diazotization with sulfanilamide for NO_2^-/NO_3^--N. Soil water content was determined gravimetrically using another aliquot (25g) of the cores, which was oven dried at 100ºC.

Net N mineralization was calculated by taking the final concentration (μg NH_4^++NO_3-N g^{-1} dry soil) of inorganic nitrogen after field incubation (T_1), minus the initial concentration at the start of the incubation (T_0). This calculation was repeated with ammonium and nitrate concentrations separately to determine net ammonification and nitrification rates, respectively. Net N mineralization, ammonification and nitrification rates were standardized by expressing them as μg N g^{-1} dry-soil d^{-1}. Cumulative annual net N mineralization (June-June), ammonification, and nitrification rates were calculated by summing daily net rates during the 30-day measurement periods and extrapolating daily rates for the intervals between incubation periods by averaging the measured rates from before and after a measurement gap. Monthly mean temperatures and precipitation were obtained from a weather station at the headquarters of the Konza LTER site to assess the qualitative relationships between climatic variables and net mineralization, ammonification, and nitrification rates.

Net N mineralization: mineral and organic horizon

The potential contribution of litter to soil net N mineralization was determined in the forest sites by leaving intact litter layers (O-horizon) in a replicate set of soil cores, concurrent with the net N mineralization assays without litter (as described previously). This assay was performed three times (June, July, and August 2004). To provide adequate space for forest litter components, longer PVC pipes (17 cm long) with an internal diameter 5 cm were driven into the ground until only the upper 7 cm was visible above the mineral soil. This longer core effectively included the top 10 cm of mineral soils and the entire intact O-horizon. These larger cores (T_{1L}) were placed within 5–10 cm of both the initial core (T_0) and the *in situ* soil net mineralization cores (T_1). This was intended to provide an estimate of net mineralization, ammonification and nitrification of the combined mineral (A-horizon) and organic soil (O-horizon), and assess any potential artifacts of litter exclusion on measured N transformation rates. This was done only in forest sites, as prairie sites did not have an O-horizon as a result of recurring spring fires. After field incubation, the forest litter was separated from the soil, and both were sieved separately (as described previously). Once the soils were sieved, the litter was then mixed with the soil and aliquots of the resulting mixture were extracted for inorganic N (as described previously), ultimately yielding net N mineralization rates of forest mineral soils with an intact O-horizon.

Potentially mineralizable C and N

Aliquots of soil were taken from the initial cores used for net N mineralization assays in July and October 2004 to determine potentially mineralizable C and N in growing and non-growing seasons, respectively. Ten g aliquots (dry wt. equivalent) of each soil were placed in 150 mL serum bottles. The soils were brought to 30% soil moisture (or 60% water filled pore space) and each serum bottle was sealed with a rubber septum and crimp-on aluminum serum

bottle closure. The sealed soil samples (n = 48) from forest and prairie soils were incubated in the dark in an environmentally controlled chamber at 25ºC for 31 d. Carbon dioxide concentrations in the headspace of the sealed serum bottles were measured every 2–3 d by inserting a 1 mL syringe through the rubber septa and withdrawing a sample of the headspace air. The air samples were immediately injected in a Shimadzu GC-8A gas chromatograph (Japan) to measure CO_2 concentrations. Peak areas were converted to μg CO_2-C g^{-1} dry soil and after accounting for injection volume, headspace volume, and dry weight of the soil. After sampling of headspace CO_2 concentrations, the serum bottles were opened for 2 h to allow equilibration with ambient O_2 and CO_2 levels (to prevent anaerobic conditions). The CO_2-C flux per gram of soil was summed over the incubation period to calculate cumulative carbon mineralization over the 31 d incubation. After 31 d the soils were extracted with 2 M KCl to determine inorganic N concentrations (as previously described). Potentially mineralizable N was determined using a formula similar to that used to calculate net field-based mineralization rates, but expressed as μg N g^{-1} dry-soil 30 d^{-1}.

Total soil C and N

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. Aliquots of T_0 soils (July and October 2004) were finely ground (pulverized) and dried at 60ºC for 48 hr, then weighed (~25 mg) into pressed silver capsules (5 x 9 mm), but not folded. The soil samples inside the silver capsules were treated with 2 M HCl to remove inorganic carbon ($CaCO_3$). Percent C and N of the treated soil was determined using dry combustion coupled with gas chromatography with a Carlo Erba model NA1500 C/N analyzer (Milano, Italy).

Microbial biomass

Microbial biomass C and N were determined using a chloroform fumigation extraction method (Jenkinson and Powlson 1976) with separate soil aliquots (~ 25 g) from T_0 samples (detailed description given in Chapter 4). Microbial biomass C was determined by subtracting the amount of CO_2-C respired in non-fumigated samples from CO_2-C respired in fumigated samples. Concentrations of CO_2-C were measured by the same methods previously outlined for measuring potentially mineralizable C. Microbial biomass N was determined by extracting inorganic N with 2 M KCl from fumigated and non-fumigated samples and subtracting the difference using the same colorimetric methods previously outlined to measure inorganic N concentrations.

Litter and root C and N

Soil surface litter was collected in the forest by removing the entire O-horizon in 20 x 50 cm (0.1 m^2) areas in plots near (3–5 m) those used for field N mineralization assays. Litter samples were dried, weighed and ground for analysis of total C and N concentrations. Soil monoliths (25 x 25 cm and 10 cm deep) were collected in both forest and prairie soils (A-horizon only) to measure root biomass and C and N concentrations. Roots were extracted from the soil monoliths, washed with DI water, passed through sieves, and dried at 60ºC. Bulk grass roots (< 4 mm) and *J. virginiana* fine roots (< 2 mm) were obtained for chemical analysis. Percent C and N for surface litter and root biomass was determined using dry combustion coupled with gas chromatography with a Carlo Erba model NA1500 C/N analyzer.

Statistical methods

Measurements obtained from each of the six plots along a given transect (forest or prairie) were averaged to obtain a mean value for each site, which was then used for statistical analysis (n = 4). A randomized complete block was used as the experimental design and significant differences in treatment means for field (by period and cumulative annual total) and laboratory assays of C and N mineralization in forest and prairie soils were determined using analysis of variance (ANOVA) (alpha = 0.05). Laboratory incubations of potentially mineralizable C and N were treated statistically with the same design structure used for ecosystem comparisons. Relationships between select soil properties (soil C, soil N, soil C:N, surface litter C:N, root biomass C:N, and microbial biomass C and N) and C and N flux, as well as Cmineralization:Nmineralization ratios were analyzed using simple regression procedures.

Results

Inorganic nitrogen

Total inorganic nitrogen (ammonium and nitrate) was significantly greater in prairie soils compared to forest soils on five of fifteen sampling dates (One-way ANOVA, $P \leq 0.05$) (Figure 1a). Most of these significant differences occurred from July 2003 – May 2004. Conversely, there was one date (August 2004) when significantly greater extractable nitrogen occurred in forest soils. Overall, a trend of greater inorganic N in prairie soils was evidenced by 10 of 15 sampling dates with greater mean extractable inorganic nitrogen.

Extractable ammonium (NH_4^+) and nitrate (NO_3^-) exhibited different trends over the two-year study. Extractable NH_4^+ was significantly greater in prairie soils compared to forest soils on eight of fifteen sampling dates (One-way ANOVA, $P \leq 0.05$) (Figure 1b). Similar to trends in total inorganic N, NH_4^+ was greater in prairie soils from July 2003 – May 2004. Greater NH_4^+

concentrations in 11 of 15 sampling times accounted for differences in total inorganic N concentrations in prairie soils, which was most pronounced from July 2003 – May 2004. However, extractable NO_3^- concentrations in forest and prairie soils were nearly congruent, with only one significant difference found in July 2004, when NO_3^- concentrations were significantly greater in forest soils (One-way ANOVA, $P \leq 0.05$) (Figure 1c).

Net N mineralization

There were no significant differences in net N mineralization rates of forest and prairie soils for any sample period (One-way ANOVA, $P \geq 0.05$) (Figure 2a). 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 prairie soils occurring in spring and summer months, and the lowest rates (< 0.03 μg N g^{-1} soil d^{-1}) occurring in winter months. 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 prairie soils (-0.05 μg N g^{-1} soil d^{-1}).

Net ammonification rates (mean ± se) showed a large degree of congruence between forest and prairie soils throughout the sampling periods, with only one significant difference ($P \leq 0.05$) in April 2004, when prairie soils exhibited a large negative rate (-0.090 ± 0.02 μg NH_4-N g^{-1} soil d^{-1}) and forest soils had a very small negative rate (-0.004 ± 0.02 μg NH_4-N g^{-1} soil d^{-1}) (Figure 2b). Overall, most net ammonification rates were very low (< 0.04 μg NH_4-N g^{-1} soil d^{-1}), or were negative. More than half of sampling dates exhibited negative net ammonification rates for both forest and prairie soils, possibly indicating the importance of nitrification for both ecosystems.

Net nitrification rates (mean ± se) were also very similar on most sampling periods, in forest and prairie soils with only one significant difference in July 2004, when forest soils

exhibited greater rates (0.083 ± 0.01 μg NO_3-N g^{-1} soil d^{-1}) than prairie soils (0.040 ± 0.009 μg NO_3-N g^{-1} soil d^{-1}) (One-way ANOVA, $P \leq 0.05$) (Figure 2c). In contrast with net ammonification rates, net nitrification rates were relatively high (≥ 0.05 μg NO_3-N g^{-1} soil d^{-1}) in both forest and prairie soils on most sampling periods in non-winter months. Lowest nitrification rates (≤ 0 μg NO_3-N g^{-1} soil d^{-1}) for both forest and prairie soils generally occurred in winter months (Dec–Feb) in both 2003 and 2004, with one exception being a negative rate in only prairie soils in June 2004. Net nitrification most closely tracked monthly and seasonal trends in mean monthly temperature and precipitation (Figure 2d).

Cumulative annual net N mineralization rates for June 2003–June 2004 and June 2004–June 2005 were significantly greater in forest soils (11.52 ± 0.38 μg N g^{-1} soil y^{-1}) compared to prairie soils (7.90 ± 0.26 μg N g^{-1} soil y^{-1}) (One-way ANOVA, $F = 60.67$, $P = 0.016$). Cumulative annual net ammonification rates for the same 2 y period were not significantly different for forest (-0.83 ± 0.17 μg NH_4-N g^{-1} soil y^{-1}) and prairie soils (-3.58 ± 3.10 μg NH_4-N g^{-1} soil y^{-1}) (One-way ANOVA, $F = 0.786$, $P = 0.469$). Cumulative annual net nitrification rates were also not significantly different for forest (12.67 ± 0.01 μg NO_3-N g^{-1} soil y^{-1}) and prairie soils (11.54 ± 2.91 μg NO_3-N g^{-1} soil y^{-1}) (One-way ANOVA, $F = 0.157$, $P = 0.731$).

Net N mineralization: mineral and organic horizon

In situ net N mineralization, ammonification, and nitrification rates were not significantly affected by the inclusion of forest litter (O-horizon) in mineralization cores compared to cores with bulk soil only (A-horizon) on any sampling period (One-way ANOVA, $P \geq 0.05$). For each of the three incubation periods (June, July, and August 2004), total net N mineralization, net ammonfication and net nitrification in cores with intact litter were not significantly different from cores without litter (Table 1).

Mineralizable C and N

Per gram bulk soil

In the growing season (July) there were no significant differences in potentially mineralizable carbon (mean ± se) per gram of bulk soil between forest (563 ± 71 μg CO_2-C g^{-1} soil $30d^{-1}$) and prairie soils (591 ± 127 μg CO_2-C g^{-1} soil $30d^{-1}$) (One-way ANOVA, $F = 0.037$, $P = 0.8546$) (Figure 3a). Again, no significant differences in mineralizable C in the non-growing season (October) were detected between forest (438 ± 33 μg CO_2-C g^{-1} soil $30d^{-1}$) and prairie soils (506 ± 87 μg CO_2-C g^{-1} soil $30d^{-1}$) (One-way ANOVA, $F = 0.589$, $P = 0.4718$). Although there were no significant differences detected between the soils in different ecosystem types, there was a general tendency for lower C mineralization in forest soils.

In the growing season (July) there were no significant differences in mineralizable N (mean ± se) per gram bulk soil between the forest (8.4 ± 1.6 μg N g^{-1} soil $30d^{-1}$) and prairie soils (5.3 ± 1.7 μg N g^{-1} soil $30d^{-1}$) (One-way ANOVA, $F = 1.781$, $P = 0.2304$) (Figure 3a). Despite large differences in mineralizable N in the non-growing season (October), there were no significant differences between forest (5.6 ± 1.9 μg N g^{-1} soil $30d^{-1}$) and prairie soils (1.7 ± 0.6 μg N g^{-1} soil $30d^{-1}$) (One-way ANOVA, $F = 3.686$, $P = 0.1033$). Converse to the trends in C mineralization, large, although non-significant differences in mineralizable N were present, with forest soils having greater rates than prairie soil in both growing (61%) and non-growing seasons (240%).

Per gram C or N

When expressed on a per gram of soil C basis, no significant differences in mineralizable carbon (mean ± se) between forest (13,303 ± 1,905 μg CO_2-C g^{-1} soil-C $30d^{-1}$) and prairie soils (16,453 ± 2,565 μg CO_2-C g^{-1} soil-C $30d^{-1}$) were found in the growing season

(July) (One-way ANOVA, $F = 0.973$, $P = 0.362$) (Figure 3b). However, in the non-growing season (October) there was a significant difference in mineralizable C between forest (10,031 ± 1,046 µg CO_2-C g^{-1} soil-C $30d^{-1}$) and prairie soils (15,723 ± 2,125 µg CO_2-C g^{-1} soil-C $30d^{-1}$) (One-way ANOVA, $F = 6.144$, $P = 0.048$). Trends in potentially mineralizable C (µg C g^{-1} soil-C) indicate less soil C mineralization relative to the total SOC pool in forest soils compared to prairie soils.

There were no significant differences in mineralizable nitrogen (mean ± se) in the growing season (July) between forest (2,252 ± 462 µg N g^{-1} soil-N $30d^{-1}$) and prairie soils (1,560 ± 461 µg N g^{-1} soil-N $30d^{-1}$) (One-way ANOVA, $F = 1.057$, $P = 0.344$) (Figure 3b). Also, no significant differences were detected in mineralizable N in the non-growing season (October) between forest (1,512 ± 538 µg N g^{-1} soil-N $30d^{-1}$) and prairie soils (577 ± 270 µg N g^{-1} soil-N $30d^{-1}$) ($F = 2.414$, $P = 0.171$). Despite non-significant results, trends in potentially mineralizable N (µg N g^{-1} soil-N) suggest greater N mineralization (yield) in forest soils relative to prairie soils in both growing (45%) and non-growing seasons (162%).

Relationships between soil properties and C and N fluxes

Possible correlations between selected C and N fluxes (C mineralization (laboratory), N mineralization (laboratory) and Cmineralization:Nmineralization (C_{min}:N_{min}) ratios) and various soil properties (soil C, soil N, soil C:N, C:N of roots and surface litter, and microbial biomass C and N) were analyzed with regression analyses. These relationships were explored for both prairie and forest soils in both growing (July) and non-growing season (October). Coefficients of determination (R^2) and the direction of the relationship are provided in Tables 2 and 3 for prairie and forest soils, respectively.

In prairie soils there was only one significant relationship, which was between soil C:N ratios and C_{min}:N_{min} ratios in the growing season ($R^2 = 0.96$, $P < 0.05$) (Table 2). This did not hold for soils sampled in the non-growing season ($R^2 = 0.14$, $P \geq 0.05$). Although there were very strong negative relationships between the root C:N ratio in prairie soil versus C_{min} and N_{min}, none were significant (Table 2). These data suggest that C_{min}:N_{min} ratios, an index of substrate quality, are related to and possibly driven by soil C:N ratios in the growing season. The breakdown of this relationship in the non-growing season suggests that other soil properties such as C:N ratios of the roots that provide organic inputs to the soil may have greater influence on C and N fluxes in prairie soil during this time. Root turnover in senescing grasses may have caused this change in the relationship of soil C:N ratios and soil C_{min}:N_{min} rates, but prairie root C:N ratios were not measured in the fall.

In forest soils C_{min}:N_{min} ratios were very strongly correlated with C:N ratios of *Juniperus* roots in both growing ($R^2 = 0.98$, $P < 0.01$) and non-growing seasons ($R^2 = 0.95$, $P < 0.05$) (Table 3). This suggests that root inputs may be a significant contributor to C and N mineralization year round. The relationships between soil C:N and C_{min}:N_{min} ratios were also strong, but not significant in either growing ($R^2 = 0.80$, $P \geq 0.05$) or non-growing seasons ($R^2 = 0.84$, $P \geq 0.05$). It appears that although the role of soil C:N ratios is important in influencing C and N fluxes in forest soils, root inputs may be far stronger and probably influence soil C:N as well. Unlike prairie that has no litter layer (due to recurring fire), forest soils have a well-defined surface litter horizon. However, C:N ratios of the O-horizon ($C:N_{litter}$) of forest soils were not correlated to net N mineralization in either growing or non-growing seasons. This suggests that forest litter does not play a strong role in short-term N cycling dynamics in forest soils. This is consistent with the finding that removing the litter layer had no effect on *in situ* net N

mineralization rates. Lastly, it appears that microbial biomass plays a strong role in C mineralization as evidenced by microbial biomass N being strongly and significantly negatively related to C mineralization in the growing season (R^2 = 0.95, $P < 0.05$) and microbial biomass C being strongly and significantly negatively related to C mineralization in the non-growing season (R^2 = 0.91, $P < 0.05$). Both microbial biomass C in the growing season (R^2 = 0.70, $P \geq 0.05$) and microbial biomass N in the non-growing season (R^2 = 0.78, $P \geq 0.05$) were strongly negatively related to C mineralization, although these relationships were non-significant.

Discussion

In situ annual net N mineralization rates in *Juniperus virginiana* forest soils were significantly greater, but there were no differences in potential soil CO_2-C flux (Figure 3a) compared to adjacent prairie soils in laboratory assays. This finding of greater N mineralization in forest soils is contrary to my initial prediction, where I anticipated reduced N mineralization. These findings have important implications for whole ecosystem processes. Increased soil N availability in *J. virginiana* forests may contribute greater ANPP or C assimilation relative to the prairies they replace, which may affect plant community dynamics and ecosystem C storage. In addition, the lower soil CO_2-C flux relative to the overall pool size in *J. virginiana* soils (Figure 3b) may help explain significant C accrual in the mineral soil (see Chapter 4), an important stable C pool. *Juniperus virginiana* encroachment creates localized patches of increased soil resource availability, specifically N, but also in some other macronutrients (i.e., Ca and Mg, McKinley unpublished data). Increases in soil resource availability are commonly found with shrub/woodland encroachment in arid and semi-arid regions (Schlesinger et al. 1990, Scholes

and Archer 1997). This study demonstrates that *Juniperus* encroachment also creates similar patterns in resource availability in mesic grasslands.

Field and laboratory assays agree that N availability as indexed by net N mineralization tends to be greater in *J. virginiana* forest soils (Figures 2-3). However, *in situ* extractable inorganic N pools were generally lower in forest soils (Figure 1), despite 46% greater annual net N mineralization relative to prairie soils. The observed differences in net N mineralization at any measured period were subtle (Figure 2a); however, when cumulative annual rates are considered, differences in N mineralization become apparent and relevant. This apparent discrepancy between lower or equal extractable inorganic N pools (Figure 1), yet greater net N mineralization in forest sites (Figure 2) suggests that *J. virginiana* forests are utilizing available inorganic N pools efficiently. In addition, the discrepancy between N availability indices suggest that soil net N mineralization assays are better tools for determining N availability than measurements of extractable N pools alone, because of differences in exploitation of extractable N pools by different species in these ecosystems. Most of the significant differences in extractable inorganic N occurred in late summer to early spring 2003 (Figure 1), when differences in phenology between perennial C_4 dominated grassland and these C_3 evergreen forests may explain this phenomenon. Perennial grasses begin to senesce in late summer, while the *J. virginiana* forests may still utilize available soil nutrients to support continued growth in the non-growing season. It is interesting to note that differences in extractable N concentrations started near the end of the growing season (September) and dissipated starting in May 2004, which corresponded with early season grass growth. Also, intra-annual variation in temperature and precipitation appear to be primary drivers of seasonal differences in field net N mineralization and net nitrification rates in both ecosystems (Figure 2a,c–d). Although net nitrification appears to driven by temperature

differences across seasons, this trend could be the result of temperature changes affecting the N mineralization rates that supply NH_4^+ for nitrification (Zaman and Chang 2004).

Potential soil C mineralization measured with laboratory incubations for both growing and non-growing season soils indicated slight (11%) reductions of C mineralization in bulk soil of forest sites (Figure 3a). The lack of significant differences in soil CO_2-C flux suggests that forest and prairie soil organic C is similar in quality, despite differences in the quality of plant litter inputs. *In situ* soil CO_2 measurements in prairie and *J. virginiana* forests (Smith and Johnson 2004) indicated an average annual reduction in *J. virginiana* forest soil CO_2 flux of about 38% due primarily to reduced soil temperatures. Results from laboratory incubations in this study showed a similar reduction (~30%) in soil CO_2 flux when considered on a per gram C basis in *J. virginiana* forest soils, which corroborates Smith and Johnson's (2004) finding that soil C turnover in *J. virginiana* soils is slower. Increased soil organic C (~12%, see Chapter 4) in forest soils may mask differences in potential soil CO_2 flux when considered on a bulk soil basis only.

Net N mineralization rates were higher in forest soils, which suggests microbial immobilization of N may be less in *Juniperus* forest soils. The ratio of Cmineralization:Nmineralization(net) or C_{min}:N_{min} ratios, an index of microbial N immobilization, was much less in forest soils compared to prairie soils (see Figure 3), which indicates that microbes are immobilizing less N in *J. virginiana* forest soils (Frank et al. 2000, Barger et al. 2004). Forest soil microbes may be immobilizing less N because of differences in microbial composition, microbial populations that are in a steady state or in decline, or greater available extractable N pools.

Carbon mineralization:nitrogen mineralization (C_{min}:N_{min}) ratios can also be viewed as an indicator of the quality of substrate used (Burke et al. 1989). Relationships that were explored between C_{min}:N_{min} ratios and C:N ratios of soil, foliar litter, root litter and microbial biomass suggest that C and N mineralization is driven primarily by root inputs in forest soils in both the growing and non-growing season, and mainly by soil C:N ratios in prairie soils during the growing season, because strong and significant correlations exist between these variables (Tables 2 & 3). One caveat is that these relationships do not necessarily reflect causation; although it is likely they are meaningful in this context.

In most forest and grassland ecosystems about 50% of heterotrophic respiration originates from recent detritus and only about 20% from soil organic matter, thus differences in root litter inputs may drive differences in C and N mineralization rates if there are significant differences in litter quality and quantity (Schimel et al. 1994). The C:N ratios of forest roots (65.4 ± 4.0) and prairie roots (88.1 ± 3.5) are significantly different ($P < 0.05$). However, forest fine root biomass (< 2 mm) 562 ± 167 g m^{-2} was only slightly greater than total bulk root biomass (< 4 mm) 482 ± 80 g m^{-2} found in prairie soil (One-way ANOVA, $F = 0.18$, $P = 0.68$), in the top 10 cm. Total root biomass of *J. virginiana* is estimated to be between 4425 – 7080 g m^{-2} (based on belowground biomass being 25 – 40% (Miller et al. 1990, Cairns et al. 1997) of aboveground biomass, which averaged 17,701 g m^{-2}). This total root biomass is at least 4x greater than that measured in prairie soils, which ranges from 859 – 1086 g m^{-2} from previous reports from Konza Prairie (Seastedt and Ramundo 1990). The differences in tissue chemistry and quantity of forest roots indicate a potential to influence soil processes.

Although decomposing surface litter can be a major driver of annual net N mineralization in a range of forests and soil types, soil N mineralization was most strongly related to litter

chemistry, specifically lignin:N ratios, rather than litter quantity, N concentration, and N content (Scott and Binkley 1997). Because of poor litter quality, *J. virginiana* forest surface litter may be releasing inorganic N via leaching into the mineral soil from decomposition too slowly to have a significant effect on short-term N availability (Table 1). This is supported by a two-year litter decomposition study that found no net release of N from fresh *J. virginiana* litter even after two years (Norris et al. 2001b). However, *J. virginiana* fine roots have slightly lower C:N ratios compared to the dominant C_4 grass (*Andropogon gerardii*) roots, but *Juniperus* roots may have higher % lignin (Norris et al. 2001b), and presumably polyphenols, both of which have been demonstrated to be important in controlling decomposition and N mineralization, and forming recalcitrant C compounds (Palm and Sanchez 1991, Reich et al. 1997, Scott and Binkley 1997, Hattenschwiler and Vitousek 2000, Norris et al. 2001b, Berg and Meentemeyer 2002, Sanchez 2004). Initial litter decomposition may be relatively fast as labile fractions of the litter are readily utilized, but initial rates of decomposition may not be sustained as lignin exerts greater control on decomposition as the relative proportion of the labile fractions diminish (Melillo et al. 1982, Taylor et al. 1989, Giardina et al. 2001). Recalcitrant C sources may become more prevalent in later stages of decomposition due the formation and accumulation of more complex carbon structures, slowing C mineralization in the long term and allowing accumulation of soil organic pools (McClaugherty and Berg 1987).

Microbial biomass C and N in the forest sites was strongly related to N mineralization and C mineralization, respectively (Table 3). Microbial biomass N is an important labile pool in tallgrass prairie (Sotomayor and Rice 1996). Microbial biomass N was strongly negatively related to C mineralization in both growing and non-growing season forest soils (Table 3). The

strong negative relationship with microbial biomass N and C mineralization may reflect differences in microbial composition compared to resident microbes in prairie soils.

Possible differences in soil microbial composition (fungi:bacteria ratios) may offer a valid, but not mutually exclusive, alternative to explaining lower Cmin:Nmin ratios in *J. virginiana* soils compared to prairie soils. Giardina et al. (Giardina et al. 2001) found that pine litter with wide C:N ratios and lignin:N (low substrate quality), compared to aspen litter with narrower C:N and lignin:N ratios, exhibited greater C and N mineralization. They found that pine soil C supported greater fungi:bacteria ratios than that found in aspen soil and this microbial composition may have help cause these unexpected patterns in C and N mineralization. Fungi are well-known lignin decomposers and are less sensitive to low soil moisture than soil bacteria (Morton 1998), like conditions (ephemeral) found in *J. virginiana* forests. In addition, fungi have greater C:N ratios (7–25:1) than bacteria (5–7:1), which allows them to mineralize more C with less concurrent N immobilization (consumption) than bacteria. Bacteria require about twice the amount of N per unit C mineralized than do fungi (Morton 1998, Wagner and Wolf 1998). Soil organic matter decomposition in forest soils may be predominantly fungal driven, which when considered with lower root litter C:N ratios, could in part explain our observed finding of reduced soil C mineralization, while maintaining greater N mineralization (Paul and Clark 1996).

Differences in microbial immobilization of N may be highly influenced by altered substrate composition and microclimate produced in these *Juniperus* forests. *Juniperus* forest soils were generally drier and had much lower temperatures (up to 7ºC lower at 5 cm), which may constrain potential C and N mineralization under field conditions (see Chapter 5, Figures 1 and 2). Smith and Johnson (2004) found that soil temperatures explained most of the variance in field CO_2 flux, and soil CO_2 flux was lower because of lower mean temperatures *in J. virginiana*

soils. Similarly, nitrogen cycling in this study appears to be strongly influenced by either soil moisture or temperature as optimal incubation conditions in the lab greatly enhanced measured net N mineralization rates. Field N mineralization in the growing season was approximately 6% and 3% of potential N mineralizaion in *J. virginana* and prairie soils, respectively. In the non-growing season forest soil maintained a similar ratio (7%), while prairie soils seem to be closer to optimum conditions for N mineralization rates with *in situ* rates approximately 34% of potential rates.

The importance of new forest C in forest soils was demonstrated by Smith and Johnson (2001) who found that 65% of the soil CO_2 flux from microbial respiration was from more recent forest-derived carbon, relative to older prairie-derived carbon. If the predominate forest C inputs are roots and these new inputs exert primary control on C and N mineralization, the lower C:N ratios of forest roots relative to prairie would cause N to be less limiting during decomposition, resulting in less microbial N immobilization. New C inputs from roots in prairie soils may be most significant in the non-growing season when C inputs from senescing root biomass may be greatest. In the growing season, soil organic matter appears to drive C and N fluxes in prairie soils, when fresh C inputs from other sources (i.e., root turnover) are presumably less and exert less influence.

Greater N availability in *J. virginiana* forest soils together with greater NUE of *J. virginiana* may support greater productivity in these *Juniperus* forests relative to the prairie they replace. Apparent differences in substrate composition and sub-optimal microclimate in *J. virginiana* forests for microbial processes compared to mesic grassland do not appear to create a positive feedback loop that limits N availability and plant productivity. Despite large quantities of organic C in forests soils, it appear that much of this C is unavailable or slowly utilized by soil

microbes as evidenced by reduced C flux relative to the overall C pools size. Reduced soil CO_2 is probably due to a larger fraction of recalcitrant C accumulated in organic matter pools. In the long-term, reduced C mineralization relative to the overall soil C pool (Figure 3b) in forest soils may contribute to greater soil organic C storage (Chapter 4). *Juniperus virginiana* soils maintain greater N availability that can help support high productivity, which could make this very widespread plant genus important as a regional C sink.

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Figures and tables

Figure 2-1 Extractable inorganic nitrogen (mean ± se) from initial (T_0) net N mineralization samples over a two year period. Total inorganic N (a) was significantly greater in prairie soils on five of fifteen sampling dates, which occurred in July 2003 through May 2004. Greater total inorganic N was apparently due to significantly greater NH_4^+ concentrations in prairie soils (b). There were few differences in extractable NO_3^- during the whole sampling period. Asterisks represent significant differences between treatment (forest and prairie) means for that specific time (alpha = 0.05).

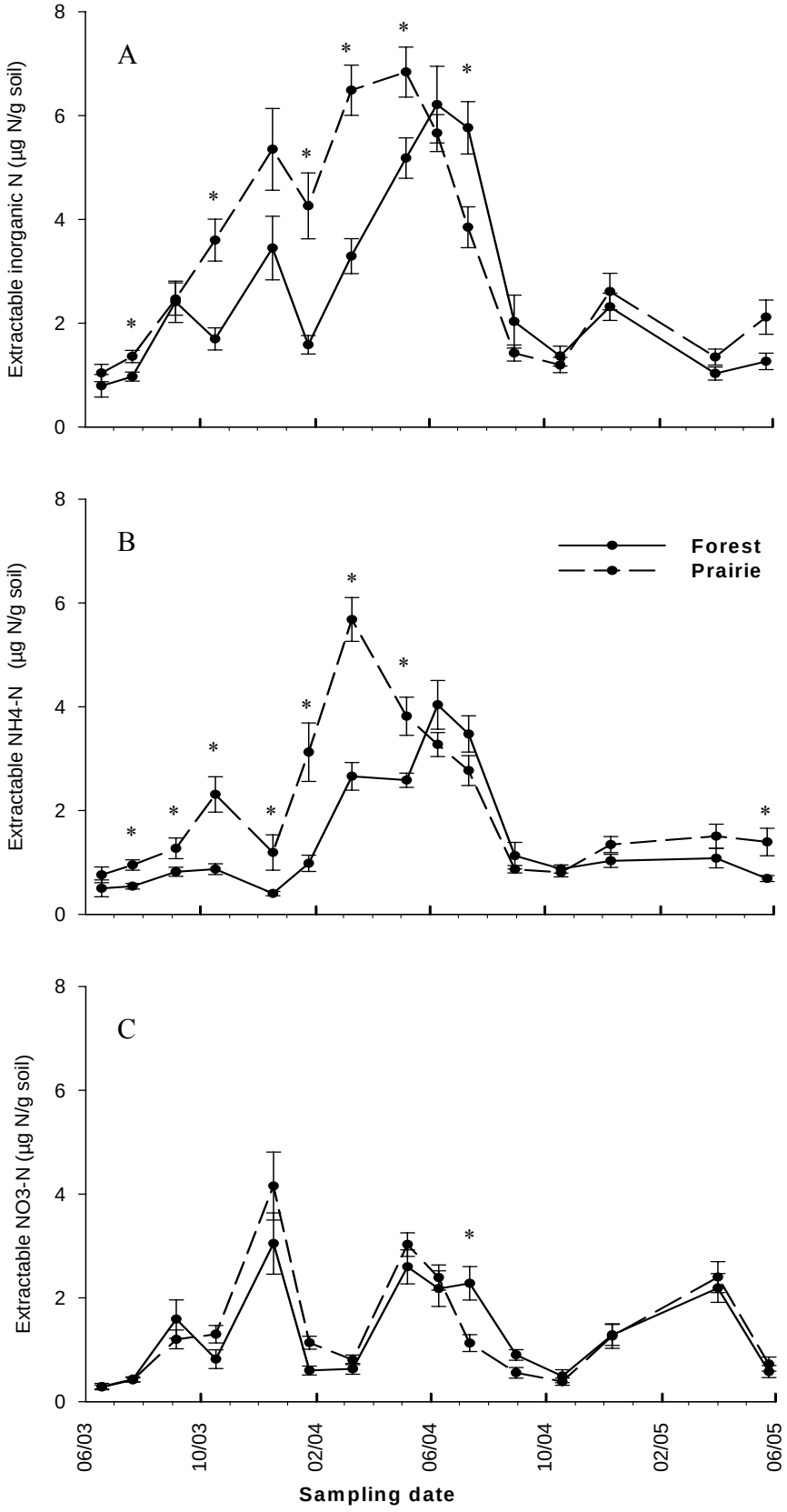
A
B
C
Forest
Prairie
Extractable inorganic N (µg N/g soil)
Extractable NH4-N (µg N/g soil)
Extractable NO3-N (µg N/g soil)
0
2
4
6
8
06/03
10/03
02/04
06/04
10/04
02/05
06/05
Sampling date

Figure 2-2 Net nitrogen mineralization rates (mean ± se) from fifteen sampling periods were determined from *in situ* assays conducted from June 2003 through June 2005. There were no significant differences between the forest and prairie soils for net N mineralization (a), and only one significant difference was found for both net ammonification (b) and net nitrification rates (c), where rates during those periods were greater in forest soils. Mean monthly temperature (dotted line, left Y axis) and precipitation (bars, right Y axis) appear to drive seasonal differences in mineralization rates (d). Asterisks represent significant differences between treatment means (forest and prairie) for that specific period (alpha = 0.05).

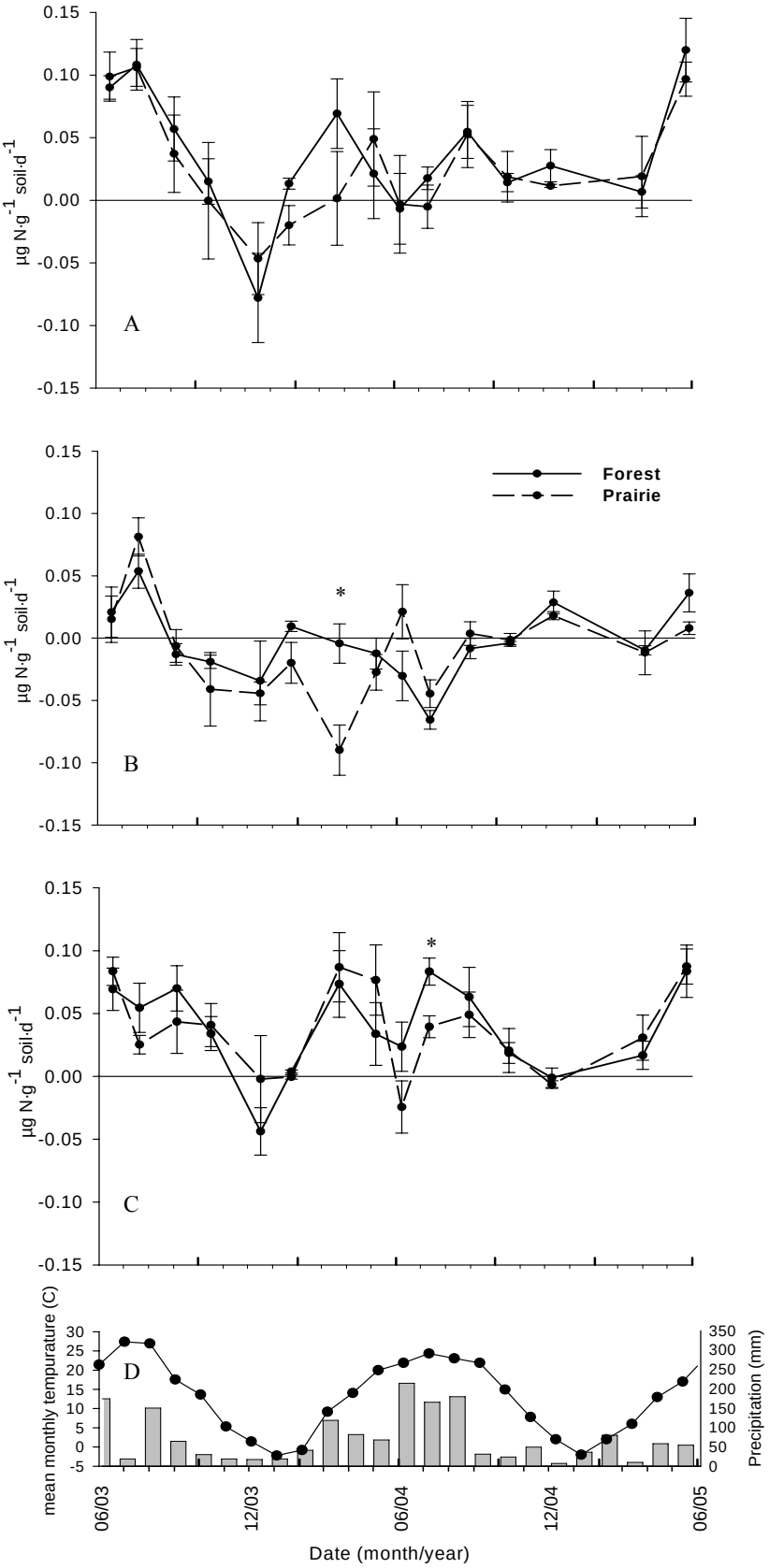
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
µg N·g-1 soil·d-1
A
B
C
Forest
Prairie
*
*
D
mean monthly tempurature (C)
Precipitation (mm)
30
25
20
15
10
5
0
-5
350
300
250
200
150
100
50
0
06/03
12/03
06/04
12/04
06/05
Date (month/year)

Figure 2-3 Potentially mineralizable carbon and nitrogen (mean ± se) were measured in July (growing season) and October (non-growing season) 2004 with 30 d laboratory incubations for forest and prairie soils. There were no significant differences in either mineralizable C or N per gram bulk soil in growing (July 2004) and non-growing (October 2004) seasons (alpha = 0.05) (a). When C mineralization rates are expressed as per unit soil C to normalize for differences in soil C content, strong differences between forest and prairie soils appear, with non-growing season C mineralization rates in the forest soils significantly less than rates found in the prairie (P<0.05) (b). Relative differences in N mineralization rates, expressed as per unit soil N, between forest and prairie soils were similar, but less than rates expressed as per gram bulk soil. Means with different letters indicate significant differences (P< 0.05).

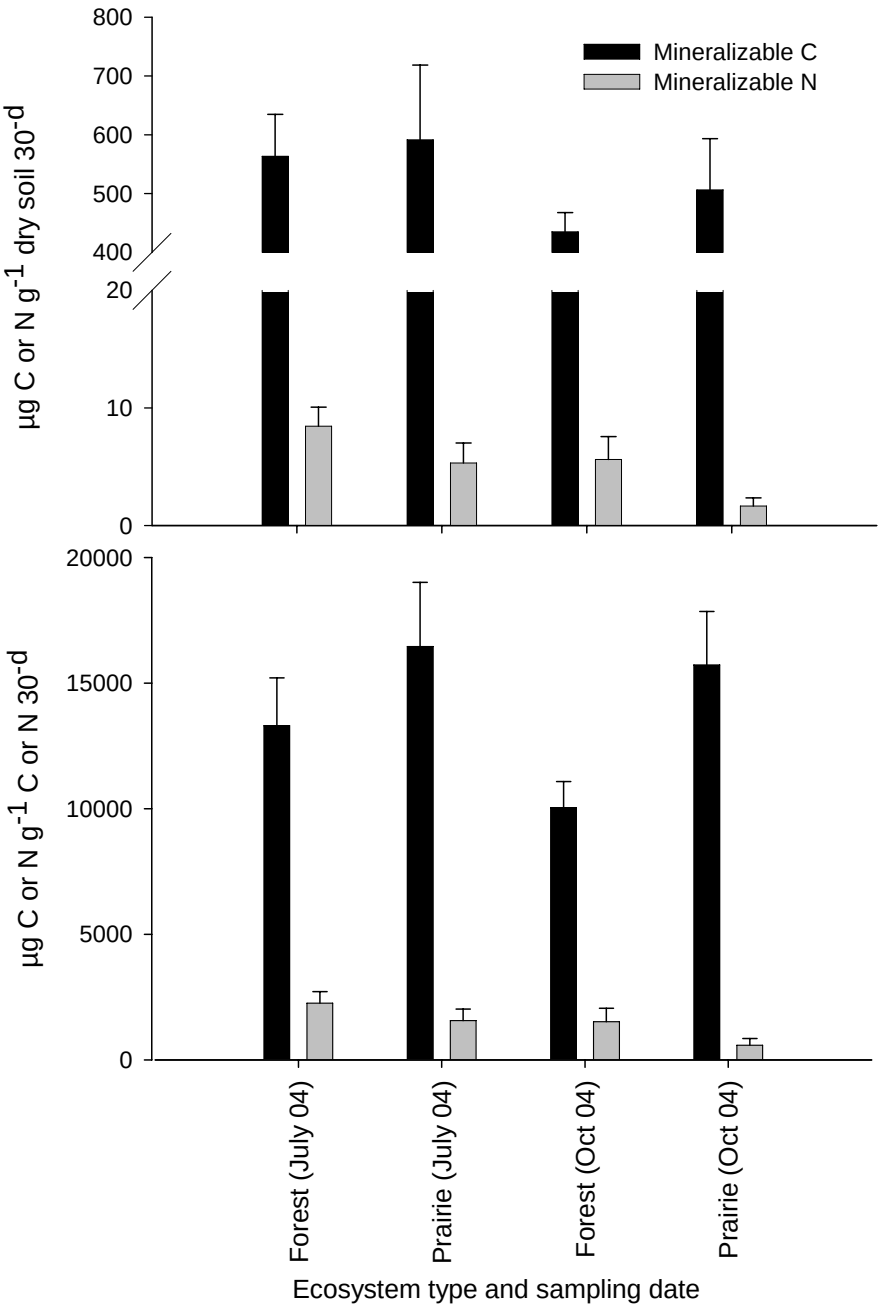
Mineralizable C
Mineralizable N
µg C or N g-1 dry soil 30-d
800
700
600
500
400
20
10
0
µg C or N g-1 C or N 30-d
20000
15000
10000
5000
0
Forest (July 04)
Prairie (July 04)
Forest (Oct 04)
Prairie (Oct 04)
Ecosystem type and sampling date

Table 2-1 Net mineralization, ammonification, and nitrification of soil cores that were incubated *in situ* with the A-horizon only (no litter) and with the A-horizon plus intact O-horizon (with litter). Each respective soil process was compared for three sampling dates with a One-way ANOVA to determine if significant differences occurred between cores incubated with and without an intact O-horizon.

Incubation period	no litter (μg N g soil^{-1} d^{-1})	with litter (μg N g soil^{-1} d^{-1})	*F*-value	*P*-value
Net N mineralization				
June	-0.020	-0.007	0.145	0.717
July	0.012	0.018	0.088	0.776
August	0.061	0.052	0.094	0.769
Net ammonification				
June	-0.029	-0.030	0.006	0.940
July	-0.066	-0.066	0.001	0.984
August	0.000	-0.009	0.676	0.442
Net nitrification				
June	0.008	0.024	0.486	0.512
July	0.076	0.083	0.189	0.679
August	0.061	0.062	0.001	0.983

Table 2-2 Relationships between various soil properties and C and N fluxes in prairie soil during growing and non-growing seasons. Coefficients of determination (R^2) are presented and their locations represent the relationship between the soil properties listed in the columns and C and N flux in the rows. Negative (–) signs indicate negative relationships between variables. Relationships between prairie litter and C and N flux could not be compared (NA) due to a lack of surface litter from recurring fire.

Soil property (prairie)	C:N	Soil C	Soil N	$C:N_{roots}$	$C:N_{litter}$	Microbial C	Microbial N
Growing season:							
C_{min}	0.27	0.36	0.11	(–)0.60	NA	0.03	0.03
$N_{min(net)}$	<0.01	0.15	0.13	(–)0.90	NA	<0.01	0.12
$C_{min}:N_{min}$	0.96*	0.58	0.18	(–)0.37	NA	0.20	0.04
Non-growing season:							
C_{min}	0.24	0.33	0.20	(–)0.66	NA	0.04	0.15
$N_{min(net)}$	0.09	0.10	<0.01	(–)0.81	NA	0.38	0.02
$C_{min}:N_{min}$	0.14	<0.01	(–)0.40	(–)0.60	NA	0.45	0.14

* $P \leq 0.05$; ** $P \leq 0.01$.

Table 2-3 Relationships between various soil properties and C and N fluxes in forest soil during growing and non-growing seasons. Coefficients of determination (R^2) are presented and their locations represent the relationship between the soil properties listed in the columns and C and N flux in the rows. Negative (–) signs indicate negative relationships between variables.

Soil property (forest)	C:N	Soil C	Soil N	$C:N_{roots}$	$C:N_{litter}$	Microbial C	Microbial N
Growing season:							
C_{min}	0.56	<0.01	(–)0.06	0.48	(–)0.14	(–)0.70	(–)0.95*
$N_{min(net)}$	(–)0.20	<0.01	0.05	(–)0.77	(–)0.50	(–)0.17	(–)<0.01
$C_{min}:N_{min}$	0.80	(–)<0.01	(–)0.25	0.98**	0.07	(–)<0.01	(–)0.36
Non-growing season:							
C_{min}	0.19	<0.01	(–)0.03	0.15	(–)0.45	(–)0.91*	(–)0.78
$N_{min(net)}$	(–)0.30	<0.01	0.02	(–)0.71	(–)0.40	(–)0.14	(–)0.46
$C_{min}:N_{min}$	0.84	(–)0.50	(–)0.47	0.95*	0.07	(–)0.34	(–)0.68

* $P \leq 0.05$; ** $P \leq 0.01$.

CHAPTER 3 - Comparison of soil N transformations and nitrifier populations in tallgrass prairie and newly established *Juniperus virginiana* forests

Abstract

Dramatic changes in ecosystem structure are associated with the conversion of grasslands to forests or woodlands. Yet, there appear to be only subtle changes in soil carbon and nitrogen cycling. Soils under *Juniperus virginiana*, an important invader in the U. S. Central Plains, have greater N availability than soils under the mesic grasslands they replace. The internal N cycling that facilitates greater N availability in these recent forest soils is not known, but may be important for understanding controls on ecosystem productivity, soil N retention and C storage in newly established *J. virginiana* stands. I examined soil N cycling processes in four sites where *J. virginiana* forests have encroached into mesic grassland (prairie). Potential gross nitrogen mineralization, nitrification, and consumption rates were determined in soils from paired forest and grassland plots using ^{15}N isotope-dilution under laboratory conditions four times during the year at the height of each season. Concurrent assays of potential nitrification rates (V_{max}) and most probable number (MPN) estimates of both NH_4^+ and NO_2^- oxidizers were also conducted. Gross N mineralization and nitrification assays revealed a slight trend of reduced gross N fluxes in forest soils relative to prairie soils. Soils of both ecosystems exhibited a great potential to immobilize inorganic N as evidenced by gross microbial consumption rates that exceeded gross production rates (negative net mineralization rates). Mean residence times (MRT) of NH_4^+ pools were very short (< 30 hr) and were not significantly different between forest and prairie soils in any season ($P > 0.05$). In contrast, MRT of NO_3^- pools varied greatly among seasons (10 – 400 hr), with the largest differences between forest and grassland soils in spring and summer.

Estimates of potential nitrification (V_{max}) and MPN counts of both NH_4^+ and NO_2^- oxidizers exhibited strong seasonal fluctuations, but no significant differences between ecosystem types in any season ($P > 0.05$). Nitrification is very important process in the cycling of N in both these ecosystems, converting about one-third of all mineralized N, most of which is subsequently immobilized by microbes. These data suggest that differences in internal N cycling and subsequent soil N availability are probably induced by differences in substrate composition or quantity rather than changes in the microbial community responsible for some key N transformations.

Introduction

Afforestation of grasslands and savannas is presently a worldwide phenomenon, occurring on every major continent except Antarctica. In North America, there are several genera of woody plants that have increased dramatically during the last 150 years relative to their historical range and distribution. Some notable genera include *Baccharis*, *Juniperus*, *Larrea*, *Pinus*, *Prosopis*, *Quercus*, and others (Van Auken 2000). Since grassland and savanna systems account for 30–35% of global terrestrial NPP, conversion of these ecosystems to woodlands and forests could have global consequences for soil or atmospheric chemistry (Field et al. 1998). In Chapter 2, I found that *J. virginiana* communities had an effect on *in situ* N availability in recently invaded mesic grasslands soils, but little is yet known about changes in internal N cycling processes, and in the microbial populations responsible for these transformations. The ability of *J. virginiana* forests to maintain high productivity compared to the grassland ecosystems they replace may result, in part, from altered availability of soil nitrogen (N), a critical limiting resource. A mechanistic understanding of how *J. virginiana* forests influence soil N availability is essential for understanding ecosystem dynamics, since soil N availability

and plant C uptake are often highly interrelated (Vitousek 1982, Reich et al. 1997, Vitousek 2004).

Juniperus virginiana is the most widely distributed species in the continental United States, occurring in every state east of the 100th meridian (Fowells 1965). Recently, in the eastern Great Plains and other parts of in its range, *J. virginiana* has encroached into adjacent grasslands at an unprecedented rapid rate, affecting millions of hectares (Schmidt and Leatherberry 1995, Briggs et al. 2002). *Juniperus virginiana*, can form dense nearly monospecific stands that modify the forest floor (or soil) microclimate with respect to light availability, soil temperature and moisture (see Chapter 5; Smith and Johnson 2004), as well as substantially decrease plant species diversity and richness below the canopy (Lassoie et al. 1983, Gehring and Bragg 1992, Briggs et al. 2002). Furthermore, *J. virginiana* encroachment leads to substantial changes in ecosystem productivity and shifts in C and N storage patterns (Norris et al. 2001, Smith and Johnson 2004). These shifts are due largely to changes in annual aboveground net plant primary productivity (ANPP), which is much higher in *J. virginiana* forests ~ 10,000 kg C ha^{-1} y^{-1} compared to mesic grassland ~3,700 kg C ha^{-1} y^{-1} and elimination of fire in the forests, leading to a substantial increase in aboveground C storage (see Chapter 4; Norris et al. 2001).

Previous studies demonstrated that annual net N mineralization rates were significantly greater on an annual basis (~ 35%) in *J. virginiana* forests compared to adjacent mesic grasslands, despite very similar intra-annual net N mineralization (see Chapter 2). Increased soil N availability, concomitant with plant greater nitrogen use efficiency (NUE) of these young forests likely drive the observed differences in primary productivity and ecosystem C storage between *J. virginiana* forests and tallgrass prairie (Norris et al. 2001). However, species with high NUE generally produce tissue of very poor quality, (i.e., high C:N ratios). The resulting

plant litter with high C:N ratios should slow decomposition, increase microbial immobilization of N, and reduce N availability. But, it is possible that inputs of *J. virginiana* root may drive soil N dynamics in *J. virginiana* forests and promote increased N availability, because of the relatively high tissue quality (low C:N ratios) and potentially quantity of fine roots (see Chapter 2). A detailed mechanistic study of the internal N cycling in the soils of these two ecosystems may be paramount for elucidating processes that cause the differences in N availability.

Net N mineralization and nitrification rates are defined as the difference between gross N production and consumption processes occurring simultaneously over time in the soil. Net N mineralization rates, together with extractable inorganic N pools, can serve as an indicator of N availability in soils. In many N-limited ecosystems, nitrogen availability is highly correlated with ANPP (Vitousek 2004). The positive relationship between N availability and ANPP holds true in many forest ecosystem types across the United States (Reich et al. 1997). Gross rates of N transformations (internal N cycles) may not be directly relevant to plant available N or necessarily related to ANPP of an ecosystem, but rather provide insights into the magnitude of N flow pathways and microbial processes that lead to observed plant-available soil N pools. Measuring gross N transformations allows for a limited mechanistic understanding of the soil microbial community, and may reveal responses of the soil community to changing environmental conditions, which can have disparate effects on concurrent gross production on consumption processes. For example, a pulse of low quality (high C:N ratio) C input to the soil of one ecosystem may affect available N status, by increasing gross microbial N immobilization, lowering net N mineralization rates and available pools, assuming gross production remains constant. Gross rates may also reveal the potential for soil N losses via microbially-mediated N

transformations (i.e., nitrification and denitrification) or leaching losses, which might affect current and long-term plant community composition and productivity.

The establishment of *Juniperus virginiana* forests on grassland soils is thought to influence many factors that affect production and consumption processes for ammonification and nitrification. Both of these processes are highly influenced by abiotic factors, such as substrate quality, soil moisture, temperature, pH, and soil organic matter accessibility (Booth et al. 2005). Conversion of grasslands to *Juniperus virginana* stands has at least some ephemeral effects, perhaps seasonal, on some of these factors that influence microbially driven N transformations (substrate quality, soil temperature and moisture), which alter internal N cycling and ultimately N availability in these forests. In addition, year round growth of *J. virginiana* may lower extractable N pool size, further altering production or consumption processes that affect ammonification or nitrification.

Measurements of annual net nitrification over two years in mineral soils of *J. virginiana* and prairie revealed no significant differences in net nitrification rates or in the observed small extractable NO_3^- pools, suggesting that there are only small differences in NO_3^- pool dynamics with ecosystem conversion to *J. virginiana* forest (Chapter 2). Small NO_3^- pools have been interpreted as an indication that NO_3^- is unimportant in the internal N cycles of grassland ecosystems (Woodmansee et al. 1981). However, most of the N mineralized (net) in both forest and grassland sites was subsequently converted to NO_3^- by nitrification in absence of plant uptake, which suggest nitrification may be important in the soils of these ecosystems (Chapter 2). Despite very similar net nitrification rates and extractable pools, these two ecosystems may differ greatly in gross nitrification, but this may not be seen if gross consumption rates (NO_3^- immobilization) vary with gross nitrification. The nitrification pathway is important because of

potential soil-evolved-gaseous N losses associated with nitrification and denitrification (Davidson et al. 1991), and potential losses of NO_3^- in the soil solution through leaching. The longer the NO_3^- molecule remains in this form (not utilized for assimilatory or dissimilatory pathways) the greater the potential for N loss through these pathways.

Recently, NO_3^- consumption in coniferous forests has received considerable attention as ^{15}N pool dilution and tracer studies have shown that NO_3^- consumption by microbial immobilization, once thought to be negligible, is significant and often equals or exceeds gross nitrification (Davidson et al. 1990, Davidson et al. 1991, Davidson et al. 1992, Hart et al. 1994b, Booth et al. 2005). Gross nitrification estimates have illuminated the importance of nitrification in systems such as an old growth coniferous forest (Davidson et al. 1992, Hart et al. 1994a) where nitrification previously was thought to be of minimum importance, because of low net nitrification rates and small NO_3^- pools like those found in *J. virginiana* soils. Part of the reason nitrification is poorly understood in coniferous forests is that factors such as low soil pH, low N availability (NH_4^+), higher available C, and strong competition for NH_4^+ with higher plants were thought to inhibit nitrification in these ecosystems, and thus nitrification was not intensively studied.

The objectives of this study were to assess changes in internal cycling of N in soils following forest encroachment into mesic grassland (prairie). I focused on gross rates of ammonium and nitrate production and consumption using laboratory based ^{15}N isotope dilution approach. I also included other concurrent measures of nitrification that may give further insights into limitations of nitrification caused by differences in nitrifier populations and substrate availability. Changes in internal N cycling and resulting N availability in forest soils relative to the grasslands they replaced may help explain the observed increase in ANPP and

ecosystem C storage in *Juniperus* forests, as well as identify possible changes in soil N loss pathways that might contribute to ecosystem N accrual in newly established *Juniperus* forests.

Materials and methods

Experimental design

Four paired sites comprised of contiguous or nearly contiguous *Juniperus virginiana* forest adjacent to native tallgrass prairie were chosen in close proximity (<1 km) to the Konza Prairie Biological Station (KPBS) (39º05'N, 96º35'W). Proximity to the KPBS, the primary location of the Konza Prairie Long-Term Ecological Research (LTER) program, allows the use of a variety of baseline data on ecological processes in native tallgrass prairie. Historical aerial photographs and analysis of soil organic carbon (SOC) isotopic composition were used to verify the recent establishment of these *J. virginiana* forests on sites that were historically grassland (see Chapter 4). Each paired forest and grassland site shared similar soil type, slope, position, and aspect. Each *J. virginiana* forest (at least 0.5 ha) was approximately 30–55 yrs old creating dense (680–1,360 trees ha^{-1}) stands with complete, or nearly complete, canopy cover. Recurring controlled or natural fires in adjacent grasslands since initial encroachment presumably formed the current distinct boundaries with adjacent prairie. These prairie sites have not been recently grazed (>15 yrs, personal communication) and currently have an average fire return interval of 1–2 yrs, as a result of controlled burns conducted in early spring. One 50 m transect was established in each vegetation type (*Juniperus* forest or prairie) at each of the four-paired study sites (n = 4), and soil and other ecosystem measurements were made in randomly assigned plots along the transects. Six soil cores (10 cm by 5 cm id.) were taken along these transects in October 2003 (fall), March 2004 (spring), July 2004 (summer), and October 2004 (fall). Gross mineralization (ammonification) and nitrification assays were performed on three of six

randomly chosen soil samples from each transect. Nitrification potential and enumeration (MPN) of nitrifying bacteria was performed concurrently with gross mineralization and nitrification assays, but were conducted with composite samples, composed of aliquots from all six soil samples collected along the same transect. There were a total of eight composite samples, one from each vegetation type from four sites (n = 4).

Site description

The native vegetation in the northern Flint Hills is primarily tallgrass prairie, dominated by a matrix of perennial, warm-season (C_4) grasses including big bluestem (*Andropogon gerardii* Vit.), little bluestem (*Schizachyrium scoparium* Michx.), indiangrass (*Sorghastrum nutans* Nash) (Anderson et al.) and switchgrass (*Panicum virgatum* L.) (Kuchler 1967; Freeman and Hulbert 1985). These C_4 grasses contribute the majority of annual aboveground net primary productivity (ANPP) (Knapp et al. 1998). However, a wide array of less abundant species, including C_3 grasses and sedges and a diverse array of forbs, contribute to the high floristic diversity of these grasslands (Freeman and Hulbert 1985). The native tallgrass prairie flora also includes a smaller number of native woody plants, such as buckbrush (*Symphoricarpos orbiculatus* Moench.), New Jersey tea (*Caenothus herbaceous* Raf.), smooth sumac (*Rhus glabra* L.) and rough-leaved dogwood (*Cornus drummondii* CA May), which can be locally abundant, especially in prairie that is infrequently burned (Briggs et al. 2005). Average annual total precipitation is 835 mm with 75% falling during the growing season (Bark 1987). Topographic relief divides the landscape into upland plateaus with mostly shallow soils, slopes with outcrops of limestone, and lowlands with deeper alluvial and colluvial soils (Oviatt 1998). Three of the study sites had silty clay loam soil; fine, mixed, active, mesic Udothentic Haplustols. The fourth site had a silt loam soil; a fine, mixed, superactive, mesic Udertic Argiustolls (United States Department of

Agriculture Soil Conservation Service, 1975). The soils at these sites were generally low in nutrients and relatively high in organic carbon (2.0–5.0%).

Gross ammonification and nitrification

Of the six soil cores (5 cm i.d. x 10 cm) collected on each date from each site (n=4 for both forest and prairie), three were randomly selected for gross ammonification and nitrification assays. All six soil samples were placed in an iced cooler and eventually placed in a refrigerator at 4ºC. Each soil core was sieved (4 mm) and mixed thoroughly by hand. Within 48 hr, 15 g of moist homogenized soil from three separate samples at each of the four sites and two vegetation types (forest and prairie) were placed in ninety-six 20 mL glass scintillation vials (Fisher Scientific®) (48 for each assay type, with half of each designated for T_0 and T_1 sample times). Soil water content was determined gravimetrically using another aliquot (25 g) of the original cores, which was oven dried at 100ºC. Soil samples were brought up to 30% gravimetric soil moisture (60% water filled pore space), to later include additions of 0.5 mL of labeled nitrogen suspension, then covered with one thin layer of polyethylene (Glad® Cling Wrap) and secured with rubber bands. Soil samples were then pre-incubated at 4ºC for 14 d to minimize the disturbance effects on soil processes. Previous soil incubations showed stabilization in soil CO_2 flux after 14 d. After the pre-incubation period, half of the soils (n = 48) were labeled with $(^{15}NH_4)_2SO_4$, and the remaining soils with $K^{15}NO_3$ (n = 48) (both highly enriched 98–99% ^{15}N). Additions of the labeled solutions were made with eight injections with a micro-syringe (totaling 0.5 mL) applied at 15 mg of nitrogen compound L^{-1} for both types of inorganic N totaling 1.95 µg $(^{15}NH_4)_2SO_4$-N and 1.29 µg $K^{15}NO_3$-N per sample (15 g soil). Generally, these ^{15}N additions did not exceed 10% of the ambient inorganic N pools. Duplicate samples (24 for each N type)

were extracted after 15 min to serve as initial (T_0) samples, and the remaining (T_1) samples were extracted after incubation in the dark in an environmentally controlled chamber at 25ºC for 24 hr.

Ammonium and NO_3^- from all T_0 and T_1 samples was extracted using the entire sample, ~ 15 g of field moist soil with 75 mL of 2 M KCl, agitated for 1 h on an orbital shaker (200 rpm), and filtered through a 0.4 µm polycarbonate membranes. The extracted samples were stored at 0ºC until analysis. Inorganic nitrogen (NH_4^+-N and $NO_3^- + NO_2^-$-N) concentrations were determined colormetrically with an Alpkem® Flow Solution autoanalyzer (Wilsonville, Oregon) using the indophenol blue method for NH_4^+-N and cadmium reduction followed by diazotization with sulfanilamide for NO_2^-/NO_3^--N. Preparations of the soil extracts for ^{15}N isotopic analysis were done using modifications to the diffusion method described by Davidson et al. (1992), and described below.

Diffusion method

The masses of NH_4^+-N or NO_3^--N in aliquots of the 2 M KCL extracts were calculated in each extract (based on prior colorimetric analysis). A carrier solution (10 mL) containing 50 µg of KNO_3-N or $(NH_4)_2SO_4$-N was used to bring concentrations of inorganic N to a detectable level for the mass spectrometer. Twenty mL of soil extract (2 M KCl) and 10 mL of the carrier solution (also in 2 M KCl) was placed in 500 mL mason jars (acid washed), with 2 glass beads (acid washed). Paper filter disks (Whatman #3, 7-mm) were made with a clean hole-punch, and were preleached three times with 2 M KCl, then rinsed with DI water. Mason jar lids were modified by installing a rubber septum with a nickel-plated T-pin inserted through the septum in a pre-punched hole (2 mm) in the middle of the lid. Each filter disk was then placed on the end of the T-pin. For samples that needed to be extracted for $^{15}NH_4^+$, ~ 0.4 g of MgO was added to the KCl extracts. The filter disk, recently acidified with 10 µL of 2.5 M $KHSO_4$ with the

modified lid assembly was placed over the extract (suspending the disk) immediately after the addition of MgO. The solution was gently swirled to disperse the MgO until a white cloud was visible in the solution. The disks were removed after 6 d, placed in new 20 mL scintillation vials, and dried for at least 72 hr in a desiccator over concentrated H_2SO_4. Pre-trial tests indicated that the N capture efficiency was at least 95%.

For samples that contained $^{15}NO_3^-$, ~ 0.4 g of MgO were added to the 2 M KCl extracts in the same manner as described for capturing NH_4^+. However, these samples were not covered until after 6 d to allow the volatilization and escape of NH_3 derived from NH_4^+. On the seventh day, ~3.0 g of pulverized Devarda's metal was added to convert NO_3^- in solution to NH_4^+, and then the same procedure described previously for capturing evolved N gas from these liquid samples was followed. The disks were removed after 6 d, placed in new 20 mL scintillation vials, and dried for at least 72 hr in a desiccator over concentrated H_2SO_4. Isotopic fractionation was thought to be minimal because of high capture efficiency of total N (~ 95%).

All samples, now captured on dried paper disks, were placed in standard 5 x 9 mm silver capsules for isotopic analysis. At the Kansas State University stable isotope lab, a Thermo Finnnigan Delta Plus mass spectrometer (samples combusted with a CE Elemental Analyzer with ConfloII), with a continuous flow setup was used to determined the isotopic ratio (atom percent excess ^{15}N) of the samples. Gross rates of mineralization (ammonification) and nitrification, as well as consumption were calculated using standard published formulae (Hart et al. 1994a).

Potential nitrification

Nitrification potentials were determined by a shaken soil-slurry procedure following the methodology outlined by Hart et al. (1994b), because it is considered to be the easiest to interpret and most reproducible measure of nitrification assays potentials. Soils for the nitrification

potential assay were taken from other aliquots from the same initial (T_0) samples used for gross nitrification assays (as previously described), from samples collected in spring, summer and fall 2004. Unlike gross mineralization and nitrification assays that used 3 of 6 replicate soil samples per transect, 60 g aliquots of each of the original six soil samples along a transect were made into one composite sample. There were eight composite soil samples, four from each vegetation type (forest and prairie), comprising four replicate samples (n = 4). These same composite soil samples were also used for enumeration of nitrifiers using a most probable number technique (described later).

Wet sieved composite soil samples (15 g) were placed into 250 mL Erlenmeyer flasks, with six subsamples taken from each composite sample to produce three ammonium-amended replicates and three controls for each composite sample. Soil water content for the composite samples was determined gravimetrically using a different sub-sample. One hundred mL of a dilute ammonium phosphate solution was added to each flask. The ammonium phosphate solution contained 0.2 M KH_2PO_4, 0.2 M K_2HPO_4, and 50 mM $(NH_4)_2SO_4$ solutions, adjusted to pH 7.2 by adding dilute H_2SO_4 or NaOH drop wise (resulted in a solution containing 1.5 mM of NH_4^+ and 1 mM of $PO^3{}_4^-$).

The shaken soil slurry procedure uses NO_3^- production measured over time to determine rates of potential nitrification (V_{max}). Nitrate consumption (measured in control samples) was determined by inhibiting nitrification using an acetylene block, and measuring potential declines in the NO_3^- pools over time. Controls (n = 3) for each composite sample were made by injecting acetylene (15 mL) with a 20 mL capacity syringe into half of the flasks through a tightly sealed double layer of Parafilm®, which was immediately sealed with a third peace of Parafilm®. All flasks were placed on an orbital shaker at ~180 rpm for 24 hr. Nitrate immobilization was

inhibited by high NH_4^+ concentrations, and denitrification was inhibited, because vigorous shaking from the orbital shaker continuously aerated the slurry.

Small aliquots (~10–15 mL) were taken at 1, 8, 16, and 24 h after the start of incubation. The sample aliquots were not allowed to settle before sampling. Each aliquot was immediately filtered through a 0.4 µm polycarbonate membrane into 20 mL plastic scintillation vials, capped and frozen until analysis. Analyses of all solutions for NO_3^- plus NO_2^- were done colorimetrically, as previously described. Nitrification potential (V_{max}) was determined by a linear regression of NO_3^- concentration over time for each soil (vegetation type and site). Since NO_3^- consumption is almost eliminated by the incubation conditions, net rates are equivalent to potential gross nitrification rates. Thus, rates from this procedure represents maximum (V_{max}) nitrification rates since presumably there were no substrate limitations.

Enumeration of nitrifiers

The most probable number (MPN) technique is the most widely used method to enumerate nitrifiers in soil. This technique was applied to both NH_4^+ and NO_2^- oxidizers. Ammonium and NO_2^- oxidizer medium was prepared with the chemical constitutes listed in Weaver et al. (1994), following the methodology outlined by Schmidt and Belser (1994). Both the NH_4^+ and NO_2^- oxidizer media were adjusted to pH 7–7.2 with 2 M NaOH prior to autoclaving. Four mL of each medium was placed into individual 20 mL glass culture tubes with threaded caps. Each culture tube was then capped and autoclaved at 15 lb/in^{-2} for 15 min.

Stock solutions were used to prepare 1nM phosphate buffer for dilution blank media. Dilution blank media was prepared by adding 4 mL of potassium monohydrogen phosphate (K_2HPO_4) and 1 mL of potassium dihydrogen phosphate (KH_2PO_4) stock solutions per L of distilled water (pH 7.1–7.4). Serum bottles (10 oz) with rubber septum caps were used for

dilution blanks, in which 90 mL of the phosphate buffer was placed, and autoclaved at 15 lb/in^{-2} for 15 min.

Moist soil (10g) was placed into a clean blender with 95 mL of sterile buffer, and blended for 60 s. The resulting solution (10^{-1} dilution) was transferred, via pipette, to a 10 oz serum bottle containing 90 mL of the phosphate buffer, further diluting the sample 10^{-2}. This serum bottle was shaken vigorously by hand, and then 10 mL of this solution was transferred, via pipette, to a serum bottle containing 90 mL of the phosphate buffer bottle (a 10^{-3} dilution). This was continued until a final dilution of 10^{-7} was achieved. With a new pipette tip, 1 mL aliquots of the highest serial dilutions to the lowest (10^{-7} – 10^{-2}) were transferred to each of five (or 10 including NO_2^- oxidizers) culture tubes for each media type, resulting in a total volume of 5 mL in the culture tubes. An equal number of tubes from the next five lower dilutions were then inoculated from the appropriate dilution blank. All culture tubes were incubated at 25°C in the dark in an environmentally controlled incubation chamber.

Modified Griess-Ilosvay reagents (diazotizing and coupling reagent) were made to test for the presence of NO_2^-. The diazotizing reagent was made by dissolving 0.5 g of sulfanilamide in 100 mL of 2.4 M HCl. The coupling reagent was made by dissolving 0.3 g of N-(1-naphthyl)-ethylenediamine hydrochloride in 100 mL of 0.12 M HCl. Both reagents were stored in amber colored bottles at 2°C. The NO_3^- spot test reagent was made by dissolving 50 mg diphenylamine in 25 mL concentrated (H_2SO_4). The NO_3^- spot test reagent was stored in a glass stopper-dropping bottle protected from light.

Initial observations were made and scored at 21 d and every 7–10 d thereafter until there were no changes in the number of negative culture tubes for at least two consecutive weeks. Preliminary checks were done visually by noting a color change in the pH indicator from blue-

green to yellow. All cultures were then spot checked (for NO_2^-) for growth determination. The samples were spot checked by transferring 0.1 mL aliquots from the culture tubes to a spot plate. Once on the spot plate one drop of diazotizing reagent and then one drop of coupling reagent were placed on the culture aliquot. A positive result was indicated by a bright pink color indicating the presence of NO_2^-. Further checks of the NO_2^- negative tubes in these end dilutions for NO_3^- were done by diphylamine spot test. Spot tests for NO_2^- oxidizers were performed as described earlier (for NH_4^+ oxidizers), however positive tubes were indicated by the disappearance of NO_2^- (which was indicated by no color change). Further checks of the NO_2^- oxidizer culture tubes for NO_3^- were done by diphylamine spot test.

The number of positive tubes in each of the appropriate end dilutions was recorded. The assay was continued with weekly checks for 3–4 months until there was no change in the number of negative tubes (tubes with no change) for a minimum of two consecutive weeks. The populations of NH_4^+ and NO_2^- oxidizers were estimated using published MPN tables (Woomer 1994) (replicates=5, dilution 10), with a further adjustment to the obtained MPN values to account for a modified initial dilution (10^{-2} rather than 10^{-1}).

Statistical methods and calculations

A randomized complete block was used as the experimental design. Each paired site composed of both forest and prairie transects was considered to be a replicate for a given vegetation type (n = 4). Means for each transect were derived from three experimental plots for gross mineralization and nitrification assays. However, nitrification potential (V_{max}) and MPN estimates were based on a composite sample from each transect, requiring that each site represent a replicate (n = 4). Typically estimates of variability for MPN counts are derived from one composite sample. However, in order to obtain greater precision, replicate composite

samples from each site (n = 4) were used to obtain estimates of means and variability. Means obtained from gross mineralization, gross nitrification, potential nitrification, MPN assays, and subsequent calculations (below) were tested with one or two-way analysis of variance (ANOVA) tests to determine if significant differences between means existed between ecosystem types and season if applicable (alpha = 0.05). Linear correlations between extractable soil ammonium and nitrate and the MRTs of ammonium and nitrate in the soil were also examined.

Ammonium and NO_3^- mean residence times (MRT) were calculated taking the initial (T_0) concentrations for each pool, and dividing this value by gross production rates for each respective pool (consumption rates were not used because of possible substrate stimulation). Microbial immobilization of N was calculated by subtracting gross NH_4^+ and NO_3^+ immobilization rates from gross mineralization rates. Ammonium immobilization was calculated by subtracting gross nitrification rates from gross NH_4^+consumption rates. Nitrate immobilization rate estimates equaled gross NO_3^+ consumption rates, since there was no significant competing process (i.e., plant uptake or denitrification). Microbial growth efficiency (Y_c) was calculated using a method developed by Schimel (Schimel 1989). Microbial growth efficiency was calculated as the product of the C:N ratio of microbial biomass and gross N immobilization rate, divided by the sum of the product of the C:N ratio of microbial biomass and gross N immobilization rate and the CO_2-C evolution rate. The estimates of microbial biomass (chloroform-fumigation incubation technique) and CO_2-C evolution rates (potential C mineralization) required for this calculation were obtained from concurrent assays (Chapters 2&4).

Results

Gross ammonification and nitrification

Gross ammonification rates were significantly greater in prairie soil (4.10 ± 0.35 μg N g^{-1} soil d^{-1}) compared to forest soil (2.86 ± 0.17 μg N g^{-1} soil d^{-1}) during spring 2004 (One-way ANOVA, $F = 10.10$, $P = 0.019$, Figure 1a). In addition, NH_4^+ consumption was also significantly greater in prairie soil (4.96 ± 0.55 μg N g^{-1} soil d^{-1}) compared to forest soil (3.31 ± 0.39 μg N g^{-1} soil d^{-1}) for the spring (One-way ANOVA, $F = 6.07$, $P = 0.049$). Neither gross ammonification nor consumption was significantly different between forest and prairie at any other measured time (One-way ANOVA, $P > 0.05$).

Seasonally, gross ammonification rates were relatively similar from fall 2003-summer 2004, and rates for both forest and prairie soils were significantly less in fall 2004 compared to the other seasons (Two-way ANOVA, $F = 29.56$, $P < 0.001$, Tukey's HSD, Figure 1a), suggesting common environmental controls on gross ammonification rates across ecosystem types. Despite a significant decrease in gross ammonification in the fall 04, NH_4^+ consumption rates in both forest and prairie rates maintained rates similar to other seasons (Figure 1b). Ammonium consumption rates were generally greater than gross ammonification (indicating negative net mineralization rates) for both forest and prairie soils at all seasons, except one measurement in the prairie in fall 2003.

Gross nitrification rates were significantly greater in prairie soil (1.55 ± 0.40 μg N g^{-1} soil d^{-1}) compared to forest soil (0.51 ± 0.13 μg N g^{-1} soil d^{-1}) during fall 2003 (One-way ANOVA, $F = 6.08$, $P = 0.049$, Figure 2a). In addition, NO_3^- consumption (Figure 2b) was also significantly greater in prairie soil (1.77 ± 0.26 μg N g^{-1} soil d^{-1}) compared to forest soil (0.71 ± 0.17 μg N g^{-1} soil d^{-1}) during fall 2003 (One-way ANOVA, $F = 11.56$, $P = 0.014$). Neither

gross nitrification nor consumption was significantly different for forests and grasslands at any other measured time ($P > 0.05$). However, in fall 2004 gross nitrification (One-way ANOVA, $F = 5.38$, $P = 0.059$) and consumption (One-way ANOVA, $F = 5.23$, $P = 0.062$) rates were greater in forest soils although these differences were only marginally significant. Nitrate consumption rates remained greater than gross nitrification rates (indicating negative net rates) for both forest and prairie soils in all seasons.

Seasonally, gross nitrification rates were much more variable than ammonification rates from fall 2003–summer 2004. A significant interaction was found between ecosystem type and season (Two-way ANOVA, $F = 4.358$, $P = 0.014$), indicating the seasons in which these measurements were taken have varying effects on the nitrification rates of these two ecosystem types. Neither of the main effects of vegetation type or season was significant (One-way ANOVAs, $P > 0.05$). Similar to gross nitrification, NO_3^- consumption also had a significant interaction between ecosystem type and season (Two-way ANOVA, $F = 4.04$, $P = 0.020$). Gross nitrification and consumption rates in the fall 2004 showed a reversal compared to rates in fall 2003 (Figure 2 a&b), with relatively high rates of nitrification in the forest in 2004 compared to high rates in prairie in 2003.

Ammonium mean residence times (MRT) (Figure 3a) were not significantly different in forest and prairie soils in any season (One-way ANOVA, $P > 0.05$). However, NH_4^+ MRT was significantly greater in both forest and prairie soils in fall 2004 compared with the other seasonal measurements (Two-way ANOVA, $F = 17.09$, $P \leq 0.05$, Tukey's HSD). Very low MRT (fast turnover rates) were found in all seasons with the fastest rates of less than 10 hr in fall 2004 and the highest MRT never exceeding 30 hr (fall 2004) in both forest and prairie soils. Also, the

NH_4^+ MRT for both the forest and prairie soils had no significant linear correlation with the size of extractable NH_4^+ pools ($P \geq 0.05$).

In contrast to NH_4^+ MRT, NO_3^- MRT exhibited large differences between ecosystem types in some seasons (Figure 3 b). The NO_3^- MRTs were relatively low in both the fall measurements for both ecosystem types with turnover rates of 25 ± 9 and 16 ± 8 hr for forest and prairie NO_3^- pools in 2004, respectively. In fall 2003, NO_3^- MRT in prairie soils 60 ± 12 were about half that in forest soils (120 ± 48), but the differences were not significant (One-way ANOVA, $F = 0.127$, $P = 0.734$). In spring 2004, NO_3^- MRT in the forest (34 ± 16 hr) was significantly less compared to prairie soils (242 ± 63 hr) (One-way ANOVA, $F = 10.45$, $P = 0.018$, Figure 3b). Conversely, in summer 2004, NO_3^- MRT in the forest (442 ± 311 hr) were much greater than those found in prairie soils (98 ± 54 hr), but the differences were not significant (One-way ANOVA, $F = 2.03$, $P = 0.250$) due to high variation in the forest estimates. In contrast to NH_4^+ MRT, NO_3^- MRT for both the forest and prairie soils had very strong significant positive linear relationships with extractable NO_3^- pools across the seasons with R^2 values of (0.50, $P = 0.005$) and (0.63, $P > 0.001$) for forest and prairie soils, respectively.

Potential nitrification (V_{max}) rates

Potential nitrification rates (Figure 4) were not significantly different in forest and prairie soils at any season (One-way ANOVAs, $P > 0.05$ for all comparisons). However, there was a significant affect of seasonal sample time, where both forest and prairie soil exhibited significantly higher rates in fall 2004 (One-way ANOVA, $F = 22.65$, $P < 0.001$, Tukey's HSD, Figure 4). Potential nitrification in the spring 2004 was 10.69 μg N g $soil^{-1}$ d^{-1} and 7.439 μg N g $soil^{-1}$ d^{-1} for forest and prairie soils, respectively (One-way ANOVA, $F = 1.018$, $P = 0.352$). Potential nitrification in the summer 2004 were the lowest of the measured seasons with 4.629

μg N g soil^{-1} d^{-1} and 5.38 μg N g soil^{-1} d^{-1} for forest and prairie soils respectively (One-way ANOVA, $F = 0.928$, $P = 0.333$). The potential nitrification in the fall 2004, although significantly greater for both ecosystems than in the other seasons (One-way ANOVA, $P < 0.001$, Tukey's HSD), was not significantly different between ecosystem types with 38.71 μg N g soil^{-1} d^{-1} and 27.876 μg N g soil^{-1} d^{-1} for forest and prairie soils, respectively (One-way ANOVA, $F = 1.032$, $P = 0.349$).

Most probable number

Most probable number counts revealed no significant differences in either NH_4^+ or NO_2^- oxidizing bacteria between forest and prairie soil in any season measured (Figure 5a&b, Table 1). Although there were no intra-season differences in forest and prairie soils for NH_4^+ and NO_2^- oxidizers, there were significant seasonal differences. Ammonium oxidizer had the greatest concentrations in spring 2004, with about 97,000 (cells per g soil) for forest soil and 64,000 for prairie soil, which dropped to less than 10,000 for both forest and prairie soils in summer and fall 2004 (Two-way ANOVA, $F = 22.09$, $P \leq 0.001$). Nitrite oxidizers had the greatest MPN estimated in the spring 2004 with approximately 2.5 million for forest soils and 1.7 million for prairie soils. Nitrite oxidizer populations in the fall 2004 were also very high with about 1.8 million in forest soils and about 0.9 million in prairie soils. The summer 2004 had the lowest counts with about 150,000 for forest soils and 170,000 for prairie soils. The forest and prairie MPN estimates for NO_2^- oxidizers in the summer 2004 were the only estimates found to be significantly different (less) than both the spring 2004 and fall 2004 estimates (Two-way ANOVA, $F = 4.41$, $P = 0.027$, Tukey's HSD). The MPN estimates for NO_2^- oxidizers were higher in all seasons compared to NH_4^+ oxidizers with the greatest differences occurring in the spring and fall 2004 with up to 1000x greater concentrations.

Discussion

With conversion of mesic grassland to *J. virginiana* forest there are subtle yet important changes in the internal cycling of N that may contribute to greater N availability in forest soils. The microbial activity in soils of these divergent ecosystems was surprisingly similar given the previously observed differences in soil temperature, soil water content, litter composition and placement, and C content of the soils in the field (see Chapters 4&5). I found reduced gross N mineralization in forest soil the spring 2004, and a general trend of reduced gross nitrification in forest soils in fall 2003 and summer 2004 (Figures 1a & 2a). Strong seasonal differences affected the magnitude of gross ammonification and nitrification rates. Despite differences (up to 3x) in gross ammonification or nitrification, consumption of NH_4^+ or NO_3^- was commensurate with production, which led to small and similar estimates of net N mineralization in both ecosystems. Similar to studies in other ecosystems, gross NH_4^+ and NO_3^- (Figures 1b & 2b) consumption rates usually exceeded production rates, possibly as a result of some substrate stimulation with the addition of the ^{15}N label or increased microbial immobilization from greater C availability caused by soil disturbance (Davidson et al. 1991, Hart et al. 1994a). Laboratory estimates of net N mineralization and nitrification derived from these ^{15}N pool dilution assays closely resemble patterns found in concurrent *in situ* assays, which also indicated small (*in situ*) to large (laboratory), but non-significant, differences in net N mineralization in 30-day field incubations (see Chapter 2). However, these small differences tended to be consistently greater in *J. virginiana* forests, which lead to significant cumulative differences in net N mineralization rates over long-time periods, as evidenced by greater *in situ* annual net N mineralization in forest soils (see Chapter 2).

Gross N mineralization in both forest and prairie soils was not related to either microbial biomass N or bulk soil C and N concentrations (presented in Chapter 4), as has been found in a

wide range of ecosystems (Booth et al. 2005). Others have found that soil temperature and moisture, rather than the soil microbial N pool were the most important factors influencing gross N mineralization (Puri and Ashman 1998). However, both soil temperature and moisture were held constant in these laboratory incubations, indicating that any differences in gross rates were caused by differences in C availability or quality and/or influenced by microbial composition. The significant difference in gross mineralization in spring 2004 in prairie soils compared to forest soils (Figure 1a) may reflect an increase in labile C, which may be highest in early spring. The decrease in gross mineralization in the fall 2004 (Figure 1a) for both forest and prairie soils may have been a result of decreased substrate quality (greater C:N ratios) of new litter inputs induced by exceedingly high precipitation in the preceding summer (60% of the annual total falling between June–August 2004) driving very high productivity, that could have widened C:N ratios of new soil inputs.

Mean residence times (MRT), a better measure for the true dynamics of inorganic N pools, indicated that there were no differences in the turnover rates of NH_4^+ in forest and prairie pools in any season (Figure 3a). The MRT NH_4^+ in both forest and prairie soils was very short (< 30 hr), indicating rapid uptake and strong microbial demands for NH_4^+. Most of the NH_4^+ was immobilized (gross NH_4^+ consumption – gross nitrification) (per g soil) in the microbial biomass in forest 79.7 ± 12.6% and prairie soils 72.4 ± 14.3%, respectively. Despite potential differences in microbial composition and substrate quality and quantity, these data suggest that the dynamics of forest and prairie NH_4^+ pools were very similar. Although the extractable NH_4^+ pools in forests are small, the lower MRT (faster turnover) suggests the opportunity for a ready supply of NH_4^+-N for plant uptake or for nitrifiers. It appears that nitrification has a role in the fate of a significant portion of the NH_4^+ pool that is not immobilized by microbes.

Similar to other grassland studies, gross nitrification in both forest and prairie soils accounted for approximately one-third of all gross N mineralization, and the NO_3^- formed from this process was rapidly immobilized in some seasons, as indicated by relatively large NO_3^- immobilization rates (Figure 2b) and low MRT (Figure 3b) (Schimel et al. 1989, Davidson et al. 1990). Deluca and Keeney (1995) found that NO_3^- was rapidly immobilized in prairie soils and after 72 hr, about 60% of the immobilized NO_3^- was found in plant roots and the remaining portion in microbial tissues. Forest soils have also demonstrated rapid NO_3^- immobilization (Woodmansee et al. 1981, Davidson et al. 1992, Hart et al. 1994a, Berntson and Aber 2000). The large magnitude of NO_3^- immobilization in both forest and prairie soil in this study demonstrates a great potential for microbes to immobilize NO_3^-, an important N retention mechanism.

Gross nitrification (autotrophic) should be very dependent on available NH_4^+ derived from heterotrophic ammonifcation. However, gross nitrification was significantly reduced in forest soils in fall 2003 and tended to be less in both spring and summer 2004 (Figure 2a) despite only small differences in extractable NH_4^+ pools (Figure 3a). The condition of similar extractable NH_4^+ concentrations during the start of the incubation may be an artifact, because initial NH_4^+ concentrations before the pre-incubation were significantly greater in prairie soils in both fall 2003 and spring 2004. Nevertheless, this created an opportunity to examine gross nitrification rates with similar levels of substrate availability (Figure 3b). This finding suggests that factors other than NH_4^+ availability (i.e., microbial community composition or size) may have a stronger influence gross nitrification rates. In addition, the reversal of the trend of reduced gross nitrification in forests in fall 2004 (Figure 2a) suggests that reduced gross nitrification in forest soils may be ephemeral and conditions causing this difference may not be

related to a specific season. Reduced nitrification in the prairie in fall 2004 may be due to increased C inputs caused by high productivity in the preceding summer, which may have decreased substrate availability (NH_4^+) for nitrification.

Gross nitrification:V_{max} ratios indicate that gross nitrification in both ecosystem types is severely limited by substrate availability as these soils only reach on average approximately 7% and 10% of their potential nitrification (V_{max}) rates across all seasons for forest and prairie soils, respectively. Low ratios of gross nitrification:V_{max} suggest that the maintenance energy requirements of existing nitrifier populations may not be met in all seasons (Davidson et al. 1990). Oscillations in substrate availability or other environmental constraints may help explain variations in NH_4^+ and NO_3^-oxidizer populations over the year (Figure 5, Table 1). Also autotrophic nitrifier sensitivity to water stress may influence nitrifier population size (Killham 1994), and soil water content tended to be less in forest soils (Chapter 5).

Nitrate MRTs varied dramatically across seasons, but were relatively rapid (< 50 hr), with the exception of the spring and summer 2004. In spring 2004, forest soils had very small NO_3^- pool, and very short turnover times (34 hr). In contrast, prairie soils had NO_3^- pools that were approximately 3x larger, and MRT close to 250 hr. In summer 2004 forest soils had only about 30% greater extractable NO_3^-, but MRT exceeded 440 hr, while MRT in prairie soils were about 100 hr. These results suggest that ephemeral conditions exists that might profoundly affect NO_3^- MRT in both forest and prairie soils. Short NO_3^- MRT and small pool sizes reduce potential N losses, by limiting NO_3^- availability for denitrification and leaching (Firestone and Davidson 1989, Paul and Clark 1996). The significant positive linear relationship between extractable NO_3^- pools and MRT of these pools suggest that potential NO_3^- immobilization may have a limited capability to immobilize relative large NO_3^- pools in the short-term. The slow

turnover of these NO_3^- pools in the soils of both these ecosystems in the spring and summer, would initially appear to increase the potential for N loss. However, in the field, plants would likely immobilize much of these NO_3^- pools during these seasons (spring and summer), thus potentially mitigating N loss. The slow MRT of these pools during the growing season for warm season grasses that coincide with increased plant demand for N might also suggest a residual effect of plant/microbe interactions, since plants were removed during this assay.

The observed phenomenon of extremely slow NO_3^- turnover despite large measured NO_3^- immobilization rates in some seasons may indicate that there could be recycling of the NO_3^- pools that short-circuit pathways requiring microbial immobilization. For example, in anaerobic microsites, NO_3^- may be used as an electron acceptor, reducing NO_3^- to NO_2^-, where NO_2^- may subsequently diffuse into aerobic microsites and be reduced to NO_3^- again (Giambiagi et al. 1993). This may occur because denitrifiers may maintain denitrifing enzymes in the soil matrix to allow them to quickly use NO_3^- as a terminal electron acceptor giving them a potential advantage over other microbes (Tiedje et al. 1982). Belser (1977) suggested that recycling of NO_3^- to NO_2^- might support large NO_2^- oxidizer populations. Short-term NO_3^- recycling might be an important N conservation mechanism in these soils.

Other indirect evidence for NO_3^- recycling is suggested by the disproportional population sizes of NH_4^+ and NO_2^- oxidizers and the lack of significant relationships between NH_4^+ and NO_2^- oxidizers in forest and prairie soils in any season. Nitrite oxidizer populations should be approximately one-third that of NH_4^+ oxidizers if their energy is derived solely from NO_2^- supplied by NH_4^+ oxidizers (Nicholas 1978). However, NO_2^- oxidizers populations in this study were up to three orders of magnitude greater than NH_4^+ oxidizers (Figure 5, Table 1). In contrast with other studies that have related MPN estimates of nitrifiers to V_{max}, MPN estimates of NO_2^-

oxidizers were positively correlated ($r = 0.94$, $P \leq 0.001$) with V_{max}, while NH_4^+ oxidizers showed no significant relationship with V_{max} (Belser 1979). In addition, there were no significant relationships between NH_4^+ and NO_2^- oxidizers, suggesting that NO_2^- oxidizers are least partially independent of NO_2^- supply from the oxidation of NH_4^+ by NH_4^+ oxidizers. These findings suggest that NO_2^- oxidizer populations may influence nitrification rates by controlling the last step in nitrification ($NO_2^- > NO_3^-$). Although large populations of NO_2^- oxidizers compared to NH_4^+ oxidizers in these soils suggest the possibility that there is some NO_3^- recycling, it does not necessarily indicate this process is occurring, because unlike NH_4^+ oxidizing bacteria, NO_2^- oxidizers are not obligate chemoautotrophs (Bock et al. 1986). In addition, NO_2^- may be supplied through heterotrophic nitrification rather that autotrophic nitrification as suggested by the strong relationship NO_2^- oxidizers have with V_{max}. In some young and mature conifer soils heterotrophic nitrification has been found to be the dominant NO_3^- forming process (Pedersen et al. 1999) and could be occurring in *J. virginiana* and prairie soils.

Reduced gross nitrification in forests with these assays does not initially appear to be caused by differences in substrate availability (NH_4^+) or nitrifier population size (Figures 2a & 3a). Allelopathy has been strongly implicated in conifer communities as inhibiting nitrification via several mechanisms that include inhibition of nitrifying enzymes or stimulation of NH_4^+ immobilization by phenolics, such as monoterpenes (Bremmer and McCarty 1988, White 1991, Fisher et al. 1994). The reversal of the trend of reduced gross nitrification in the fall 2004 indicates that if allelopathy exists, there is no sustained suppression of gross nitrification in forest soils. Rather, since gross nitrification:V_{max} nitrification ratios indicate that nitrification is strongly resource limited in both forest and prairie soils throughout the year, reduced gross

nitrification is probably due to rapid and short term changes in NH_4^+ pools. Previous research has found a strong trend of reduced NH_4^+ pools in forest soils (from plant uptake), which likely influences the activity of nitrifiers in these soils, resulting in reduced gross nitrification in these short-term laboratory incubations despite similar substrate availability at the initial start time of the assay (see Chapter 2).

In previous work I suggested that greater net N mineralization in forest soils was due to less microbial immobilization of N, as a result of differences in substrate composition (lower C:N) and quantity, or the presence of microbes with greater microbial growth efficiencies (Chapter 2). Based on low Cmin:Nmin ratios (Chapter 2), an index of microbial N immobilization, as well as the relationship of this index to C:N ratios of *J. virginiana* root biomass, I speculated that inputs from *J. virginiana* fine roots with relatively low C:N ratios (high tissue quality) led to less microbial immobilization of N and greater net N mineralization. Evidence from these assays does not support this speculation. Using estimates of gross mineralization minus immobilization processes (NH_4^+ immobilization + NO_3^- immobilization) I found that microbes in forest soils (102.3 ± 10.0 μg N g^{-1} soil-C d^{-1}) are immobilizing less N (gross) per gram of organic soil C than prairie soils (126.7 ± 12.1 μg N g^{-1} soil-C d^{-1}) across all measurements (One-way ANOVA, $F = 3.97$, $P = 0.056$), but gross ammonification (production) was also significantly less in forest soils on a per gram of organic soil C basis (One-way ANOVA, $F = 7.2346$, $p = 0.013$). Also, specific microbial N immobilization, the amount of N immobilized per unit microbial biomass N, also showed no significant differences between these two ecosystems (One-way ANOVA, $F = 0.18$, $P = 0.68$). Lastly, using the microbial growth efficiency (Y_c) calculation developed by Schimel (1988), which utilized CO_2-C flux rates and microbial biomass C:N ratios estimates from a concurrent study with the same soils, I found that

microbes in forest soils (0.68 ± 0.04) have only slightly different growth efficiencies than prairie soils (0.64 ± 0.04) across summer and fall 2004 (Two-way ANOVA, $F = 0.93$, $P = 0.352$). These high microbial growth efficiencies (> 50%) suggest that the microbial populations in both of these soils are dominated by fungi (Holland and Coleman 1987). It appears that in both forest and prairie soils, microbial immobilization of N is simply proportional to the amount produced, and there are no differences in microbial community function that would lead to less N immobilization. Yet, N mineralization was found to be greater in forest soils in field and laboratory assays; lack of evidence in support less microbial N immobilization may be due to limitations of these very short-term (one day) assays that may not be sensitive enough to detect long-term trends in N mineralization.

At first glance the internal N cycling in the soil and microbial populations responsible for nitrification in forest and grassland soils seem very similar. Most of these assays are plagued with inherent high variability, even with this well-replicated design. However, these assays do show small and consistent trends that are ecologically significant as evidenced by consistent result in parallel field studies. The internal rates of N transformations in forest soil are similar in most seasons to prairie soils. However, if these one-day rate estimates are indicative of the majority of season they are designed to represent, then these small differences may accumulate to large differences over that season, which may account for the differences in long-term patterns of net N mineralization observed under field conditions.

Juniperus virginana forests are able to maintain very conservative internal N cycling in mineral soils, as well in plant biomass (Norris et al. 2001). In these *J. virginana* forests, the internal cycling of N in the mineral soil and portions of the microbial populations responsible for nitrification remain relatively similar compared to prairie soils. The cycling of soil N in *J.*

virginana forests, much like prairie, minimizes the potential for significant N loss via soil pathways. Although *J. virginiana* plants were not explicitly considered when looking at internal N cycling in the soil, these plants likely have a strong interaction with soil processes. For example, field measurements have shown significantly lower concentrations of extractable NH_4^+ in forest soils (see Chapter 2). Competition between *J. virginana* and microbes for soil N is likely more intense than prairie species, because of the much longer growing season of this evergreen. Gross nitrification potential could be reduced in certain seasons, due to substrate limitation imposed by greater N uptake by forest biomass and reduction in nitrifier populations. *Juniperus virginana* forests are able to exert enough positive influence on soil properties and soil microbial communities to create conditions of greater N availability while limiting potential soil N loss.

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Figures and tables

Figure 3-1 Seasonal measurements of potential gross ammonification and consumption (mean ± se) in forest and prairie soils. Gross ammonification rates (a) were significantly greater in prairie soil (4.10 ± 0.35 μg N g^{-1} soil d^{-1}) compared to forest soil (2.86 ± 0.17 μg N g^{-1} soil d^{-1}) during Spring 2004 (F = 10.10, P = 0.019). In addition, NH_4^+ consumption (b) was also significantly greater in prairie soil (4.96 ± 0.55 μg N g^{-1} soil d^{-1}) compared to forest soil (3.31 ± 0.39 μg N g^{-1} soil d^{-1}) during Spring 2004 (F = 6.07, P = 0.049). Neither gross ammonification nor consumption was significantly different at any other measured time (P > 0.05). Different letters indicated significant differences (P < 0.05).

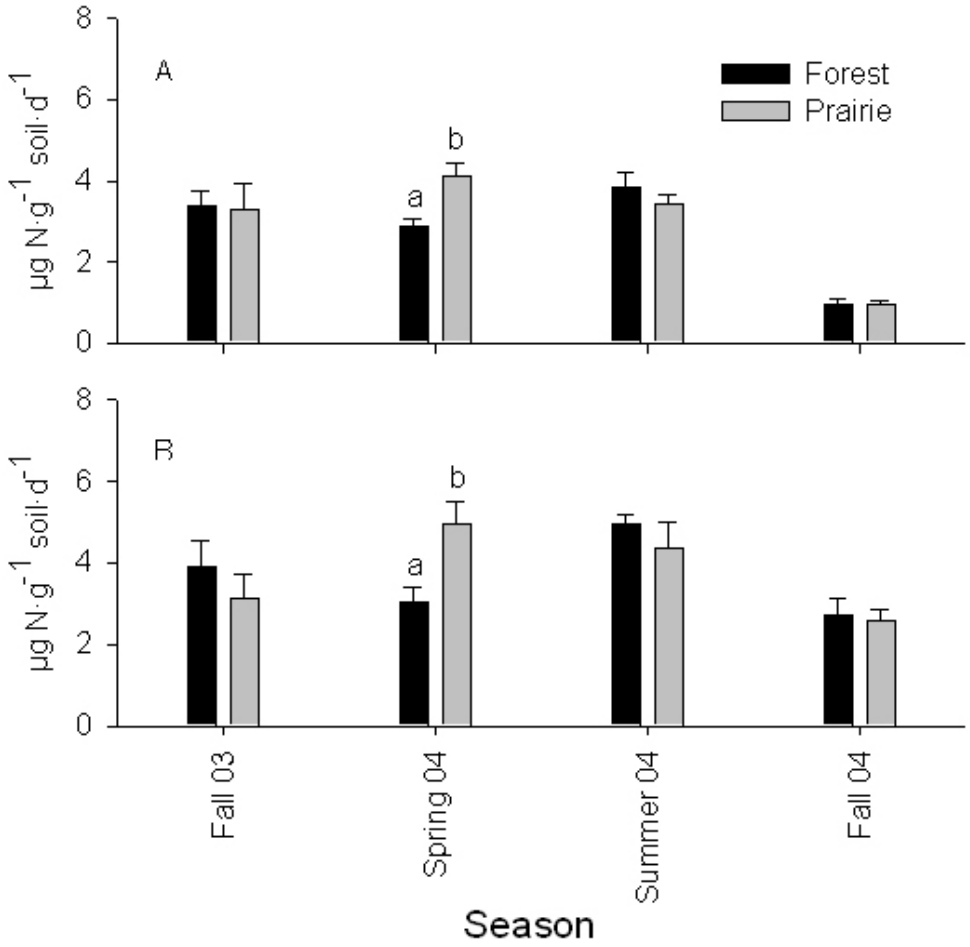

Figure 3-2 Seasonal measurements of potential gross nitrification and consumption (mean ± se) in forest and prairie soils. Gross nitrification rates (a) were significantly greater in prairie soil (1.55 ± 0.40 µg N g^{-1} soil d^{-1}) compared to forest soil (0.51 ± 0.13 µg N g^{-1} soil d^{-1}) during Fall 2003 (F = 6.075, P = 0.049). In addition, NO_3^- consumption (b) was also significantly greater in prairie soil (1.77 ± 0.26 µg N g^{-1} soil d^{-1}) compared to forest soil (0.71 ± 0.17 µg N g^{-1} soil d^{-1}) during Fall 2002 (F = 11.56, P = 0.014). Neither gross nitrification nor consumption was significantly different at any other measured time (P > 0.05). However, in Fall 2004 gross nitrification (F = 5.38, P = 0.059) and consumption (F = 5.23, P = 0.062) rates appeared to be greater in forest soils although these differences were not significant. Different letters indicated significant differences (P < 0.05).

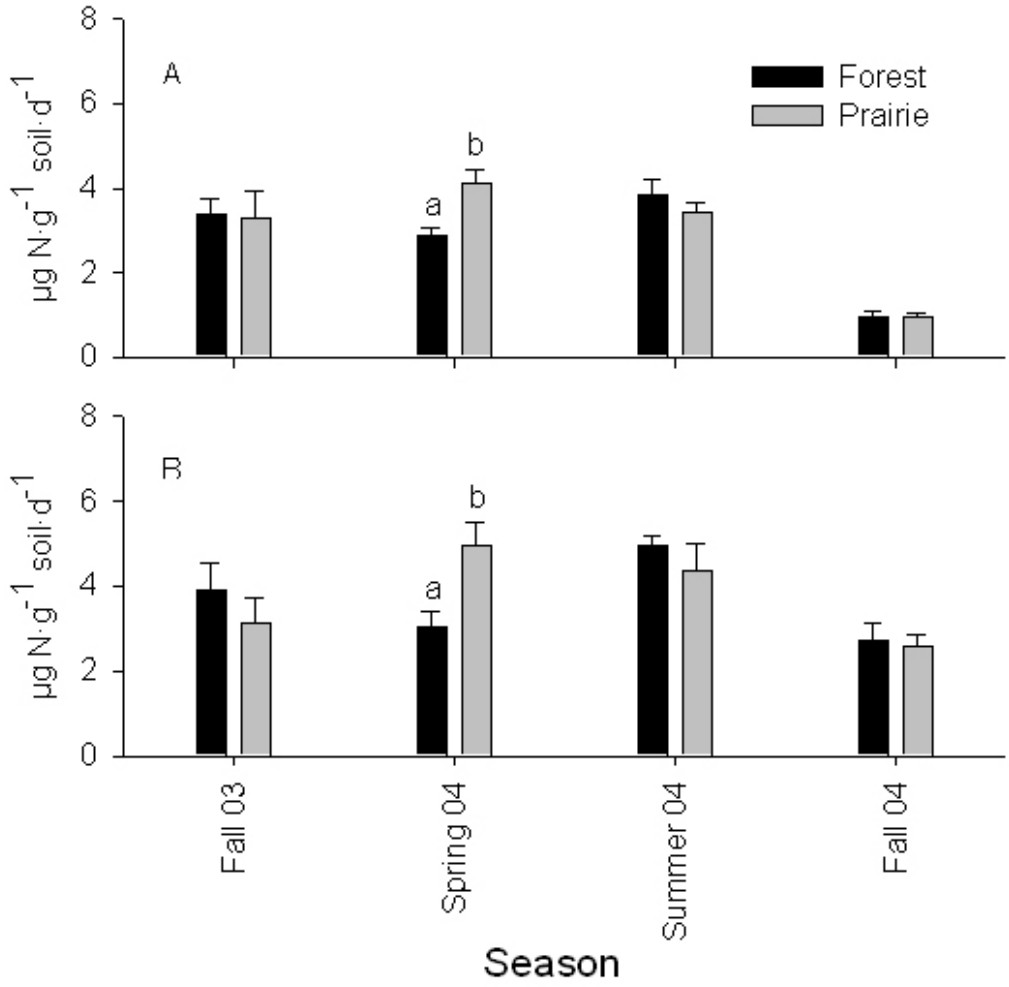

Figure 3-3 Seasonal measurements (mean ± se) of extractable NH_4^+ (a) and NO_3^- (b) (left y-axis) and mean residence time (MRT) of NH_4^+ (a) and NO_3^- (b) (right y-axis) in forest and prairie soils. No significant differences in NH_4^+ mean residence time (MRT) of forest and prairie soil were found in any season. In spring 2004, NO_3^- MRT in the forest (34 ± 16 hr) was significantly less compared to prairie soils (242 ± 63 hr.) ($F = 10.45$, $P = 0.018$, One-way ANOVA). Different letters indicated significant differences ($P < 0.05$).

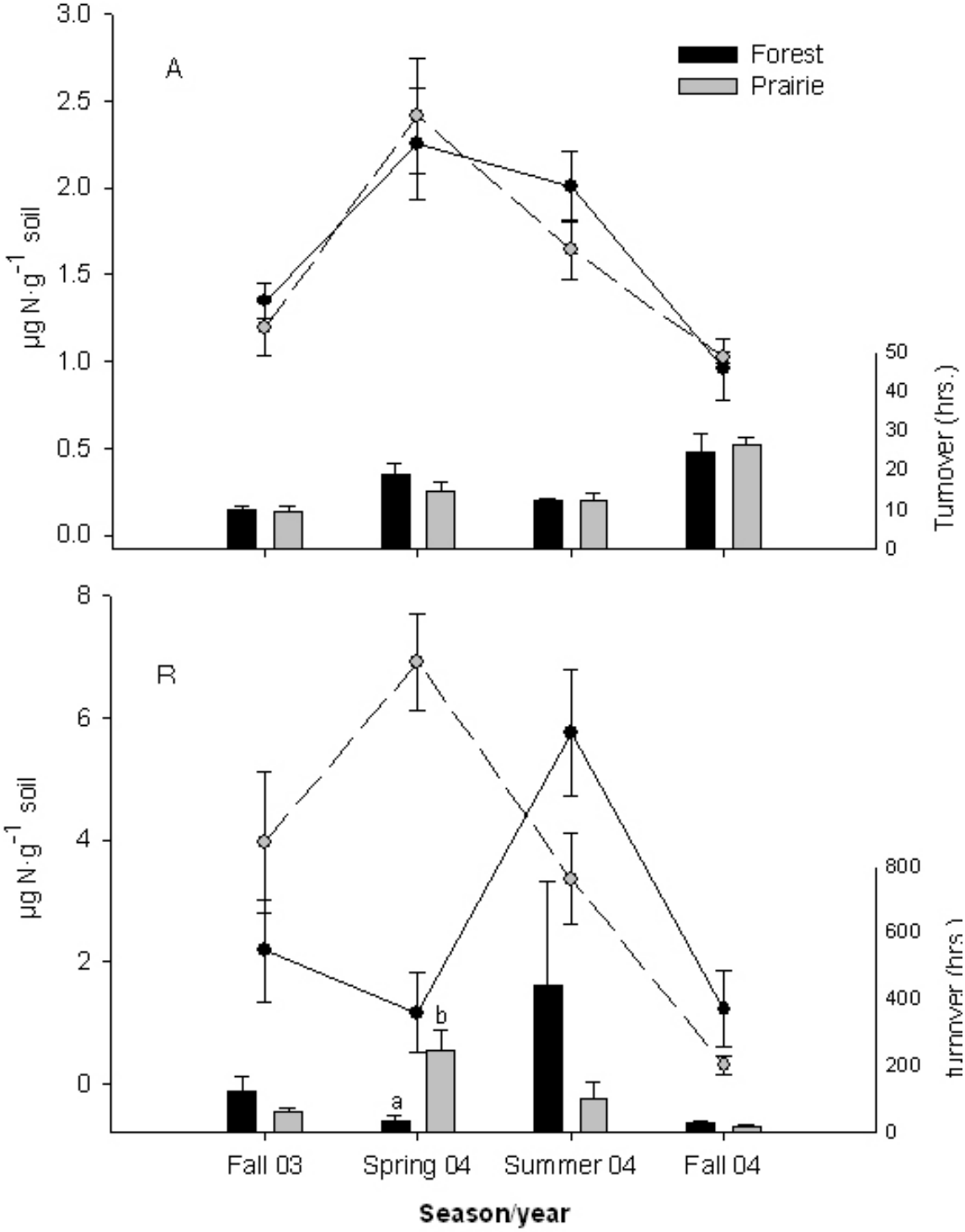
A
Forest
Prairie
μg N·g-1 soil
Turnover (hrs.)
R
a
b
Fall 03
Spring 04
Summer 04
Fall 04
Season/year

Figure 3-4 Seasonal measurements (V_{max}) of potential nitrification (mean ± se) in forest and prairie soil, measured with a shaken soil slurry technique. There were no significant differences between forest and prairie soil at any measured season ($P > 0.05$). Extremely high rates of potential nitrification were found in both forest (38.71 ± 9.12 μg N g^{-1} soil d^{-1}) and prairie soil (27.88 ± 5.52 μg N g^{-1} soil d^{-1}) in Fall 2004.

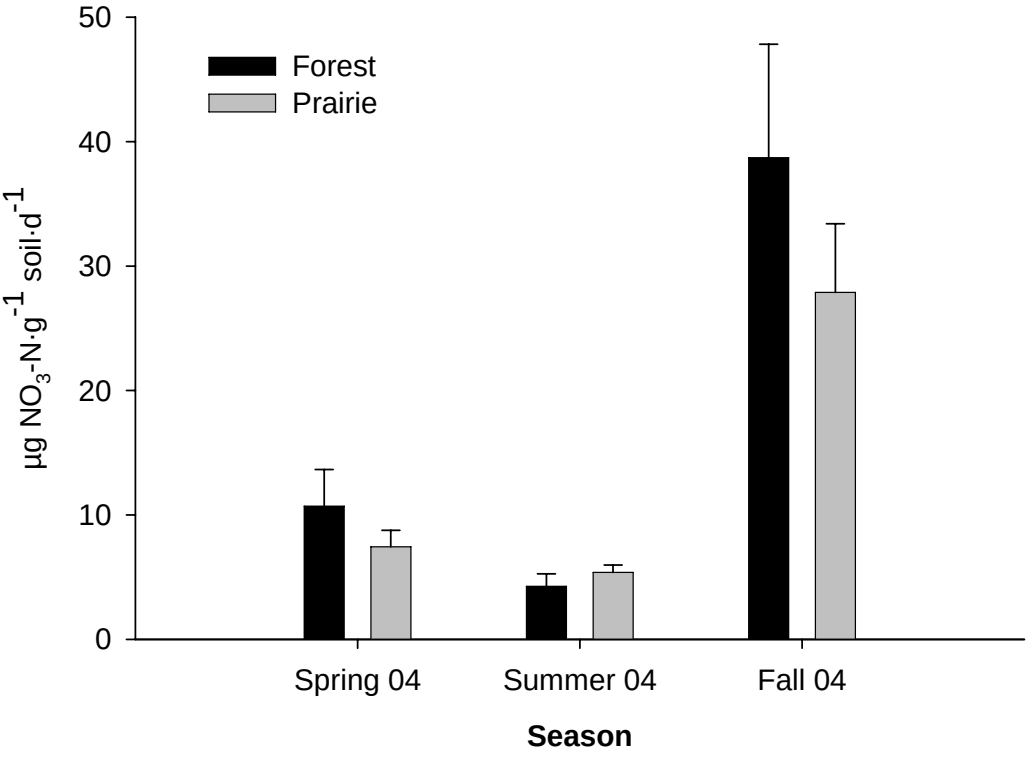

Figure 3-5 Seasonal measurements of ammonium and nitrite oxidizers (log mean) in forest and prairie soil, measured with a most probable number (MPN) technique. Although the bar represent (log) mean MPN counts for all sites in this figure, actual comparisons were done at the site level. At each site there were no significant differences between forest and prairie soils in the numbers of ammonium (a) or nitrate oxidizers (b) at any measured season. The MPN estimates for nitrite oxidizers were higher in all seasons compared to ammonium oxidizers with the greatest differences occurring in the spring and fall (up to 1000x)

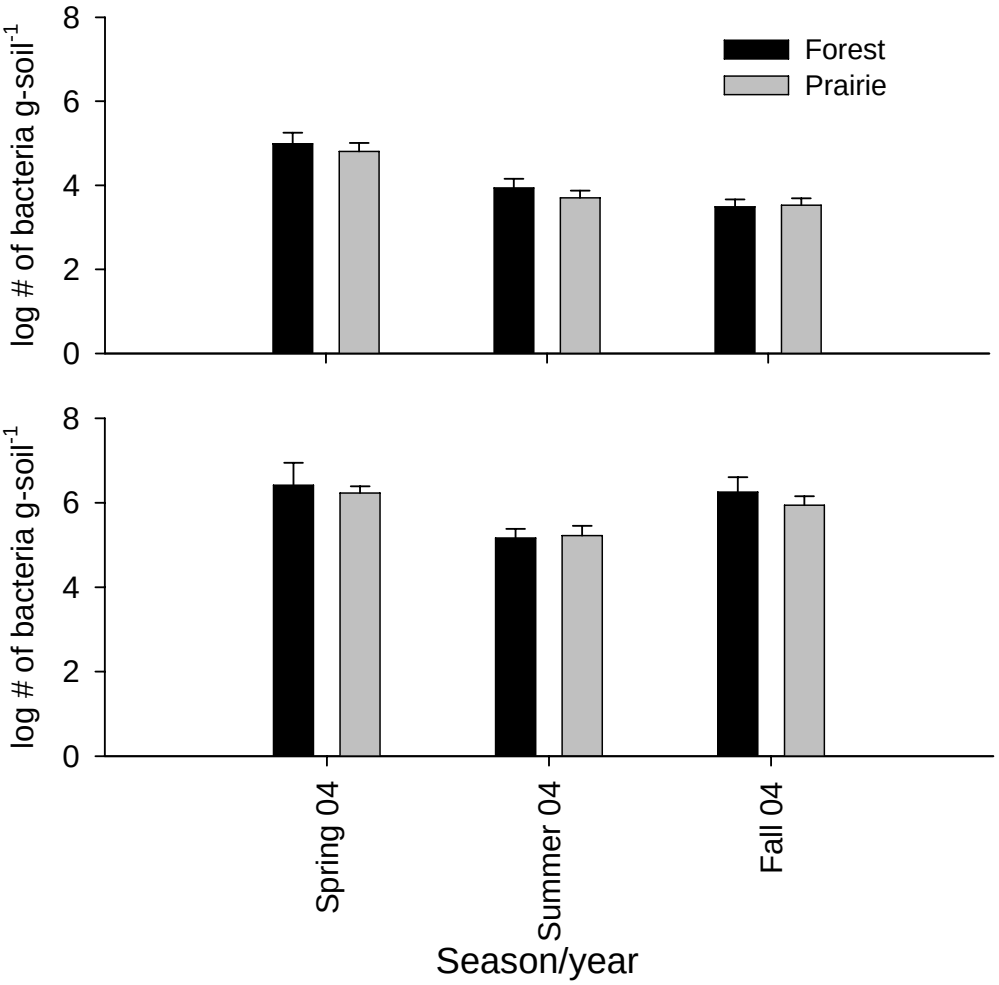

Table 3-1 Most probable number (MPN) raw counts (mean ± se) for NH_4^+ and NO_2^- oxidizers in forest and prairie soils (per gram dry soil) measured in the spring, summer, and fall 2004. Comparisons were only made for MPN estimate of like bacteria measured in the same season. There were no significant differences found with any MPN estimate in the same season, but significant season differences existed. Although there were not significant differences with the same bacteria type, NO_2^- oxidizers were present in much greater numbers (up to 3 orders of magnitude) at all sampling dates than NH_4^+ oxidizers. Means with different letters indicate significant differences, upper case for NH_4^+ oxidizers and lower case for NO_2^- oxidizers.

Season	Ecosystem	Bacteria	MPN (mean)	Sign(alpha =0.05)
Spring 04	Forest	NH_4^+ oxidizer	97,476	A
Spring 04	Prairie	NH_4^+ oxidizer	64,305	A
Spring 04	Forest	NO_2^- oxidizer	2,583,237	a
Spring 04	Prairie	NO_2^- oxidizer	1,689,214	a
Summer 04	Forest	NH_4^+ oxidizer	8,663	B
Summer 04	Prairie	NH_4^+ oxidizer	5,044	B
Summer 04	Forest	NO_2^- oxidizer	145,578	b
Summer 04	Prairie	NO_2^- oxidizer	166,302	ab
Fall 04	Forest	NH_4^+ oxidizer	5,878	B
Fall 04	Prairie	NH_4^+ oxidizer	4,200	B
Fall 04	Forest	NO_2^- oxidizer	1,786,011	a
Fall 04	Prairie	NO_2^- oxidizer	876,093	ab

CHAPTER 4 - Do plant traits and plant-soil nutrient feedback loops promote rapid C accrual following conversion of tallgrass prairie to *Juniperus virginiana* forests?

Abstract

Juniperus virginiana, an important woody plant invader in the U.S. Central Plains, has increased dramatically in density and cover in large areas previously dominated by highly diverse prairie communities, causing changes in key ecosystem properties and processes. These ecosystem responses may include changes in plant and soil C and N stores, as well as changes in plant-soil feedback loops that affect N availability and C uptake. Ecosystem stores of C and N in plant and soil pools, as well as rates and patterns of C uptake and leaf-level nitrogen use efficiency (NUE) were assessed in both native tallgrass prairies and recently established *J. virginiana* forests to better understand potential plant-soil feedback loops that may promote the high productivity of *J. virginiana* communities after encroachment into tallgrass prairie. Leaf-level photosynthetic NUE of *J. virginiana* was about 10x less than that of the dominant C_4 grass species (*Andropogon gerardii*) in prairie, but the ability of *J. virginiana* to photosynthesize when grasses are senescent allows greater annual C uptake. Increased plant productivity and the absence of fire in *J. virginiana* forests have allowed an increase of almost 8,000 g m^{-2} in ecosystem C storage relative to prairie in about half a century. The observed high productivity of *J. virginiana* may be possible, in part, because of greater soil N availability, which was not expected given hypothesized plant-soil feedback loops associated with conversion of a grass-dominated to conifer-dominated ecosystem. Conservation of N due to the elimination of fire in *J. virginiana* forests, coupled with possible access to other sources of available nitrogen, as

suggested by a substantial increase (~44%) in ecosystem N in measured pools and greater N uptake, likely maintains adequate soil N availability and prevents N limitation in the time that these forests have developed. Although *J. virginiana* forests may provide strong regional carbon sinks, the large allocation of new C aboveground make these sinks vulnerable to significant losses through volatilization in fire, and through soil potential erosion caused by reduced herbaceous cover in these forests.

Introduction

Woody plant encroachment into grasslands and savannas is a worldwide phenomenon (Van Auken 2000, Archer 2001). In the U.S. Central Plains, *Juniperus* and several other genera of woody plants have increased dramatically beyond their historical range and distribution (Schmidt and Leatherberry 1995, Briggs et al. 2002) and pose a significant threat to grassland conservation. Conversion of native tallgrass prairie to closed-canopy *J. virginiana* forest can occur in as little as four decades (Briggs et al. 2002). When grassland ecosystems are converted to *J. virginiana* forests there are steep decreases in plant species richness (Gehring and Bragg 1992, Briggs et al. 2001) and changes in ecosystem properties and processes (Norris et al. 2001a, Norris et al. 2001b, Smith and Johnson 2003, Smith and Johnson 2004). Grassland and savanna ecosystems account for 30-35% of global terrestrial NPP and have substantial stores of C and N belowground. Compared to other ecosystems, savannas have some of the greatest potentials for C gain and loss (ORNL 1998). Substantial changes in the rates and magnitude of C accrual, as well as C allocation, in *J. virginiana* forests could have substantial consequences for both soil chemistry and regional ecosystem-exchange processes (Moiser 2001).

Juniperus virginiana, an important encroaching species in the eastern U.S. Central Plains, causes substantial increases in ecosystem productivity and shifts in the magnitude and location of

C and N storage in ecosystem pools (Norris et al. 2001a, Smith and Johnson 2004). For example, the vast majority (~96%) of C is stored belowground in prairie, whereas most of the ecosystem C is stored in aboveground biomass in *J. virginiana* stands (Norris et al. 2001a). Shifts in the magnitude of ecosystem C and N stocks occur rapidly and are driven in part by changes in plant productivity, because aboveground net primary productivity is much greater in *J. virginiana* forests ~10,000 kg C ha^{-1} yr^{-1} compared to the mesic grasslands they replace ~3,700 kg C ha^{-1} yr^{-1} (Norris et al. 2001a).

Recent studies in *J. virginiana* communities have found that N availability was slightly increased in shallow soils (A-horizon) of *J. virginiana* forests compared to the grassland ecosystems they replaced (see Chapter 2), and this increase in N availability may contribute to differences in productivity of *J. virginiana* forest compared to tallgrass prairie (Norris et al. 2001a). However, greater N availability is contrary to what is expected, since hypothesized plant-soil N feedback dynamics should reduce N availability in *J. virginiana* forests relative to prairie. This is because increased plant productivity in *J. virginiana* forests should elicit immobilization of significant quantities of N, both in the plant biomass and litter, as well as in the SOM, as a result of increased plant C assimilation and soil C inputs. Immobilization of N in these plant and soil pools coupled with changes in litter chemistry, which make this litter input less decomposable than prairie litter (Norris et al. 2001b), should reduce N availability. Reduced N availability would be expected to lower plant productivity if N is limiting and if NUE is similar for prairie plants and *J. virginiana*. Species with greater nitrogen use efficiency could maintain high productivity under conditions of reduced N availability, but this would be expected to reinforce plant-soil feedback loops and maintain low soil N availability.

Coniferous species, like *J. virginiana*, generally have greater ecosystem nitrogen use efficiency (NUE) than most other plants, which may allow greater productivity with limited N availability. Species with greater NUE are able to assimilate more CO_2 per unit nitrogen in plant tissues (Chapin 1980, Aerts 1995, Lambers et al. 1998). However, plant tissue produced by species with high NUE is usually of poor quality, as tissue quality (as indexed by C:N ratios) is inversely related to NUE (Seastedt 1991). Litter produced by species with high NUE typically has a high C:N ratio that slows decomposition, reduces labile N pools and decreases soil N availability (Vitousek 1982). These reductions in soil N availability can, in turn, promote greater NUE of species developing under these conditions, thus creating a self-reinforcing plant-soil feedback loop.

Plant-soil N feedback dynamics that result in decreased N availability in *J. virginiana* soils could be alleviated or avoided by several potential mechanisms, which are not mutually exclusive. *Juniperus virginiana* forests may be able to better conserve existing stocks of N, exploit additional exogenous N sources (i.e., atmospheric N scavenging), or forage for N deeper in the soil profile relative to prairie species. Obtaining new sources of N or enhancing conservation of N may compensate for a greater N immobilization potential in plant biomass and soil organic matter, and allow maintenance of labile soil N pools and greater soil N availability than would otherwise be possible during conversion of grasslands to *J. virginiana* forests.

In contrast to expected lower litter quality in *J. virginiana*, McKinley (see Chapter 2) found 50% greater root tissue N concentrations and lower C:N ratios of *J. virginiana* fine roots (< 2mm) compared to bulk roots of mixed species in prairie. These relatively labile subsurface litter inputs may drive observed greater N availability in forests. While short-term soil N-cycling dynamics in *J. virginiana* forests may be partially explained by this phenomenon, it does not

address how fine root tissue quality (relatively low C:N ratios) was developed under potentially strong soil N limitations that were hypothesized.

Observations of higher soil N availability in the shallow A-horizon (see Chapter 2) despite large amounts of N immobilized in *J. virginiana* plant tissue and organic soil would suggest significant total N accrual in *J. virginiana* forests. Nitrogen accrual in *J. virginiana* forests may occur with either increased N inputs or a reduction of losses of current N pools relative to prairie. Previous work by McKinley (see Chapter 3) demonstrated that both forest and prairie ecosystems can minimize soil N losses by quickly immobilizing inorganic N. Furthermore, there were few differences in soil N cycling between these ecosystems that would suggest greater loss of N in either ecosystem, as evidenced by little differences in gross nitrification and high NO_3^- consumption rates. Therefore, determining if there is N accrual in the *J. virginiana* forests, and identifying possible causal factors are germane for understanding the underlying reasons that N availability is not decreased by plant-soil interactions in *J. virginiana* soils. The role of N in plant productivity, patterns of magnitude of ecosystem C and N storage, and potential sources and losses of N was examined in both *J. virginiana* and prairie ecosystems to gain an understanding of the underlying drivers that cause differences in soil N availability and ecosystem productivity.

Material and methods

Experimental design

Four paired sites comprised of contiguous or nearly contiguous *Juniperus virginiana* forest adjacent to native prairie were chosen in close proximity (<1 km) to the Konza Prairie Biological Station (KPBS) (39º05'N, 96º35'W). Proximity to the KPBS, the primary location of the Konza Prairie Long-Term Ecological Research (LTER) program, allows the use of a variety

of baseline data on ecological processes in native tallgrass prairie. Historical aerial photographs and analysis of soil organic carbon (SOC) isotopic composition were used to verify recent replacement of these grasslands with *J. virginiana* forests (detailed later). Each paired site shared similar soil type, slope, position, and aspect. Each *J. virginiana* forest (at least 0.5 ha) was relatively mature (~30-55 yrs) creating dense (680-1,360 trees ha^{-1}), complete or nearly complete canopy cover. Recurring controlled or natural fires since initial encroachment presumably formed the current distinct boundaries with adjacent prairie. These prairie sites have not been grazed recently (>15 yrs, personal communication) and have an average fire return interval of 1-2 yrs, which is currently a result of controlled burns conducted in early spring. One 50 m transect was established in each vegetation type (*Juniperus* forest or prairie) at each of the four-paired study sites (n = 4), along which soil and other ecosystem measurements were made in randomly assigned plots.

Site description

The prevalent native vegetation in the northern Flint Hills is tallgrass prairie, dominated by a matrix of perennial, warm-season C_4 grasses including big bluestem (*Andropogon gerardii* Vit.), little bluestem (*Schizachyrium scoparium* Michx.), indiangrass (*Sorghastrum nutans* Nash) (Anderson et al.) and switchgrass (*Panicum virgatum* L.) (Kuchler 1967; Freeman and Hulbert 1985). These C_4 grasses contribute the majority of net primary productivity (NPP) (Knapp et al. 1998). However, a highly diverse mixture of less abundant species, including C_3 grasses and sedges and a diverse array of forbs, contribute to the high floristic diversity of these grasslands (Freeman and Hulbert 1985). The native tallgrass prairie flora also includes a smaller number of native woody plants, such as buckbrush (*Symphoricarpos orbiculatus* Moench.), New Jersey tea (*Caenothus herbaceous* Raf.), smooth sumac (*Rhus glabra* L.) and rough-leaved dogwood

(*Cornus drummondii* CA May), which can be locally abundant, especially in prairie that is infrequently burned (Briggs et al. 2005). Average annual total precipitation is 835 mm with 75% falling during the growing season (Bark 1987). Topographic relief divides the landscape into upland plateaus with mostly shallow soils, slopes with outcrops of limestone, and lowlands with deeper alluvial and colluvial soils (Oviatt 1998). Three of the study sites had silty clay loam soil; fine, mixed, active, mesic Udothentic Haplustols. The fourth site had a silt loam soil; a fine, mixed, superactive, mesic Udertic Argiustolls (United States Department of Agriculture 1975). The soils at these sites were generally low in nutrients and relatively high in organic carbon.

Photosynthetic nitrogen use efficiency (PNUE)

Leaf-level photosynthetic rates (A_{max}) were measured seven times in the growing and non-growing seasons with a LiCor 6400 portable infrared gas analyzer system (Li-Cor, Lincoln, Nebraska, USA). Terminal portions of exposed shoots (relatively young leaf tissue) of *J. virginiana* or a single grass leaf of *A. gerardii* was sealed in a leaf chamber equipped with a red-blue diode light source for 3-4 minutes during which CO_2 assimilation was measured. Gas exchange measurements were made under a photosynthetic photon flux density (PPFD) of 1500 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 width at the mid point of the leaf in the cuvette and then multiplying by the length of the cuvette (2 cm), to acquire leave area (cm^2). *Juniperus virginiana* leaves were measured with a LI-3100 leaf area meter (Li-Cor, Lincoln, Nebraska, USA) to acquire projected leaf area after transport to the lab. Due to variability in leaf area measurements, each *J. virginiana* leaf area was measured five times to obtain a single mean value for further calculations. Also, no attempt was made to account for the

three-dimensional shape of the leaves. Specific leaf mass was determined in May 2005 for *J. virginiana* by dividing the dry weight of the sampled leaves in the cuvette by the leaf area. A single mean value derived from all sites in May was used to estimate specific leaf mass from measured leaf areas for all measurement times. Published values of specific leaf mass of *A. gerardii* in burned sites were used for comparisons (Knapp et al. 1998).

Following measurements of photosynthesis, grass and juniper tissues were collected in the field and placed in sealed plastic bags in an iced cooler. Once leaf area was determined, the samples were dried at 60ºC for at least 48 hr then ground (pulverized) and placed into tin capsules for analysis of tissue N content (see plant tissue analysis). Photosynthetic nitrogen use efficiency (PNUE) was calculated by dividing A_{max} (μmoles CO_2 m^{-2} s^{-1}) by the nitrogen content (moles N m^{-2}).

C and N stores: aboveground

Aboveground biomass was measured at the seasonal peak (August 2005) in prairie sites by clipping all vegetation within 20x50 cm quadrat to the soil surface and weighing the samples after drying at 60ºC for at least 72 hr. Representative subsamples of bulk plant material were collected and analyzed for C and N content.

Aboveground biomass for *J. virginiana* sites was estimated using published allometric equations for (Norris 2001a). The allometric equations required tree diameter at breast height (DBH), which was calculated by converting tree circumference measurements to diameter. Tree DBH measurements were converted to tree biomass C and N via the following published formulae: log (biomass C, kg) = -0.838 +2.050 (DBH, cm), R^2 = 0.977, P = 0.0002, and log (biomass N, kg) = -2.994 +2.093 (DBH, cm), R^2 = 0.961, P = 0.0006.

C and N stores: soil

Organic soil

Litter stocks were determined in forest sites using 0.1 m^2 quadrats (20x50 cm) placed randomly along established transects. All surface litter (including O_i, O_e and O_a soil horizons) was removed to the mineral soil, placed in plastic bags and returned to the lab for analysis. Prairie sites, since they were burned in the previous spring, had no litter accumulation. Samples were dried at 60ºC for at least 72 hr, weighed, and total C and N and isotopic composition was determined (see methods below). Soil monoliths were excavated to the dimensions of 25x25x10 cm or until bedrock was reached along the forest and prairie transects. Roots were extracted from the soil monoliths by wet sieving, then washed to remove soil, dried at 60ºC, and analyzed for C and N content and isotopic composition (see methods below).

All tissue samples were dried at 60ºC for 48 hr then finely ground (pulverized) and weighed (~ 7 mg) into tin capsules. Percent C and N was determined using dry combustion coupled with gas chromatography with a Carlo Erba model NA1500 C/N analyzer (Milano, Italy) or a mass spectrometer for isotopic values (see details below).

Mineral soil

Bulk soil was sampled with a 2x10 cm Oakfield corer. The mineral soil was divided into 5 equal 2 cm sections (which corresponded with soil depth) with a clean knife. Each soil segment was placed in separate labeled Ziploc© bags. This process was repeated 5 times for each 25 m^2 plot (n = 6) per site (n = 4). The 5 subsamples per plot were combined by soil depth to acquire one representative composite sample per depth increment for each plot. The samples were placed in an iced cooler until transit to the laboratory. At the laboratory the soils were sieved (4 mm), mixed thoroughly by hand and oven dried at 100ºC for at least 48 hr. The soil

samples were then finely ground (pulverized) and weighed (~25 mg) into silver capsules, and treated with 2M HCl daily until effervescence was no longer evident, to remove inorganic carbon ($CaCO_3$). Percent C and N was determined using dry combustion coupled with gas chromatography with a Carlo Erba model NA1500 C/N analyzer. Total soil organic C and N per square meter was calculated after adjusting for soil bulk density.

Mineral soil $\delta^{13}C$ and $\delta^{15}N$

Additional sub-samples of bulk soil samples were used to determine $\delta^{13}C$ and $\delta^{15}N$ of decarbonated bulk soil at each depth increment to assess the amount of C_4-derived SOC replaced by SOC from new C_3 forest inputs. At the Kansas State University stable isotope lab a Thermo Finnnigan Delta Plus mass spectrometer (samples combusted with a CE Elemental Analyzer with ConfloII), was used to determined the isotopic value of C ($\delta^{13}C$) in the soil samples. A mixing model (Balesdent et al. 1988, Arrouays et al. 1995) was used to determine the percent soil C converted from C_4-C to C_3-C: $f = \delta\text{-}\delta_0 / \delta_1\text{-}\delta_0$, 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 juniper litter and roots). Soil $\delta^{13}C$ from the paired grasslands was used in the mixing model because it represented the best estimate of the soil isotopic ratio before *J. virginiana* encroachment.

Microbial biomass

Soil microbial biomass C and N was determined at the peak of the growing season, Summer 2004 (July), and at the beginning of the non-growing season, Fall 2004 (October), using the chloroform fumigation-incubation technique (Jenkinson and Powlson 1976). Approximately 25 g of each sample was placed in 125-mL Erlenmeyer flasks and DI H_2O was added to bring the soils to field capacity (60% water filled pore space). The samples were covered with Parafilm®

and pre-incubated at 25ºC for 7 d. After 14 days, half of the samples (controls) were continuously incubated for the remainder of the experiment; the remaining samples were fumigated with chloroform ($CHCL_3$). The fumigated samples were placed inside vacuum desiccators with wet paper towels and evacuated until chloroform ($CHCL_3$) placed in a 100 mL beaker inside the desiccators boiled for ~30s, then air was flushed in by releasing the desiccator's valve to allow for greater dispersion. This was repeated for a total of three times. In the final vacuum sequence the valve was immediately closed then left to stand for 24 hr in the sealed desiccators. After 24 hr the desiccator was evacuated repeatedly, and flushed with air to remove chloroform residue. Fumigated and control samples were then placed into mason jars and incubated for 10 days at 25ºC. After 10 days headspace gas was sampled with a syringe through rubber septa in the top of the mason jars. The gas samples (0.3-0.5 mL, depending on CO_2 concentration) were immediately injected in a Shimadzu GC-8A gas chromatograph (Japan) to measure CO_2 concentrations. Peak areas were covert to µg CO_2-C g^{-1} dry soil with a standard curve and after accounting for injection volume, headspace volume, and dry weight of the soil. Microbial biomass C was determined by difference in the amount of CO_2-C respired from fumigated and non-fumigated samples. Microbial biomass N was determined by difference in the amount extractable inorganic N from fumigated and non-fumigated samples. Ammonium and nitrate were extracted from the entire sample, ~ 25 g of field moist soil with 100 mL of 2 N KCl, agitated for 1 h in an orbital shaker (200 rpm), and filtered through a 0.4 µm polycarbonate membrane. The extracted samples were stored at 0ºC until analyzed. Inorganic nitrogen (NH_4^+-N and $NO_3^- + NO_2^-$-N) concentrations were determined colormetrically with an Alpkem® Flow Solution autoanalyzer (Wilsonville, Oregon) using the indophenol blue method for NH_4^+-N and cadmium reduction followed by diazotization with sulfanilamide for NO_2^-/NO_3^--N. An

efficiency factor of 0.41 and 0.54 was used for C and N, respectively, (Horwarth and Paul 1994) for both prairie and *J. virginiana* soils.

Soil N mineralization

Annual rates of net N mineralization were determined from cumulative estimates from 15 different N mineralization assays, from June 2003- June 2005, with intact *in situ* soil cores. Soil cores were taken at six plots along each transect (both forest and prairie) at four sites and averaged to obtain one value for each site. For full description of methods, see Chapter 2 of this dissertation.

Bulk density

Bulk density is a measure of the mass of the soil per unit volume (g cc^{-1}), on an oven dry basis (100ºC). A measure of bulk density is important for more accurate comparisons and standardization of metrics of soil properties and standing stocks of nutrients between different soils on an ecosystem level. A standard bulk density corer was used to determine bulk density to a depth of 10 cm. Bulk density between 0-5 cm and 5-10 cm was determined. Bulk soils were excavated by driving the bulk density soil corer to a depth of 10 cm then the split sieve was opened to obtain two stacked internal cores (5x5 cm). Each core was carefully shaved with a sharp blade to remove any extraneous soil from the dimensions of the internal cores. The cores were placed in sealed plastic Ziploc® bags and placed in a cooler until transit to the lab was complete. At the laboratory, the soils were weighed (for soil moisture measurements) and place in an oven at 100ºC for 48 hrs. After drying, the soils were reweighed. The soils were then sieved with a 2-mm soil-sieve screen; with rocks or any other matter in the soil they were removed and accounted for by subtracting the mass and volume of the rocks (or other matter)

from the total mass. Bulk density was calculated by dividing the soil dry weight by the adjusted volume of the core.

Statistical methods and calculations

A randomized complete block was used as the statistical design structure. A mean from each plot (6) along each vegetation transect at each site was obtained for statistical analysis for all soil analyses. Each paired site, composed of both forest and prairie transects, was considered to be a replicate (n = 4). One and two-way ANOVAs were used to determined significant differences between mean values for forest and prairie, where applicable. A simple linear regression model was used to determine the relationship between the percentage of SOC of forest origin in forest soils by soil depth. Linear regression was also used to assess the relationship of leaf N content and net photosynthetic rates in *A. gerardii* and *J. virginiana*.

Results

PNUE

Leaf-level photosynthetic rates were usually greater in *A. gerardii* compared to *J. virginiana* for much of the growing season (Figure 1a). However, there was no C uptake by *A. gerardii* in the non-growing season (Nov-April) when the 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 just over 1.5% in *J. virginiana* shoots over the entire year (Figure 1b). However, leaf tissue N in *A. gerardii* reached a peak of 2.52% in the beginning of the growing season, and then decreased steadily during the growing season, leveling off at ~1.5% from July through early October. There was a precipitous drop in leaf N concentrations in recently senesced *A. gerardii* to less than 0.5% in November, as much of the leaf N was resorbed prior to senescing. Photosynthetic nitrogen use efficiency 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 1c). 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 4a) relative to other months, while leaf N remained at moderate levels (Figure 1b).

Ecosystem C and N

There were large accumulations of both C and N in *J. virginiana* ecosystems relative to the grasslands that they replaced (Table 1). Total ecosystem carbon accumulation increased from 3,606 g C m^{-2} in prairie to 11,476 g C m^{-2} in *J. virginiana* stands. Ecosystem N accumulation in *J. virginiana* was also substantial, 433 g N m^{-2} in *J. virginiana* forests compared to 301 g N m^{-2} in prairie, but this increase of about 44% was not proportional with C accrual. Allocation of new C and N stocks also has shifted in *J. virginana* forests relative to grasslands. Total ecosystem C stock in prairie was about 95% belowground, in stark contrast with about 48% stored belowground in *J. virginiana* forests. However, the majority (89%) of total ecosystem N in forest was still stored belowground in soil pools. Alterations of soil C and N pools are particularly important because of the potential to influence key soil processes.

Changes in C and N in the organic and mineral soil were also evident with conversion to *J. virginiana* forests. The most noticeable difference was in the soil organic horizon (O-horizon), which accumulated a significant C and N pool in *J. virginiana* soils and was absent in prairie due to frequent fire-return intervals. Carbon and N in the organic soil horizon of *J. virginiana* forests was 1,540 ± 51 g C m^{-2} and 56 ± 3.8 g N m^{-2}. Organic C and N were also significantly greater in mineral soils (A-horizon) of *J. virginiana* forests compared to prairie (Figure 2a&b). Soil organic carbon (SOC) had increased about 12% in *J. virginiana* forests,

which was significantly greater 3,871 ± 119 g C m^{-2} compared to prairie with 3,443 ± 188 g C m^{-2} (One-way ANOVA, $F = 7.42$, $P = 0.01$, Figure 2a). Soil organic nitrogen (SON) had a similar significant increase in *Juniperus* forest (10%), 329 ± 9.4 g N m^{-2} compared to 298 ± 12.8 g N m^{-2} in prairie (One-way ANOVA, $F = 8.27$, $P = 0.01$, Figure 2b). Concurrent increases in both soil organic C and N led to no significant changes in soil C:N ratios in *J. virginiana* (11.77 ± 0.17) compared to prairie soils (11.41 ± 0.20) (One-way ANOVA, $F = 1.87$, $P = 0.18$ Figure 2c)

The accumulation of SOC was relatively uniformly with depth in the A-horizon of *J. virginiana* soils relative to prairie soils (Figure 2a), as evidence by no significant interaction between soil depth and vegetation type (Two-way ANOVA, $F = 0.25$, $P = 0.91$), despite significantly greater SOC (One-way ANOVA, $F = 6.48$, $P = 0.02$). However, the origin of the SOC in forest soils was not uniform (Figure 3b). Although about 34% of the SOC in *J. virginiana* soils was of C_3 (forest) origin), the greatest accumulation (48%) occurred in the 0-2 cm soil increment, and SOC of forest origin decreased predictably and linearly (R^2=0.97, y = 49.33 +3.6245x, $P \leq 0.01$, (using a single mean site values for each depth increment)) with depth to about 17% in the 8-10 cm soil increment. However, there were no significant differences in the proportion of SOC from forest origin by depth when using mean site values, due to high variance between sites (One-way ANOVA, $F = 0.94$, $P = 0.47$).

Microbial biomass C and N pools, which compose a small portion (3% C and 5-6% N) of the total soil organic pools of the A-horizon, were not different (One-way ANOVA, $P \geq 0.05$) in either the growing or non-growing seasons in *J. virginiana* and prairie soils (Figure 4). Microbial biomass C pools (A-horizon) were 141 ± 18 g C m^{-2} and 117 ± 12 g C m^{-2} in *J. virginiana* soils, and 112 ± 14 g C m^{-2} and 123 ± 8 g C m^{-2} in prairie soil in the growing and

non-growing season, respectively. Microbial biomass N pools (A-horizon) were 17 ± 1 g C m^{-2} and 16 ± 2 g C m^{-2} in *J. virginiana* soils, and 18 ± 3 g C m^{-2} and 16 ± 2 g C m^{-2} in prairie soil in the growing and non-growing season, respectively.

Discussion

Substantial changes in ecosystem productivity, as well as in the allocation and magnitude of plant and soil C and N stocks are evident following *J. virginiana* replacement of native prairie. Norris (2000) found that *J. virginiana's* efficient long-term use of N in C uptake may allow greater ecosystem productivity, despite leaf-level N use inefficiencies found in this study. Despite hypothesized changes in plant-soil feedback loops with conversion of prairie to *J. virginiana* forests, there was no evidence of changes in soil N labile pools (e.g., microbial biomass) and N availability. Understanding this phenomenon requires answering two questions; 1: Are there compensations for large amounts of N immobilization in plant and soil pools, which might otherwise lead to decreased potential soil N availability, and 2: How do plant-soil interactions allow greater soil N mineralization?

Differences in ecosystem level NUE of *J. virginiana* may contribute to high productivity of these forests, despite limited soil N availability. However, PNUE of *J. virginiana* was over an order of magnitude less than that of the dominant C_4 grass, *A. gerardii*, in the prairie (Figure 1c). *Andropogon gerardii* has greater PNUE because of greater photosynthetic rates (Figure 1a) and less N in the leaf on a 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 ($R^2 = 0.646$, $P \leq 0.01$). 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 ($R^2 \leq 0.01$).

These instantaneous measurements of nitrogen use efficiency at the leaf level demonstrate that *J. virginiana* cannot assimilate C at nearly the same rate as a dominant C_4 grass in the prairie per unit N over short time periods. So how do the forests achieve 3x greater ANPP (Norris et al. 2001a) compared to prairie species considering the relatively inefficient use of N on the leaf level?

Although PNUE was at least 10x lower in *Juniperus* forests relative to one of the dominant grass species they replace, leaf longevity, greater canopy leaf area, and most notably the ability to photosythesize year round when grasses are senescent allows greater overall annual ecosystem NUE and primary productivity (Field and Mooney 1983, Miller et al. 1987, Escudero and Mediavilla 2003). Ecosystem nitrogen use efficiency (NUE), defined as the ratio of ANPP:litterfall N, provides and index of the amount of aboveground biomass produced per unit of N lost in senesced litter (Chapin 1980, Vitousek 1982). Norris's (Norris 2000) calculation of annual NUE of 223 for *J. virginiana* and 93 for adjacent grassland, indicates that *J. virginiana* was about 2.5 times more efficient at producing biomass with each unit N in plant tissue. The large differences in NUE between *J. virginiana* and the grassland community it replaces help explain the observed greater ANPP and large plant biomass and C accumulation.

Ecosystem C and N

Large differences in the productivity of *J. virginiana* forests compared to prairie (Norris 2001a) have contributed to large accumulations of C and N in all major ecosystem pools in relatively short periods of time. Ecosystem C accrual in *J. virginana* forests was significant; with 2.2 times the amount of C, or an additional 7,870 g C m^{-2}, in measured pools of *J. virginiana* forests compared to paired grassland sites (Table 1). If estimates of C stored in root biomass of *J. virginiana* are included, total ecosystem C accrual could be much greater.

Root:shoot ratios of *J. virginiana* are probably around 25% as a general percent for trees (Cairns et al. 1997), but could be as high as 40% as seen in *Juniperus occidentalis* (Miller et al. 1990). Considering the aboveground biomass of these forests (9,462 – 15,000 g m^{-2}) there could be as much as 2,366 – 3,750 g m^{-2} of belowground biomass. Assuming a root tissue C concentration of ~50% there could be between 1,183 – 1,875 g m^{-2} of additional C not accounted for in my ecosystem estimates. These estimates of *J. virginiana* belowground C allocation are much greater than comparable estimates of prairie belowground biomass C, which range between 430 – 543 g C m^{-2} based on the same root tissue C concentration (Seastedt and Ramundo 1990).

Large ecosystem accumulation of C has also occurred in *J. occidentalis* communities, with striking similarities in overall pool sizes with *J. virginiana*. For example, total ecosystem C of a mature (108-231 yrs old) *J. occidentalis* forest was 13,622 g C m^{-2} compared to 11,605 g C m^{-2} in mature *J. virginiana* forests (Table 1) when considering mineral soil, organic soil (O-horizon), and aboveground biomass pools (Tiedemann and Klemmedson 2000). However, ecosystem C allocation differed between these species, with more C being allocated to aboveground biomass and less to mineral soil in *J. occidentalis*, while litter layer C amounts were nearly identical for both *Juniperus* species. Total ecosystem N accumulation and allocation patterns were also similar between *J. virginiana* = 433 g N m^{-2} and *J. occidentalis* =368 g N m^{-2} (a 44% and 51% increase, respectively, over adjacent grassland ecosystems), which suggests that N accrual (Tiedemann and Klemmedson 2000, Norris et al. 2001a, Norris et al. 2001b) may be a ubiquitous change in ecosystem properties with *Juniperus* encroachment into grassland communities.

One of the most important ecosystem changes has been the allocation of new C accrued in *J. virginiana* forests relative to the grasslands they replace. *Juniperus virginiana* forests shift

the bulk C allocation, formerly 96% belowground in prairie, to about 52% aboveground (Table 1). Although much N shifts to aboveground plant biomass in *J. virginiana* forests relative to prairie where >99% is stored belowground, the majority (89%) still remains in soil pools in these forests. The redistribution of C in *J. virginana* ecosystems maximizes potential C sequestration in the short-term (decades), but aboveground C stocks are vulnerable to significant losses with the inevitable return of fire.

Although *J. virginiana* forests store the bulk of newly accrued C aboveground, there are also significant increases of bulk soil organic C, as well as N. Changes in the amount and origin of SOC, if it has different chemical composition, can substantially alter soils processes, such as C and N mineralization. The accrual of total SOC is relatively uniform with depth; however, replacement of old SOC from new forest inputs is greatest at the mineral soil surface (48%) and decreased rapidly with depth (Figure 3 a&b). However, these differences in SOC from forest origin were not significantly different with depth because of high variance between sites. The relative evenness of SOC with depth and large amounts of forest SOC accrual in mineral soil, suggest that increased SOC is a result of increased C input from forest litter. Carbon accrual in *J. virginiana* forest mineral soils (Figure 2 a&b) is important in reducing potential losses during fire, thus allowing long-term sequestration. Also, increased organic matter in the soil increases potential site fertility as these large organic stocks may allow greater potential N mineralization, particularly with no significant differences in soil C:N ratios (Figure 2c).

Significant ecosystem N accumulation (~44%) has occurred in *J. virginiana* forests since initial establishment, amounting to over 132 g N m^{-2}. Accrual of ecosystem N of this magnitude in plant biomass and shallow soil pools provides strong evidence that *J. virginina* communities are able to compensate for greater productivity and subsequently greater N immobilization in

plant and soil organic matter by enhanced N acquisition or conservation. The resulting plant-soil feedback loops do not result in increased soil N limitations, but rather may slightly alleviate N constraints. Maintenance of labile N pools (e.g., microbial biomass N) (Figure 4) and greater N availability (annual net N mineralization) in shallow mineral soils of *J. virginiana* forests provide evidence that plant feedbacks maintain or even enhance soil N availability. Allocation of N by *J. virginiana* in fine roots may have provided sufficient opportunity and substrate quality to allow greater soil N availability by soil microbes (see Chapter 2).

Microbial biomass in these soils is an important short-term source and sink of plant available nutrients, and is important for conserving N in tallgrass prairie (Garcia and Rice 1994). Microbial biomass pools are sensitive indicators of changes in soils processes as a result of changes in substrate quality and/or environmental conditions (Bohlen et al. 2001). Microbial biomass C and N was not significantly different in *J. virginiana* forest soils compared to prairie (Figure 4), which suggests that both forest and prairie soils have similar sized liable C and N pools.

How does N accrual occur?

Conservation of N

In *J. virginiana* forests, the elimination of fire and concomitant reduction in ecosystem N losses by volatilization resulting from the elimination of fire probably plays a large role in the observed increased total ecosystem N storage. Depending on aboveground standing stocks, prairies lose substantial amounts of N (1-4 g N m^{-2}) when burned (Ojima et al. 1994, Blair 1997), while the elimination of fire allows conservation of N that would otherwise be volatilized in *J. virginiana* forests. The average standing stock of N in prairie at these experimental sites was about 3 g N m^{-2} during the mid-growing season, but senescent stocks were estimated (based

on tissue samples taken in November) at only 1.76 g m^{-2} that could be potentially volatilized by fire in a single year. If these forests have been present for an average of approximately 45 years, the elimination of fire may account for at least half of the N that has accumulated in *J. virginiana* forests, based on the amount that could be volatilized from prairie ecosystems every year.

The $\delta^{15}N$ values of the mineral soil may provide an integrated measure of N loss, potentially via NO_3^- leaching, ammonia volatilization, or dentrification (Högberg and Johannison 1993, Vitousek 2004). The difference in $\delta^{15}N$ of the bulk soils of *J. virginiana* and prairie soils was not statistically significant, but there was a trend for ^{15}N enrichment at shallower depths in prairie soil (Figure 5). The $\delta^{15}N$ decreased significantly with depth in both prairie and *J. virginiana* soils (one-way ANOVA, $F = 6.86$, $P \leq 0.01$), which is consistent with trends in most soils and is caused by isotopic fractions of the SOM (Nadelhoffer and Fry 1988). The modest trend for N isotopic enrichment in shallow prairie soil might suggest greater N losses in prairie soil near the soil surface. However, there are several alternative hypotheses for this small enrichment in prairie soils, such as differences in where grasses and trees access N in the soil profile, or differences in plant preference for NH_4^+ and NO_3^- (Högberg 1997). Nitrogen losses through ammonia volatilization and denitrification in prairie are relatively small (Sotomayor and Rice 1996, Blair 1997) and are likely similar in *J. virginiana* forests because of small ammonium and nitrate pools. Overall, the lack of substantial differences in $\delta^{15}N$ values in the soils of both of these ecosystems indicate the absence of large differences in N losses in the top 10 cm of soil, which is consistent with findings in Chapters 2 and 3.

Use of other sources of N

Estimates of annual plant N uptake in *J. virginiana* forests, derived from Norris's (2000) data, are about 3.5x greater than in prairie ecosystems. However, annual cumulative estimates of net N mineralization in *J. virginiana* soils (1.4 g N m^{-2} y^{-1}, A-horizon), an index of the annual rate of supply to plants, falls short of *J. virginiana's* requirement for N (5.85 g N m^{-2} y^{-1}) (N uptake was calculated by summing the mean annual N gain in bolewood (1.74 g N m^{-2} y^{-1}) and N loss in litterfall N (4.11 g N m^{-2} y^{-1}), from Norris 2000). Conversely, annual cumulative soil N mineralization in prairie (1.69 g N m^{-2} y^{-1}) does appear adequate to satisfy annual plant N requirements of prairie vegetation (1.66 g N m^{-2} y^{-1}) (N uptake was calculated from estimates of senesced standing stock N, from Norris 2000). The apparent inability of soil N mineralization in the top 10 cm (A-horizon) to meet plant requirements suggests acquisition of N from other N pools or inputs not explicitly quantified in this study.

Ecosystem N accrual via exploitation of other N sources may occur by differences in the ability of *J. virginiana* to scavenge atmospheric N deposition, or translocation of N from other locations within the ecosystem. *Juniperus virginiana* plant architecture and year-round photosynthetic capacity may allow for greater atmospheric N uptake from wet and dry deposition. Average annual inorganic N deposition through bulk precipitation has reached a recent high of ~1 g N m^{-2} yr^{-1} (Blair et al. 1998), and additional dry deposition are likely, but not quantified. The differences in the amount of N that enters either the forest or prairie ecosystem after accounting for uptake efficiencies from wet and dry atmospheric deposition is unknown. Nitrogen translocation into *J. virginiana* forests from adjacent grasslands (lateral foraging hypothesis) is not likely to be of quantitative importance. Translocation of N from deeper soils horizons is also possible, but has not been investigated for these forests. Ecosystem N accumulation following the encroachment of other woody species has been observed,

particularly due to N_2-fixation by leguminous shrubs, but *J. virginiana* is not an N-fixer and substantially suppresses understory growth, including legumes that could co-occur. Other sources of N input into *Juniperus* forests, such as animal inputs or N fixation from lichens (Foreman and Dowden 1977) are possible, but probably do to not contribute enough to account for the size and rapid accumulation ecosystem N pools.

Conclusions

Two keys for understanding lack of the hypothesized decrease in soil N availability due to plant-soil feedback loops in *J. virginiana* forests are; 1: increases in plant and soil N pools, which provide evidence that forests are able to accrue additional N by conservation of N and/or acquisition of N from other sources, and 2: increased N availability in forests allows some production of relatively high quality substrates (i.e., fine roots) despite most other plant tissues being poor quality, which may contribute to increased soil N availability. Significant accrual of N in *J. virginiana* ecosystems may have resulted from conservation of N from the elimination of fire and potential exploitation of N not available to most prairie species. Ecosystem N accrual allows *J. virginiana* to allocate and maintain relatively high concentrations of N in fine root biomass, which in turn allows production of root tissue with relatively high substrate quality (as indexed by C:N ratios) compared to prairie species (Chapter 2).

Accrual of ecosystem N in *J. virginiana* forests, as well as greater soil N availability, similar soil microbial biomass N (labile pools), and high productivity of *J. virginiana* forests provides evidence of avoidance of more severe N constraints that were expected relative to native prairie. *Juniperus virginiana* may be less depended on shallow soil N availability than prairie vegetation, because of its potential ability to obtain N from other sources. Greater conservation and exploitation of N in the absence of fire have allowed an almost 8,000 g C m^{-2}

increase in total measured ecosystem C stocks in *J. virginiana* forests compared to prairie (Table 1), which has significant implications for regional C sequestration potential when considering the scale of *J. virginiana* encroachment in the Central Plains. These finding suggest that protracted fire return intervals in grassland and savanna ecosystems are not only the primary reason for *J. virginiana* encroachment (Briggs et al. 2002, Briggs et al. 2005), but rapid growth after initial establishment may be explained by conservation of N in these forests in the absence of fire, which avoids a plant-soil feedback loop that could limit potential productivity.

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Figures and tables

Figure 4-1 Net photosynthetic rates (A_{max}) (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. Net photosynthetic rates tended to be higher in *A. gerardii* than *J. virginiana* at most times in the growing season (a). However, *J. virginiana* was able to photosynthesize year round when *A. gerardii* could only photosynthesize May-October. Leaf N content (b) varied dramatically in *A. gerardii* during the growing season, which started at a high in the beginning of the growing season and steadily declined until the end. Leaf N content exhibited little difference over the year in *J. virginiana*. Photosynthetic nitrogen use efficiency (c) was always at least an order of magnitude greater in *A. gerardii* compared to *J. virginiana*. Means with asterisks (*) indicate significant differences ($P<0.05$) between *J. virginiana* and *A. gerardii* for that specific time interval.

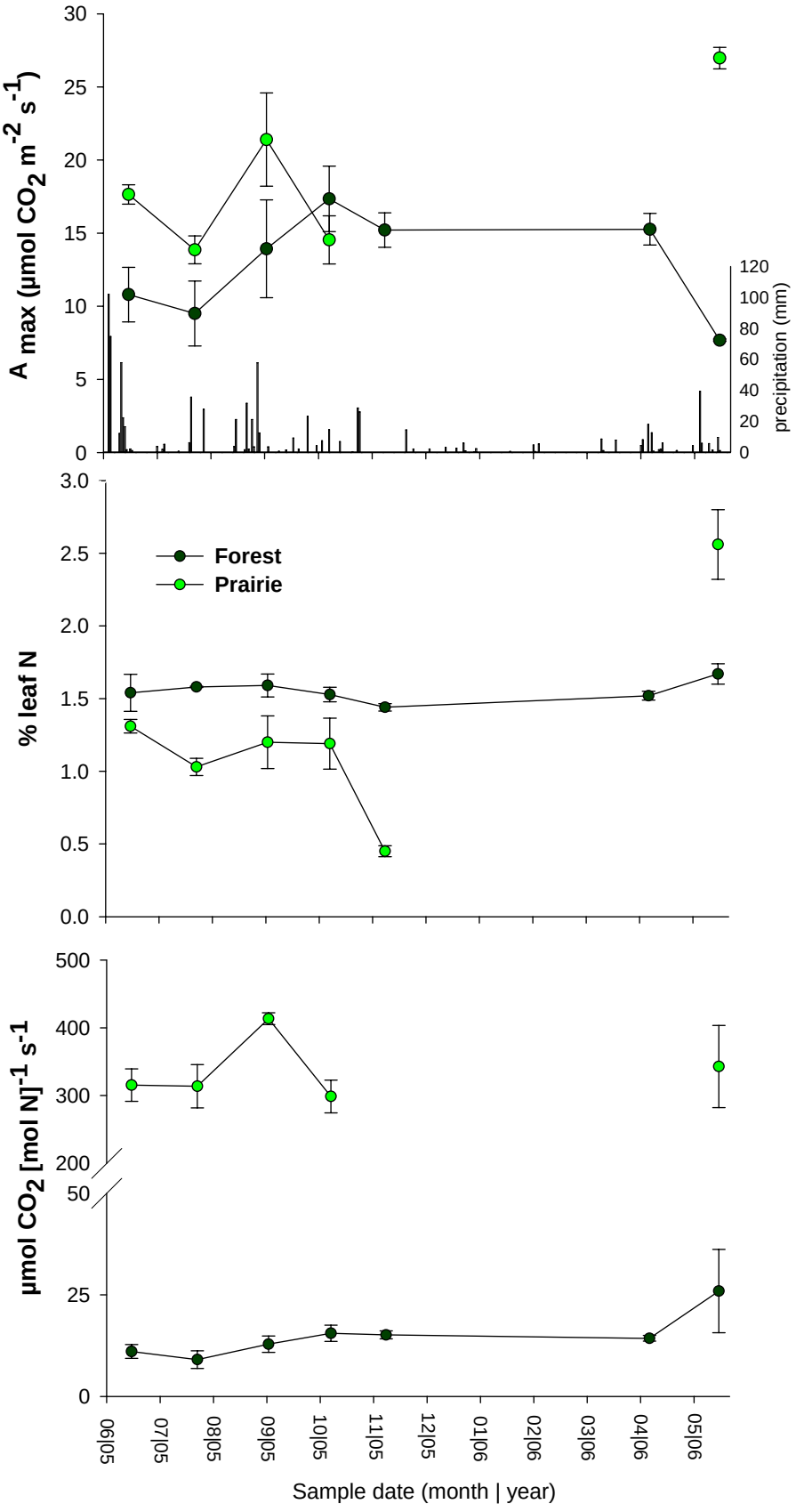
A max (µmol CO2 m-2 s-1)
precipitation (mm)
Forest
Prairie
% leaf N
µmol CO2 [mol N]-1 s-1
Sample date (month | year)
06|05
07|05
08|05
09|05
10|05
11|05
12|05
01|06
02|06
03|06
04|06
05|06

Figure 4-2 a-c Mean ± se for total soil organic carbon (SOC) (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 prairie soil. Total SOC was significantly greater in forest soils compared to prairie soils (One-way ANOVA, $F = 7.42$, $P = 0.01$). Total SON was also significantly greater in forest soils compared to prairie soils (One-way ANOVA, $F = 8.27$, $P = 0.01$). The changes C:N ratios in forest soils compared to prairies soils were small and not significantly different (One-way ANOVA, $F = 1.87$, $P = 0.18$).

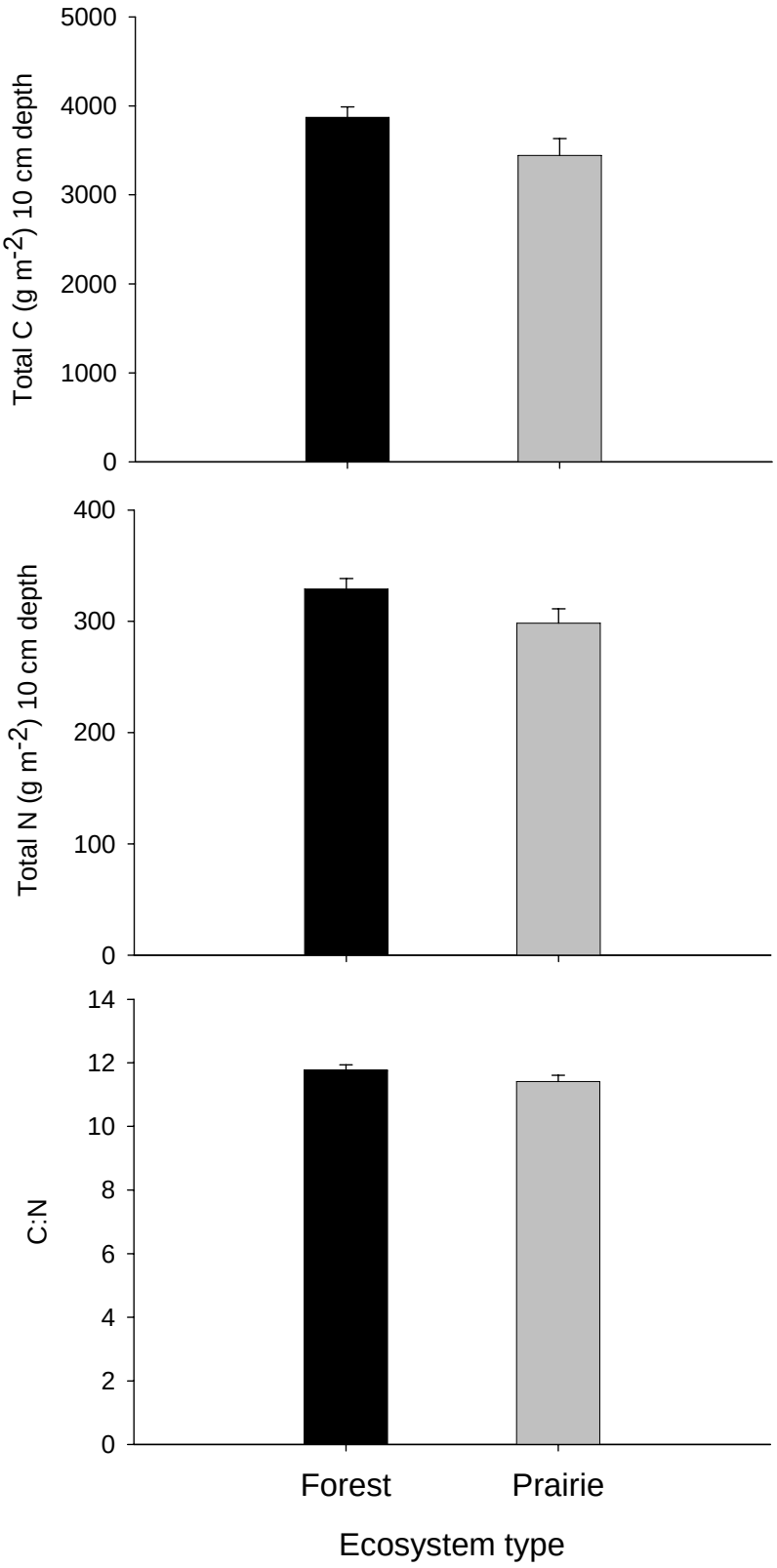
5000
4000
3000
2000
1000
0
Total C (g m-2) 10 cm depth
400
300
200
100
0
Total N (g m-2) 10 cm depth
14
12
10
8
6
4
2
0
C:N
Forest
Prairie
Ecosystem type

Figure 4-3 Total SOC (mean ± se) by depth in forest and prairie soils (a), and the percent replacement of SOC from forest origin in forest soil by depth (b). Total SOC carbon in forest soil was greater than in prairie soils at each depth (a), but there were no significant differences in SOC in any specific depth ($P \geq 0.05$). The greatest percentage (48%) of SOC from forest origin in forest soils was at the 0-2cm-depth increment, which decreased predictably (linearly, $R^2 = 0.97$, $y = 49.33 + 3.6245x$) with depth to the lowest percentage from forest origin of 17% at the 8-10 depth increment. Total SOC replacement in forest soils was 34% to a depth of 10 cm.

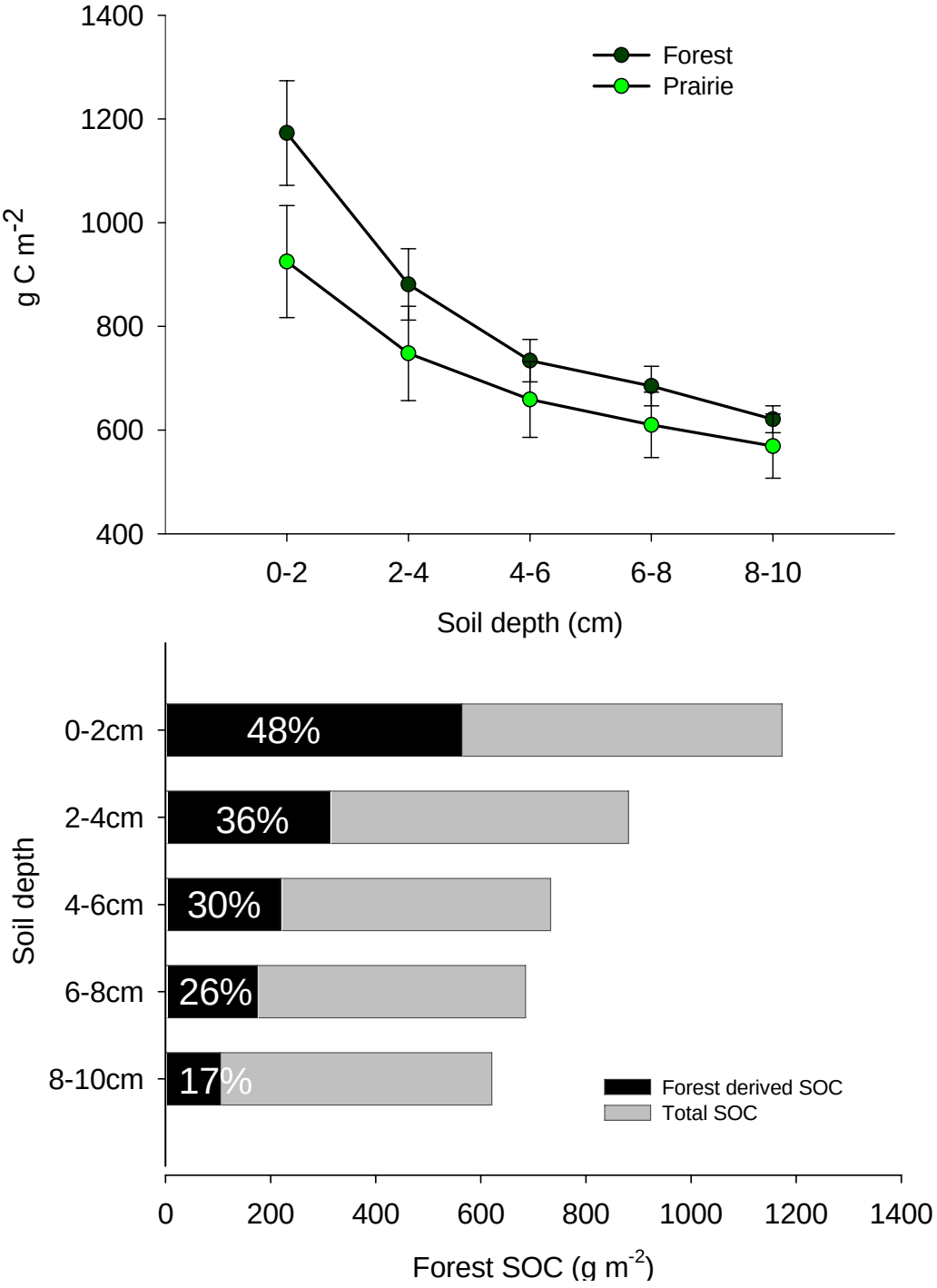
1400
1200
1000
800
600
400
g C m-2
Forest
Prairie
0-2
2-4
4-6
6-8
8-10
Soil depth (cm)
0-2cm
2-4cm
4-6cm
6-8cm
8-10cm
Soil depth
48%
36%
30%
26%
17%
Forest derived SOC
Total SOC
0
200
400
600
800
1000
1200
1400
Forest SOC (g m-2)

Figure 4-4 Microbial biomass C and N (mean ± se) expressed as g m^{-2} for *J. virginiana* forest and prairie soils collected in the growing season (July) and non-growing season (October). There were no significant differences in microbial biomass C and N between the forest and prairie soils in either season ($P \geq 0.05$). Note: a scale break between 50-100 on the y-axis.

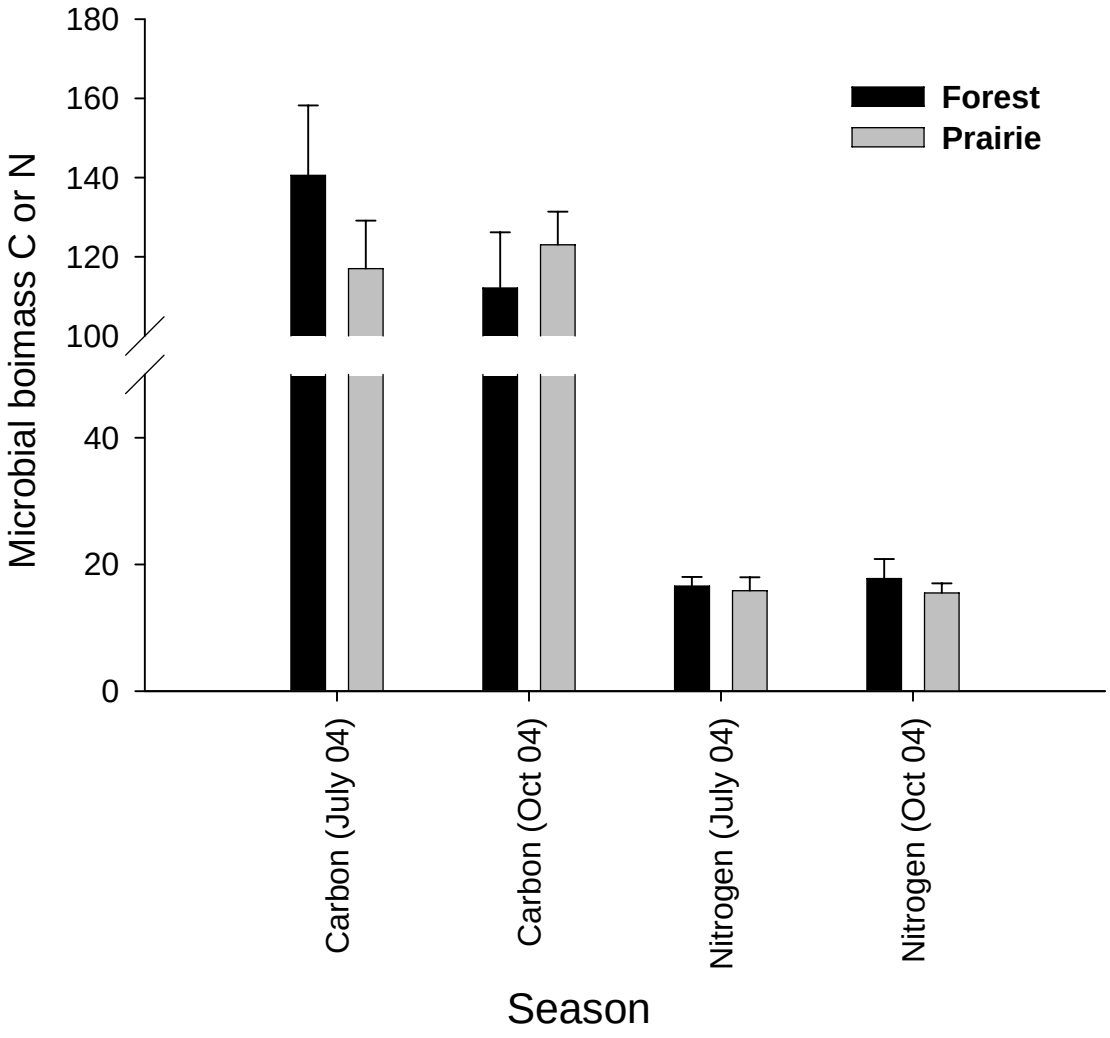

Figure 4-5 The $\delta^{15}N$ in the bulk mineral soil (mean ± se) by 2 cm depth increments in *J. virginiana* and prairie soils. There was no significant interaction between the two ecosystems with depth (Two-way ANOVA, $F = 0.25$, $P = 0.91$). However, there was a significant difference in the $\delta^{15}N$ values in both these ecosystem with depth (One-way ANOVA, $F = 6.86$, $P < 0.01$). There was no significant difference in the $\delta^{15}N$ values between *J. virginiana* forest and prairie ecosystems (One-way ANOVA, $F = 0.99$, $P = 0.33$).

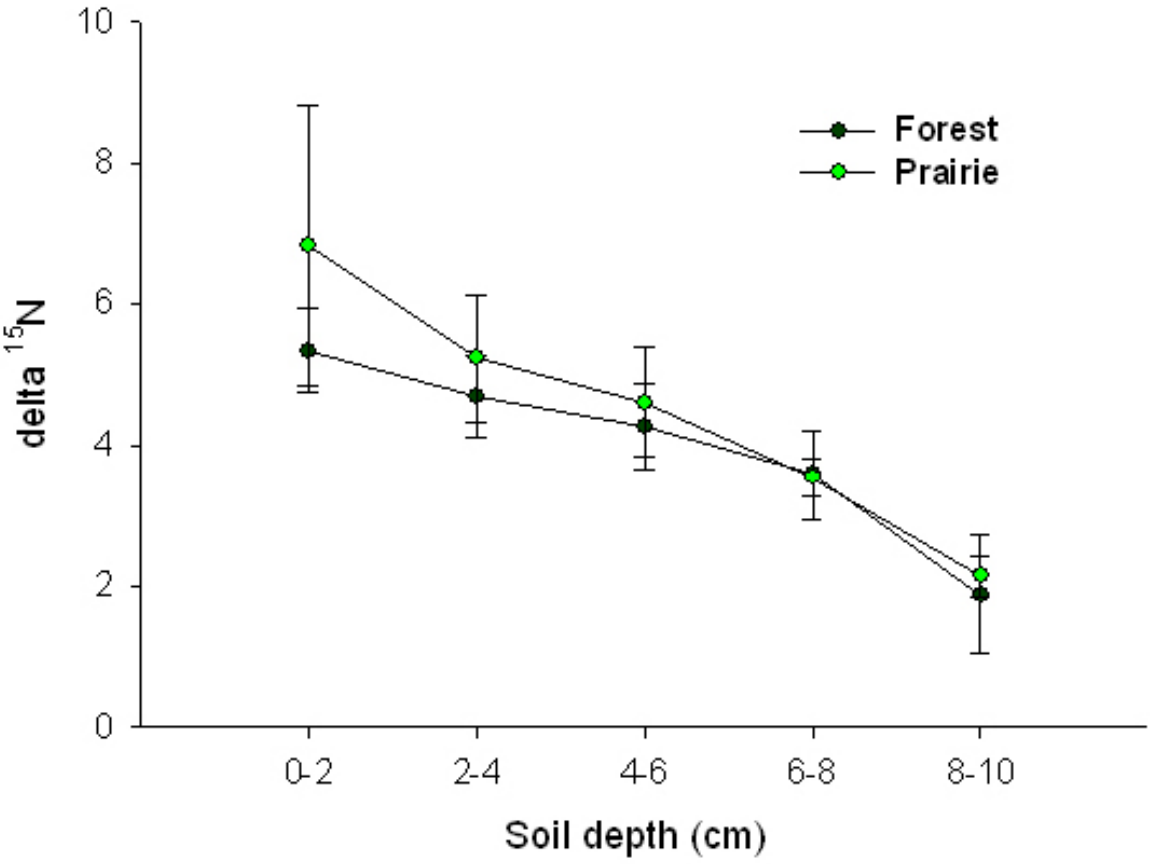

Table 4-1 Carbon and nitrogen stocks in major pools in *J. virginiana* forest and prairie ecosystems. Total ecosystem N in measured pools were about 44% greater in forest ecosystems compared to prairie with most of the increases occurring in the soil organic horizon and aboveground plant biomass. Carbon stocks have increased about 218% in forest pools compared to prairie, with the vast majority of new accumulation in aboveground plant stocks.

Ecosystem pool	**Nitrogen** ($g\ m^{-2}$)	**Carbon** ($g\ m^{-2}$)
Forest		
Aboveground biomass	48 ± 7	6,065 ± 743
Soil		
O-horizon	56 ± 4	1,540 ± 51
A-horizon (10 cm)	329 ± 9	3,871 ± 119
Microbial biomass	16 ± 1	129 ± 11
Extractable N	0.1-0.6	
Total	**433**	**11,476**
Prairie		
Aboveground biomass	2.95 ± 0.64	163 ± 35
Soil		
O-horizon	NA	NA
A-horizon (10 cm)	298 ± 13	3,443 ± 188
Microbial biomass	17 ± 2	118 ± 8
Extractable N	0.1-0.6	
Total	**301**	**3,606**

CHAPTER 5 - SYNTHESIS: Altered ecosystem processes as a consequence of *Juniperus virginiana* L. encroachment into North American tallgrass prairie[1]

[1] ***In*** **Ecology and Management of Western North American *Juniperus* Communities: A Dynamic Vegetation Type, ed. O.W. Van Auken, Springer, Ecological Studies (In Press).**

Abstract

Juniperus virginiana, an important woody plant invader in the U.S. Central Plains, has increased dramatically in density and cover in large areas previously dominated by highly diverse prairie communities. This change in plant cover has the potential to significantly alter key ecosystem properties and processes. Here we summarize results from a variety of studies, mostly conducted in the Central Plains, which have assessed potential changes in ecosystem C and N pools and fluxes as *J. virginiana* expands into native grasslands. Differences in plant growth form, biomass and phenology of *J. virginiana* forests, relative to grasslands, alter the soil microclimate as well as litter quality and quantity, which influence soil microbial activity and key soil processes. Changes induced by the shift from grasslands to *J. virginiana* forests include increased aboveground net primary productivity and litterfall, increased organic carbon (C) accrual in surface litter and soil, reductions in soil respiration, and replacement of C_4 grass-derived soil C with new C from *J. virginiana* trees. These aggrading forests also exhibit significant total ecosystem N accumulation, and a trend for increased soil N availability compared to grasslands. Although leaf-level instantaneous photosynthetic nitrogen use efficiency (NUE) was over a magnitude higher in the dominant grass, *Andropogon gerardii*, ecosystem-level NUE (the ratio of ANPP:litterfall N) was about 2.5 times greater in *J. virginiana* forests. This high ecosystem-level NUE and greater soil N availability may contribute to the

rapid accrual of C in newly established *J. virginiana* forests. Although *J. virginiana* forests may provide strong-regional carbon sinks, these sinks are vulnerable to significant losses through volatilization in fire, as well as losses through soil erosion caused by reduced herbaceous cover in these forests.

Introduction

Expanding cover and abundance of woody plants in grasslands and savannas (afforestation) is a worldwide phenomenon with the potential to alter ecosystem structure and function in a variety of important ways. Increases in woody plant cover, or conversion of grasslands to woodlands, may alter ecosystem processes such as nutrient cycling and availability, which influence primary productivity, resource competition, species richness and composition, as well as the interactions between plants, animals, and microorganisms (Vitousek 2004). Nutrient cycling dynamics and the long-term stability of carbon (C) and nitrogen (N) pools may also change as a result of a shift in the allocation of plant biomass and ecosystem C and N stocks from largely belowground in grasslands to aboveground in woodlands. These ecosystem changes may, in turn, alter regional terrestrial and atmospheric biogeochemistry if newly established woodlands act as a sink for C and N (Moiser 2001).

An important form of woody plant expansion in grasslands of the U.S. Central Plains is an increase in the cover and abundance of *Juniperus virginiana*, or eastern redcedar. *Juniperus virginiana* L. (hereafter redcedar) is the most widely distributed *Juniperus* species in the continental United States, occurring in every state east of the 100th meridian (Fowells 1965). In the eastern Great Plains and other areas, redcedar has encroached into adjacent grasslands at an unprecedented rate, affecting approximately seven million hectares in western portions of its range (Schmidt and Leatherberry 1995; Briggs, Hoch and Johnson 2002). Redcedar, like most

other *Juniperus* species in North America, forms both dispersed community associations, or often, very dense (130-3,500 trees ha^{-1}), nearly monospecific stands (Norris et al. 2001; Briggs, Hoch and Johnson 2002; Rhoades, Miller and Shae 2004; McKinley 2006). Redcedar is typically found in more mesic areas than most western *Juniperus* species and in a very broad range of soils across the eastern United States.

Juniperus expansion in North America began in the late 1800's as European settlement and associated land management practices and other anthropogenic activity became more extensive (Smeins 1983). Historically, due to their sensitivity to fire, *Juniperus* trees mainly grew in areas that were sheltered from intense fire, such as rocky outcrops or areas with shallow soils. Fire suppression and reduced fuel loads due to grazing by domestic ungulates have been widely implicated in promoting the rapid expansion of these native trees beyond their historical distribution (Van Auken 2000; Briggs, Hoch and Johnson 2002). The ecosystem consequences of conversion from grassland to woodland are often significant (Archer, Scifres and Bassham 1988; Archer 1990; Belsky 1994; Hester 1996; Van Auken 2000; Norris et al. 2001; Jackson et al. 2002; Smith and Johnson 2003, 2004; Briggs et al. 2005), and the encroachment of *Juniperus* into areas of native grassland might be expected to cause substantial changes in key ecosystem properties and processes, given the change in dominant plant life form from C_4 grasses to a C_3 coniferous tree species. Here we summarize results from a variety of studies, mostly conducted in the Central Plains, which have assessed potential changes in ecosystem C and N pools and fluxes as redcedar expands into native grasslands.

Site description

The majority of studies reviewed here utilized native tallgrass prairie sites paired with adjacent redcedar stands developed in areas that until recently were grassland. These studies (Norris et al. 2001; Norris, Blair and Johnson 2001; Briggs, Hoch and Johnson 2002; Smith and Johnson 2003; 2004; McKinley 2006) were conducted in the Flint Hills region of northeastern Kansas in close proximity (<25 km) to the Konza Prairie Biological Station (KPBS) (39º05'N, 96º35'W). Proximity to the KPBS, the primary location of the Konza Prairie Long-Term Ecological Research (LTER) program, allows the use of a variety of baseline data on ecological processes in native tallgrass prairie. Average monthly temperature ranges from a January low of -2.7ºC to a July high of 26.6ºC (NOAA 2004). Average annual total precipitation is 835 mm with 75% falling during the growing season (Bark 1987). Soils in this region are highly variable, but generally consist of cherty, silty clay loams or silt loams overlaying limestone bedrock. These soils commonly have low inorganic N and available P, but are relatively high in organic matter. Topographic relief divides the landscape into upland plateaus with mostly shallow soils, slopes with outcrops of limestone, and lowlands with deeper alluvial and colluvial soils (Oviatt 1998). Redcedar stands are especially prominent in relatively shallow soil upland sites, and those sites were used in the studies reported here.

The dominant native vegetation in the northern Flint Hills is tallgrass prairie, dominated by a matrix of perennial, warm-season C_4 grasses including big bluestem (*Andropogon gerardii* Vit.), little bluestem (*Schizachyrium scoparium* Michx.), indiangrass (*Sorghastrum nutans* Nash) (Anderson, Brumbaugh and Jackson 2001) and switchgrass (*Panicum virgatum* L.) (Kuchler 1967; Freeman and Hulbert 1985). These C_4 grasses contribute the majority of annual net primary productivity (ANPP) (Knapp et al. 1998). However, a highly diverse mixture of less abundant species, including C_3 grasses and sedges and a diverse array of forbs, contribute to the

high floristic diversity of these grasslands (Freeman and Hulbert 1985). The native tallgrass prairie flora also includes a smaller number of native woody plants, such as buckbrush (*Symphoricarpos orbiculatus* Moench.), New Jersey tea (*Caenothus herbaceous* Raf.), smooth sumac (*Rhus glabra* L.) and rough-leaved dogwood (*Cornus drummondii* CA May), which can be locally abundant, especially in prairie that is infrequently burned (Briggs et al. 2005).

Assessing the effects of grassland conversion to redcedar forests was done using multiple paired sites consisting of native tallgrass prairie that was burned frequently (1-3 year fire return intervals) and either recently grazed by cattle (Norris et al. 2001; Norris, Blair and Johnson 2001; Smith and Johnson 2003; 2004) or not grazed it the recent past (McKinley 2006). Each tallgrass prairie site was paired with an adjacent redcedar stand that had developed on an area that was historically grassland, and that shared similar soil type, slope, position, and aspect. Historical aerial photographs and analysis of soil organic carbon (SOC) isotopic composition confirmed that these stands of redcedar, which utilize a C_3 photosynthetic pathway (creating more depleted $\delta^{13}C$ organic carbon), were recently established on areas historically dominated by C_4 grasslands (with accumulated SOC relatively more enriched in ^{13}C) (Smith and Johnson 2003; McKinley 2006). Each redcedar stand was at least 0.5 ha^{-1} and consisted of relatively mature trees (~30-80 yrs) creating dense (680-1,900 trees ha^{-1}) stands with complete or nearly complete canopy cover.

Potential drivers of altered ecosystem processes

Microclimate

Juniperus trees modify the microclimate beneath their canopies relative to grasslands (Breashears et al. 1997; Breashears et al. 1998; Smith and Johnson 2004). Soil temperatures were consistently higher, sometimes by as much as 8ºC, in grasslands than in comparable redcedar sites (Figure 1) (McKinley 2006), which may contribute to changes in soil processes.

For example, Smith and Johnson (2004) found a 38% reduction in soil respiration in redcedar soils compared to adjacent grassland sites, and concluded that soil temperatures, rather than soil moisture, explained most of the variability in soil respiration. An estimated Q_{10} value for soil respiration, which represents the sensitivity of soil respiration (a measure of microbial activity) to temperature, was slightly less in redcedar soils (2.2) compared to grassland soils (2.4) (Smith and Johnson 2004). Differences in soil moisture in redcedar stands and grasslands may result from differences in soil temperatures, as well as canopy interception and evapotranspiration. However, periodic measurements indicated that soil water content tended to only be slightly greater in grassland soils on a mean seasonal basis (Figure 2), and soil water content explained much less of the measured variance in soil respiration than did temperature (Smith and Johnson 2004). Therefore, differences in soil temperatures, and the microbial response to temperatures, appear to be a major driver in the alteration of some key ecosystem processes, such as soil CO_2 flux, following redcedar encroachment into grasslands.

Ecosystem productivity and biomass accumulation

Species with inherently fast growth rates like redcedar can exhaust soil nutrients by sequestering essential nutrients in plant biomass, and create feedback loops that exacerbate soil nutrient limitations (Chapin1980; Vitousek 1982; Vitousek 2004). Aboveground biomass in redcedar stands in two different studies in the northern Flint Hills ranged from 114,120 - 210,952 kg ha^{-1} in sites that were 35-80 years old (Norris et al. 2001) and 94,620 – 150,001 kg ha^{-1} in sites that were 35-55 years old (McKinley 2006). These aboveground biomass accumulations were much greater than peak biomass of grasslands in similar topographic positions (20 year mean peak from KPBS = 3,690 kg ha^{-1}, range = 1,780-5,700 kg ha^{-1} see Knapp et al. 1998), and occurred over a relatively short period of time. Greater ANPP in redcedar stands (7,250 - 10,440

kg ha^{-1} yr^{-1}) compared to annually burned upland grasslands (3,690 kg ha^{-1} yr^{-1}) (Norris et al. 2001), coupled with the elimination of fire in redcedar communities has allowed for this rapid accumulation of biomass. Differences in productivity of these communities, and presumably changes in litter quality or quantity, may alter nutrient cycles and soil nutrient availability, affecting further community changes.

Litter inputs

Litter chemistry influences decomposition rates, and consequently C and N mineralization, and N availability, which may be especially important in N limited soils such as those characteristic of tallgrass prairie (Blair et al. 1998). The quantity and quality of plant litter inputs also control the accumulation and storage of C and N as soil organic matter (SOM). Thus, changes in the quantity, quality and location (above- vs. belowground) of plant litter inputs as redcedar encroaches into grasslands may be an important driver of altered ecosystem processes such as C mineralization and N cycling, and these processes can, in turn, influence higher plant growth and subsequent nutrient feedback loops.

Foliar litter inputs, and root inputs from either exudates or root turnover, are the main sources of soil C and N (McClaugherty, Aber and Melillo 1982). Carbon to nitrogen ratios, % lignin, lignin to N ratios, and other indices of litter quality have been shown to strongly influence decomposition and the release of N from decomposing litter. Although the majority of redcedar biomass (bolewood) is of low quality (i.e., C:N >250:1), greater allocation of biomass N to foliage and roots may make these tissues, and the fine litter produced from them, relatively high quality (low C:N) (Norris, Blair and Johnson 2001). For example, foliage of both redcedar and mixed-bulk grassland vegetation has relatively low C:N ratios in mid-growing season (July), 37:1 and 56:1 respectively (McKinley 2006). However, both redcedar and *A. gerardii* resorb

significant amounts of leaf N prior to senescence, resulting in higher C:N ratios in foliar litter of both redcedar (~52:1) and *A. gerardii* (~70:1) (Norris, Blair and Johnson 2001). Reports of C:N ratios of redcedar and *A. gerardii* fine roots (≤2 mm diameter) vary, with Norris, Blair and Johnson (2001) reporting values of ~101:1 for redcedar roots (1-2 mm diameter) and ~70:1 for *A. gerardii* roots, while McKinley (2006) found that the C:N ratios of fine (<2 mm diameter) redcedar roots were significantly lower (~70:1) compared to mixed species roots excavated from an adjacent annually-burned, ungrazed prairie (~90:1). Although redcedar may provide comparable or better quality fine litter inputs as indexed by C:N ratios, lignin content, which can also strongly influence decomposition, was three times greater in litter of redcedar foliage and twice that in root biomass compared to the foliar litter and roots of the dominant grasses (Norris, Blair and Johnson 2001).

Norris, Blair and Johnson (2001) found that litterfall in redcedar stands average about 500 g m^{-2} $year^{-1}$, which was an order of magnitude greater than litterfall (52 g m^{-2} $year^{-1}$) in annually burned grasslands (Seastedt 1988). Redcedar litterfall contributed approximately 4 g N m^{-2} $year^{-1}$ to the O-horizon, and a total litter N accumulation of 25 - 56 g N m^{-2} in redcedar stands (Norris, Blair and Johnson 2001; McKinley 2006). However, the net release of N from decomposing redcedar litter is slow. In a two-year litter decomposition study, Norris, Blair and Johnson (2001) detected no net release of N. In another study, field incubations of soil cores with and without the presence of an intact O-horizon indicated no detectable contributions of the O-horizon to inorganic N production during the 30-day incubations (McKinley 2006). Despite potentially large surface litter inputs and accumulations of organic N, surface litter decomposition appears to contribute little to inorganic N in the mineral soil in short-term assays. The eventual release of inorganic N from surface redcedar litter may require long periods of time

due to differences in litter chemistry relative to grassland species (Murphy, Klopatek and Klopatek 1998; Norris, Blair and Johnson 2001). However, foliar litter inputs may contribute to the size of the SOM pool in the surface mineral soil, as evidenced by replacement of grass-derived soil organic carbon (SOC) with redcedar-derived SOC in the shallow mineral soil horizons of redcedar stands (Smith and Johnson 2003, McKinley 2006).

The turnover of redcedar root biomass may also provide significant quantities of organic matter to the mineral soil, but this has not been quantified and we know of no comparative studies of fine root productivity or turnover in grassland and redcedar sites. However, there are comparative studies that address fine root biomass in redcedar stands and grasslands and the decomposition dynamics of redcedar fine root litter. Redcedar root biomass including both fine/small roots (<2 mm diameter) and coarse roots (≥2 mm diameter) in excavated soil monoliths (25x25x10 cm) was more than double than root biomass found in adjacent grasslands (McKinley 2006). Root biomass may become concentrated in upper soil horizons in shallow soils where these redcedar communities typically develop, and this may allow greater concentrations of root inputs per unit soil volume, ultimately altering soil processes. In addition to large accumulations of redcedar roots, there were greater concentrations of N in redcedar fine roots (0.74%) measured in the late growing season, compared to roots of mixed species in adjacent grasslands (0.51%) (McKinley 2006). In contrast, Norris, Blair and Johnson (2001) reported that redcedar roots had lower concentrations of N than roots of *A. gerardii*. The apparent discrepancy between these studies may reflect seasonal and site-specific differences in root tissue N, as well as differences in comparing roots of a single grass species (*A. gerardii*, see Norris, Blair and Johnson 2001) with roots of a mixture of grassland species (McKinley 2006), or the inclusion of smaller redcedar roots (≤1 mm diameter) in tissue analyzed by McKinley.

Norris, Blair and Johnson (2001) also found that redcedar root decay rates were 35% less than *A. gerardii* roots, suggesting the potential for root litter to contribute to greater soil organic matter accumulations in redcedar stands. The contribution of root inputs in *Juniperus* stands has been largely overlooked, but given the large amount of root biomass and differences in root chemistry of redcedar and the dominant grasses they replace, belowground litter inputs may be very important in influencing soil processes, such as N cycling, in newly established redcedar stands.

Altered ecosystem processes

Carbon storage and flux

Changes in carbon allocation patterns following redcedar encroachment into grasslands are so profound that the bulk of the ecosystem C storage shifts from belowground in grasslands (~96%) to aboveground (~52%) in redcedar stands (Norris et al. 2001; McKinley 2006). The top 10 cm of mineral soils in redcedar stands has 12% greater soil organic carbon (SOC) per m^{-2} compared to adjacent grassland soils (McKinley 2006). Increased total SOC pools have been observed in other comparisons of soils under the canopies of *Juniperus* relative to adjacent grasslands or intercanopy patches (Bates, Svejcar and Miller 2002; Smith and Johnson 2003; Miller et al. 2005). Increased organic C storage in the soil and potential changes in SOM composition may be especially important factors affecting nutrient availability in redcedar stands.

Smith and Johnson (2003) took advantage of the differences in photosynthetic pathways of redcedar, a C_3 plant, and the C_4 grasses that historically dominated these grasslands, and utilized a stable isotope technique to determine the proportion of SOC in new redcedar stands that was derived from recent forest litter inputs. They found that a significant portion (~20%) of the SOC in the top 25 cm of the mineral soil originated from forest inputs, with the greatest

replacement (~40%) in the shallow mineral soil horizons (0-2.5 cm) (Figure 3a). Smith and Johnson (2003) also found that the proportion of SOC of redcedar origin decreased rapidly and predictably with depth to less than 11% below 10 cm (Figure 3b). Analysis of $\delta^{13}C$-CO_2 produced from soil respiration in laboratory incubations of the top 10 cm of mineral soil from redcedar stands revealed consistently more depleted (negative) $\delta^{13}C$ values than those of the corresponding bulk SOC (Smith 2001). These values when used in a mixing model indicated that ~65% of soil C respired was of forest origin, which indicated greater microbial utilization of new forest C, suggesting that this pool was more labile than the total soil C pool. These results indicate the important role of new forest organic inputs in soil processes such as CO_2 flux via microbial respiration.

Laboratory soil incubations performed under optimal temperature and moisture conditions can reveal differences in substrate quality or quantity, but ignore differences in potential abiotic drivers that may be important under field conditions. Laboratory soil incubations by McKinley (2006) corroborated the finding of reduced soil respiration (38%) in soils of redcedar stands by Smith and Johnson (2004), and indicated slight but non significant reductions (~5-13%) in mineralizable C in redcedar stands compared to adjacent grasslands. In contrast, mineralizable N was 2-3x greater in soils of redcedar stands compared to adjacent grassland soils (McKinley 2006). As a result C mineralization to N mineralization (Cmin:Nmin) ratios were significantly lower in redcedar soils. Lower Cmin:Nmin ratios, interpreted as an index of substrate quality, suggest that the organic matter pools of redcedar soils have a higher substrate quality compared to adjacent grasslands (McKinley 2006).

Nitrogen accumulation and availability

Frequent fires are common in highly productive grasslands such as tallgrass prairie, and volatilization of N during fire is the major avenue of N loss from ungrazed tallgrass prairie (Blair et al. 1998). In contrast, redcedar stands only develop in the absence of fire (Briggs, Hoch and Johnson 2002), and this has a significant affect on ecosystem-level N loss and retention. Both Norris et al. (2001) and McKinley (2006) reported substantially greater accumulation of total ecosystem N in redcedar stands compared to adjacent grasslands. While biomass nitrogen allocation has largely shifted to aboveground in redcedar stands, at least 85% of total ecosystem N storage remains belowground as a result of large litter and SOM pools (Smith and Johnson 2003; McKinley 2006). Especially important are changes in soil organic N, found to be 21% greater in redcedar soils than in comparable grasslands, which may contribute to greater N availability (McKinley 2006). Reduced ecosystem N losses in redcedar stands due to the elimination of N volatilization during fire probably plays a major role in observed increases (~44%) in ecosystem N storage (including aboveground biomass, organic and mineral soil to 10 cm) in redcedar stands relative to the grasslands they replaced (McKinley 2006). Depending on the amount of accumulated plant litter, grasslands lose substantial amounts of N (1-4 g N m^{-2} yr^{-1}) when burned (Blair 1997). In contrast, the absence of fire in redcedar stands allows a substantial accumulation of both C and N in aboveground biomass and surface litter (Norris et al. 2001; Norris, Blair and Johnson 2001; McKinley 2006).

Elimination of fire may be a significant contributor to N accumulation in redcedar stands, but altered N inputs may also be important. The complex redcedar plant architecture and year-round photosynthetic capacity may allow greater potential uptake of atmospheric N from wet and dry deposition. Large increases in ecosystem N in the middle as well as the edges of contiguous redcedar stands, suggest that N translocation from adjacent grasslands is not substantial

(McKinley 2006). In addition, analysis of $\delta^{15}N$ of redcedar tree rings taken from the heartwood (center rings) to the outer rings of the bolewood of the oldest trees, which provided tissues of different ages, indicated that there are no significant changes in the isotopic composition (-1 $^{0}/_{00}$ shift over at least six decades) of N in the bulk wood (McKinley unpublished data). Presumably, major shifts in the source of N that could become available to plants in these communities would be reflected by changes in the isotopic composition of bulk wood tissues, and since there are no significant differences it is likely that N accumulation in these redcedar ecosystems are due to N conservation rather than from large pulses of exogenous N inputs (Poulson, Chamberlain and Friedland 1995). Other exogenous sources of N input into *Juniperus* stands, such as animal inputs or N fixation from lichens (Foreman and Dowden 1977) are possible, but probably do not contribute enough to account for the size and the rapid accumulation of current ecosystem N pools.

Concentrations of KCl-extractable inorganic N in soils of redcedar stands and grasslands are small; measured concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) combined usually did not exceed 6 μg N g^{-1} soil (Figure 4a) and NH_4^+ was the dominant form. Mean seasonal concentrations of extractable inorganic N in redcedar stands and grasslands were usually not significantly different, with the exception of winter when significantly greater concentrations of extractable N occurred in grasslands (One-way ANOVA, F=13.39, P=0.02), a trend that started in the late fall and continued into early spring (Figure 4a). Differences in winter time extractable soil N of redcedar and grassland soils were likely due to differences in seasonal patterns of plant uptake, as redcedar may continue to utilize inorganic soil N in late fall to early spring when the grasses are dormant.

Low concentrations of extractable inorganic N suggest strong N limitations on plant growth. Extractable N pools in grasslands typically reach a maximum in the early spring that coincides with grassland "green up" (Figure 4a). This increase in extractable N may alleviate N limitations in the beginning of the growing season in grassland soils when plant demand for N begins to rapidly increase in response to plant growth. This over-winter accumulation of extractable N in grasslands appears to be a result of reduced (or eliminated) plant uptake, since measured net N mineralization during the winter was small or negative (Figure 4b). Consistently low concentrations of extractable N in redcedar stands suggest sustained N limitations throughout most of the year. Small NO_3^- pools found in both ecosystems are of particular interest, because small NO_3^- pools reduce the potential for N losses through soil leaching or denitrification that could exacerbate N limitations on plant growth.

Net N mineralization exhibited strong seasonal patterns in both redcedar and grassland soils, with the highest rates in the spring and summer months (>2 μg N g soil^{-1} d^{-1}), intermediate rates in the fall, and low or negative rates in winter months (Figure 4b). Although net N mineralization rates tended to be greater in redcedar stands compared to grassland, there were no significant differences with any average seasonal rate ($P \leq 0.05$). However, when cumulative N mineralization rates were calculated on an annual basis, redcedar soils had significantly greater annual net N mineralization rates (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}) (One-way ANOVA, F=60.67, P=0.02). Greater N mineralization rates, yet similar or lower concentrations of extractable soil N in redcedar stands compared to grassland soils, suggests substantial utilization of available inorganic N by redcedar most of the year (Figure 4 a&b). There are other reports of soil nitrogen availability under redcedar and other *Juniperus* canopies and adjacent grassland communities, measured as either net N

mineralization or extractable inorganic N (Charley and West 1977; Klopatek 1987; Klopatek, Klopatek and DeBano 1990; Miller, Eddleman and Miller 1991; Padien and Lajtha 1992; Klopatek and Klopatek 1997; Klemmedson and Tiedemann 2000; Norris 2001 et al., Norris, Blair and Johnson 2001; Roberts and Jones 2000; Bates 2002; Stark et al. 2002; Svejcar and Miller 2002; Stubbs and Pyke 2005; McKinley 2006), but most of these studies support greater N availability under *Juniperus* canopies relative to either adjacent ungrazed grasslands or intercanopy spaces in a variety of ecosystems.

Nitrogen availability can change dramatically with differences in land management, particularly with fire and grazing regimes, which affect N availability in grasslands (Schimel et al. 1986; Blair et al. 1998; Knapp, Conrad and Blair 1998; Johnson and Marchett 2001; Briggs, Knapp and Brock 2001). For example, annually burned grasslands typically have lower extractable N pools and lower net N mineralization rates compared to less frequently burned sites (Blair et al. 1998). Norris (2000) measured extractable N and net N mineralization over a two-year period, comparing redcedar stands with adjacent frequently burned cattle-grazed grasslands. Norris (2000) found only small differences in extractable N and net N mineralization (with the grasslands sites having slightly greater N availability). Although Norris (2000) reported similar rates of annual N mineralization in redcedar soils 14.4 μg N g^{-1} soil y^{-1} compared to 11.5 μg N g^{-1} soil y^{-1} in a later study (McKinley 2006), net N mineralization rates were much greater in recently-grazed grassland soils (~17 μg g^{-1} soil y^{-1}) compared to long-term ungrazed grasslands (~8 μg N g^{-1} soil y^{-1}) in a comparable study (McKinley 2006). The presence of cattle can increase net N mineralization by concentrating highly labile N inputs, affecting plant tissue quality and soil feedback loops by decreasing N immobilization potential (Schimel et al.1986). Thus, the relative changes in soil N availability due to redcedar encroachment may depend, in

part, on other land management practices, such as fire and grazing that have strong effects on N availability in native grasslands (Johnson and Marchett 2001).

Altered plant resource use

Leaf level photosynthetic nitrogen use efficiency (PNUE) is defined as the maximum carbon assimilation rate (A_{max}) relative to the amount of total nitrogen in the leaf (Lambers, Chapin and Pons 1998). Plants with high PNUE are able to achieve high photosynthetic rates with relatively small amounts of nitrogen. There are typically differences in PNUE between C_3 and C_4 species, with C_4 species generally being more efficient (Sage and Pearcy 1987). In evergreens, A_{max} and consequently PNUE may be further constrained by thick cell walls that limit gas exchange, or leaf N may be allocated to maintaining leaf longevity rather than invested in photosynthetic enzymes (Field and Mooney 1983). In redcedar, PNUE ranged between 0.5 - 1.1 μmol CO_2 g^{-1} N s^{-1} over the course of a year. The highest values were observed in early fall, and were caused by higher photosynthetic rates, as leaf N concentrations varied little throughout the year (McKinley 2006). Big bluestem (*Andropogon gerardii* Vitman) a dominant C_4 species in the paired grassland sites had much greater PNUE values, which ranged between 17.8-29.8 μmol CO_2 g^{-1} N s^{-1} (McKinley 2006).

Though instantaneous metrics of PNUE are much lower in redcedar stands relative to grasses they replace, leaf longevity, greater leaf area, and the ability to assimilate CO_2 year round particularly when grasses are senescent may allow greater annual ecosystem-level nitrogen use efficiency (NUE) and primary productivity (Field and Mooney 1983; Miller, Eddleman and Angell 1987; Escudero and Mediavilla 2003). Ecosystem-level nitrogen use efficiency (NUE), defined as the ratio of ANPP:litterfall N, provides an index of the amount of aboveground biomass produced per unit of N lost (Chapin 1980; Vitousek 1982). Norris (2000) calculated an

ecosystem NUE of 223 for redcedar and 93 for adjacent grassland, which indicated that redcedar was about 2.5 times more efficient in producing biomass per unit N lost in senesced plant tissue. Greater NUE in redcedar stands compared to grasslands is a result of differences in plant phenology that allow more conservative N use, attributable in part to longer leaf life-span and potential to photosynthesize year round, as well as stand development in consistently low soil N availability (Chapin 1980; Vitousek 1982).

Conclusions

Redcedar encroachment into native grasslands can lead to significant changes in C and N cycling, which also alters the accumulation and patterns of the storage of these elements compared to the grasslands they replace. Redcedar canopies cause substantial reductions in soil temperature, which influence soil processes, such as C mineralization. Despite alterations in abiotic conditions that reduce soil respiration, there appears to be slightly enhanced N availability in redcedar stands compared to adjacent annually burned ungrazed prairie, but this was detectable only with long-term measurements. Soil temperatures appear to drive differences in C mineralization, while substrate quality drives differences in N mineralization between ecosystem types. Nitrogen availability in both redcedar and grassland communities is characterized by low concentrations of inorganic nitrogen, and relatively low rates of net N mineralization. Changes in soil processes resulting from grassland to redcedar conversion appear to allow soil C accumulation through reduced soil CO_2 flux, while simultaneously allowing greater soil N availability to support greater ANPP in redcedar communities.

Redcedar encroachment creates localized patches of increased resource availability, specifically N, but also in some other macronutrients (i.e., Ca and Mg) (McKinley 2006). Increases in resource availability are commonly found with shrub/woodland encroachment in

arid and semi-arid regions (Schlesinger et al. 1990; Scholes and Archer 1997). Intrinsic properties of *Juniperus* stands lead to more conservative N cycling and conditions that may relieve N constraints, which allow greater maintained productivity, at least until other factors (i.e., light or perhaps other nutrients) become more limiting as the forest matures. Reduced N losses due to the elimination of fire probably play a considerable role in observed increased ecosystem N storage and mitigation against the occurrence of more severe N constraints in most *Juniperus* communities. Also, much like the grasslands these redcedar stands replace, very small soil extractable NO_3^- indicate minimal potential for nutrient losses and low contributions to terrestrial and atmospheric loss pathways (Vermes and Myrold 1991; Sotomayor and Rice 1996).

Enhanced soil N availability and greater NUE of redcedar communities may favor redcedar expansion into adjacent grassland communities. Although PNUE, an instantaneous measurement of NUE at the leaf level, indicates that redcedar are comparatively inefficient compared to the C_4 grasses (*Andropogon gerardii* Vitman) they replace, the ability to consistently photosynthesize year round allows redcedar to have much greater overall stand level NUE. The combination of greater N availability, more efficient use of N, and year-round photosynthetic capacity, allow much greater ANPP and biomass accumulation than grasslands. Eventually, redcedar plants obtain sufficient size to reduce available photosynthetically active radiation to grasses beneath the canopy, thus potentially altering the competitive balance between these life forms (Schimel et al. 1991).

Many researchers have suggested that expansion of forest ecosystems may be part of the 'missing' carbon sink, which may play a role in the mitigation of increasing greenhouse gases (Myneni et al. 2001). In redcedar, there is almost a four-fold difference in total ecosystem C storage relative to grasslands (if root estimates are included), which amounts to almost 10^4 kg

ha^{-1} of additional C (Norris et al. 2001; McKinley 2006). Redcedar dominated ecosystems act as strong C and N sinks in these converted mesic grasslands. However, these ecosystems can not maintain significant rates of C accumulation indefinitely; as redcedar communities mature, their potential role in further C sequestration become limited, as seen in older western juniper communities (Tiedemann and Klemmedson 2000). Also, given that the bulk of the new C and N allocation is aboveground, these pools are very vulnerable to significant and rapid losses, primarily through fire (Klopatek, Klopatek and DeBano 1990). In addition, long-term ecosystem stores of C and N in the soil may be lost through soil erosion (Davenport 1998), because of reduced or absent plant cover under the redcedar canopies (Briggs, Hoch and Johnson 2002). Also, redcedar communities exposed to future increases in anthropogenic N deposition may vary in rates of C and N accumulation, but the potential effects are currently unknown.

Expansion by redcedar and other *Juniperus* species will likely continue in the near future, and will have substantial consequences on the biogeochemistry, productivity, and species diversity of a variety of ecosystems (Norris 2000; Norris et al. 2001; Norris, Blair and Johnson 2001; Briggs, Hoch and Johnson 2002; Smith and Johnson 2003; Smith and Johnson 2004; McKinley 2006). The ultimate end state of these altered ecosystems in terms of structure and function, as well as the role of redcedar in community succession and ecosystem stability of redcedar communities is unclear, but seems certain to be significant. Management of redcedar must be carefully considered due to the pronounced effects of different management regimes on the expansion of existing redcedar communities and the potential for community alterations with redcedar encroachment into grasslands. *Juniperus* has a unique niche in North American grassland ecosystems, as this unprecedented recent expansion will probably continue to play a large role in changing ecosystem structure and function in many communities.

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Figures and tables

Figure 5-1 Hourly averages of mineral soil temperatures at 5 cm depth in redcedar and grassland soils measured from mid-June through mid-October (McKinley 2006). Measurements were made simultaneously at four sites, with four thermocouples per vegetation type. Grassland soil temperatures were greater at nearly all times compared to redcedar stands, with the differences reaching 8ºC. Temperatures tended to converge in nighttime and after significant rainfall events.

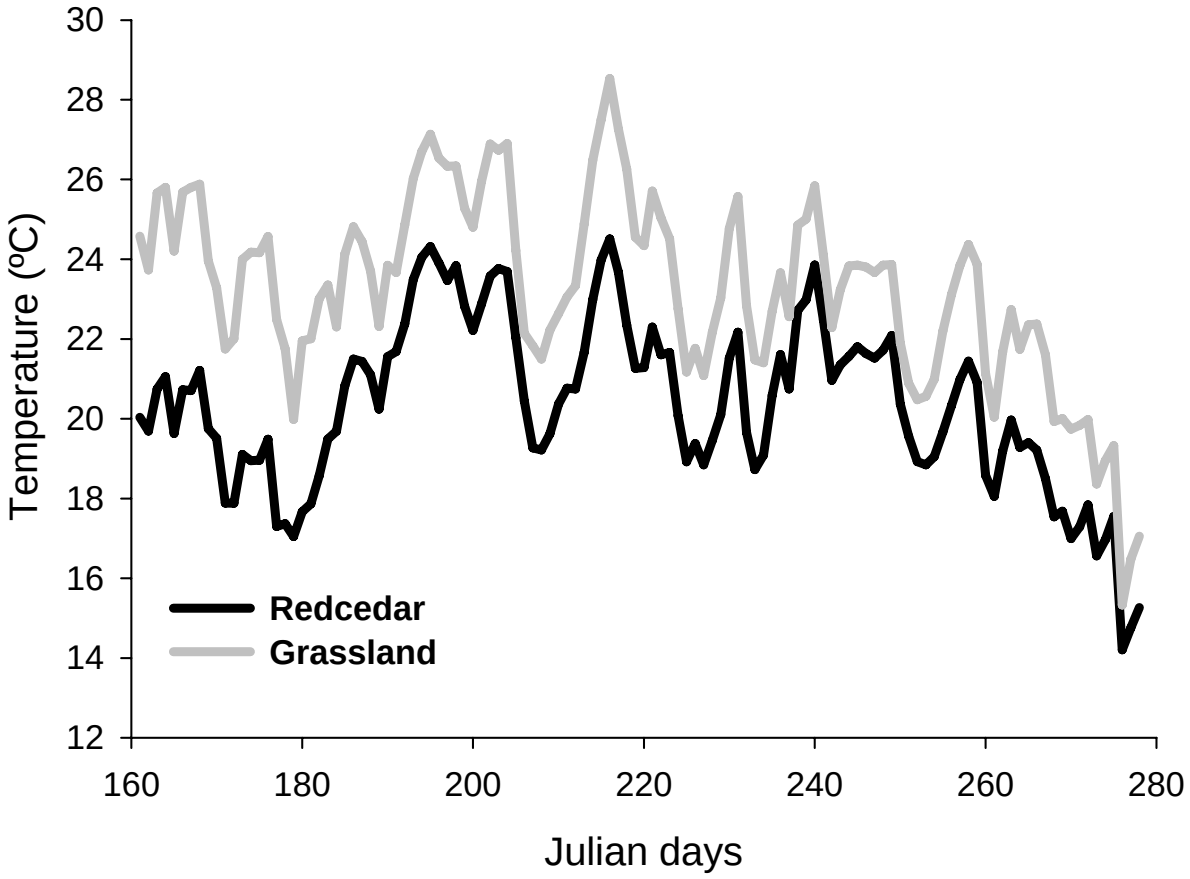


Figure 5-2 Seasonal patterns of gravimetric soil moisture (mean ± se) averaged over a two-year period at four sites from multiple measurements per season (McKinley 2006). Mean seasonal soil moisture was not significantly different in any season ($P > 0.05$), although grassland soils tended to be consistently greater.

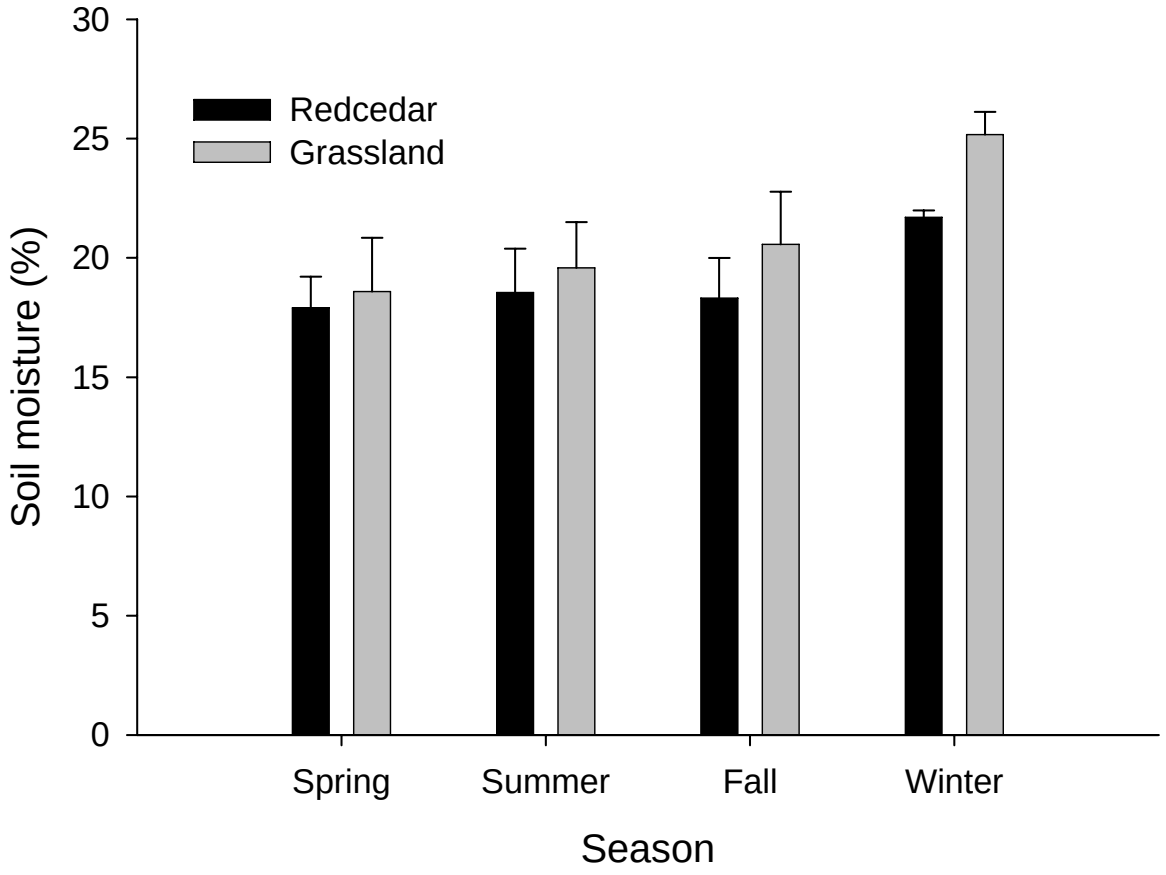
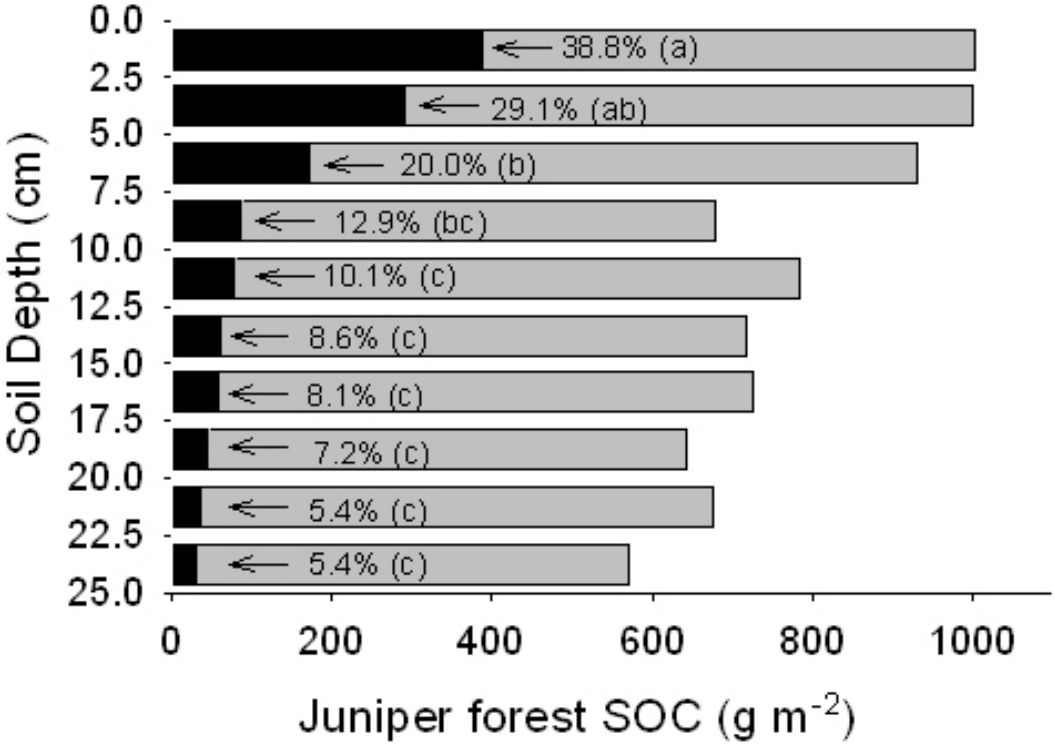


Figure 5-3 (a) Amounts and percentages of C_3 forest-derived and C_4 prairie-derived C in SOC profiles in redcedar forest. Dark bar=SOC derived from forest, gray bar=SOC derived from residual prairie carbon. A mixing model [Balesdent, Wagner and Mariotti 1988; Arrouays et al. 1995] used to calculate net C_3-SOC input into redcedar forest soils over 40-60 years. The solid portion of each bar is new C_3-SOC, while the shaded portion is prairie carbon composing the remainder of SOC. Numbers to the right of an arrow indicate the percent C_3 input at each soil depth. Different letters indicates statistically significant differences among depths in the forest profile. (b) Regression performed on the C_3-C% input data points indicating an exponential decrease of net C input with increasing depth (from Smith and Johnson 2004, reproduced by permission of American Geophysical Union).


Soil Depth (cm)
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
38.8% (a)
29.1% (ab)
20.0% (b)
12.9% (bc)
10.1% (c)
8.6% (c)
8.1% (c)
7.2% (c)
5.4% (c)
5.4% (c)
0
200
400
600
800
1000
Juniper forest SOC (g m-2)

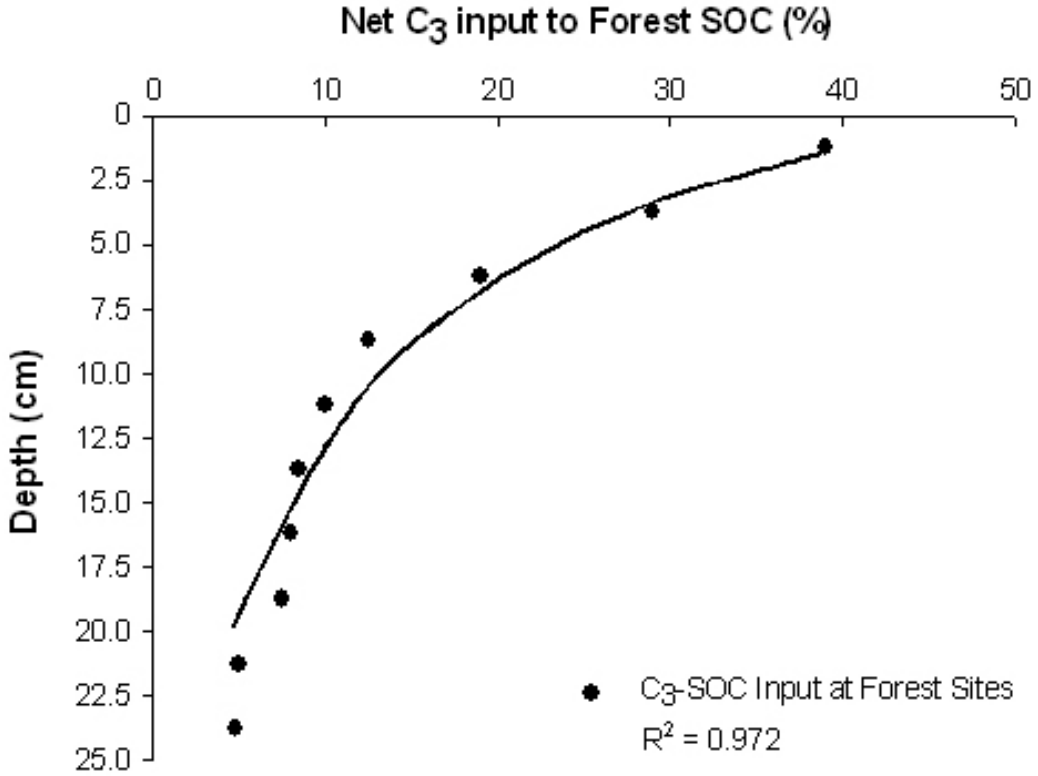
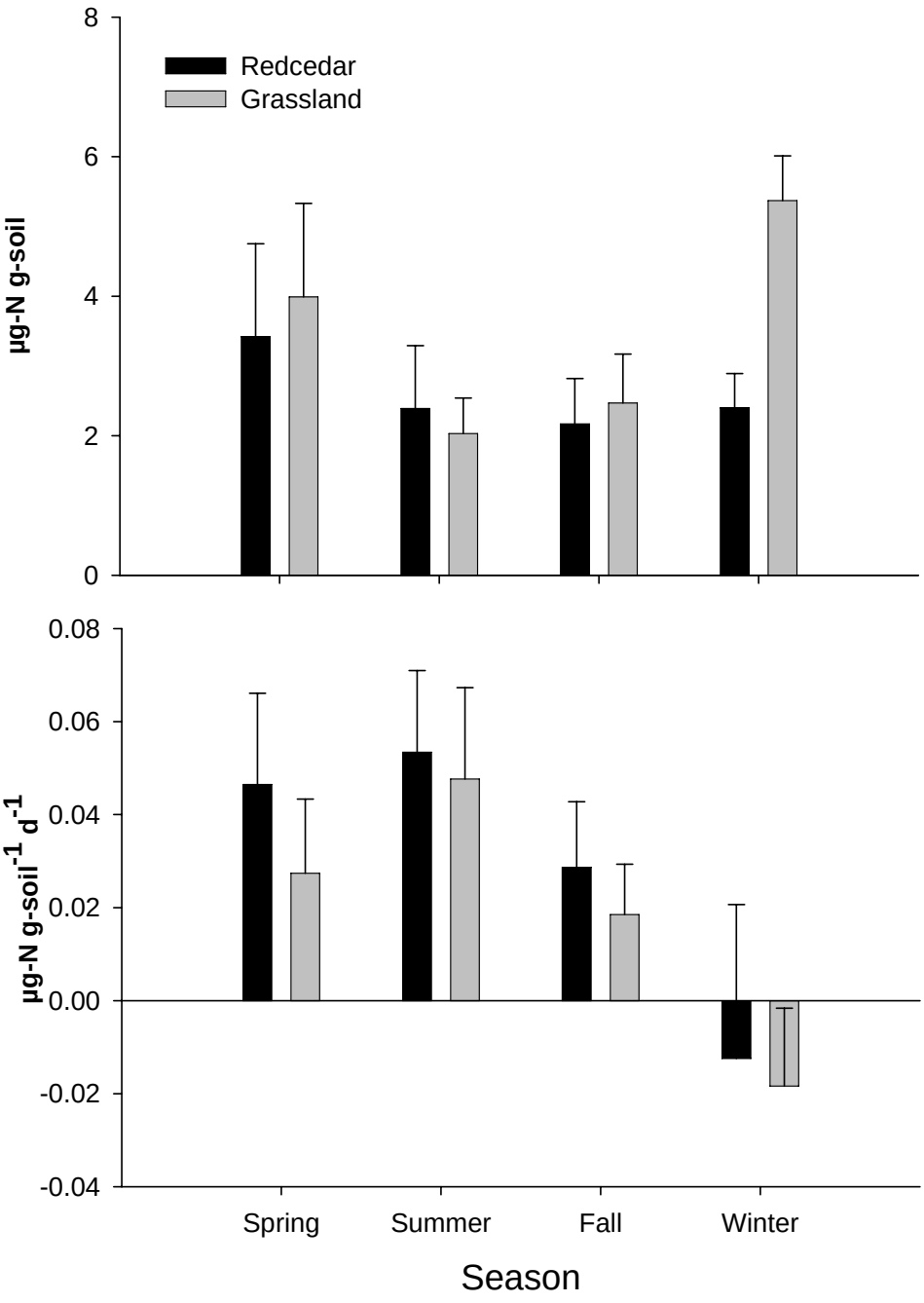

Net C3 input to Forest SOC (%)
0
10
20
30
40
50
Depth (cm)
0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
20.0
22.5
25.0
C3-SOC Input at Forest Sites
R2 = 0.972

Figure 5-4 Seasonal patterns of extractable soil inorganic nitrogen (a) and net N mineralization (b) (mean ± se) averaged over a two-year period across four-paired sites. Extractable N pools were relatively small in both redcedar and grassland soils over all seasons. There were few differences in soil extractable N pools in most seasons, with the exception of winter, when grassland soils had significantly greater concentrations (One-way ANOVA, $F = 13.39$, $P = 0.02$). Soil net N mineralization was not significantly different between redcedar and grassland soils, however there was a consistent trend of greater N mineralization rates in redcedar soils. Asterisks represent significant differences between vegetation types (redcedar and grassland) for that specific time (alpha = 0.05).


Redcedar
Grassland
µg-N g-soil
8
6
4
2
0
0.08
0.06
0.04
0.02
0.00
-0.02
-0.04
µg-N g-soil-1 d-1
Spring
Summer
Fall
Winter
Season

Appendix A - Soil characteristics

Appendix-Table 1: Soil characteristics of the A-horizon in forest and prairie soil across all sites. There were no significant differences for any soil characteristic with the exception of calcium, which was significantly greater in forest soils.

	Soil Characteristics for A-horizon (10 cm)	
	Forest (mean ± se)	**Prairie (mean ± se)**
pH	**7.32 ± 0.21**	**7.56 ± 0.17**
Bray P (µg/g)	**3.03 ± 0.41**	**3.33 ± 0.80**
K (µg/g)	**5,620 ± 320**	**5,530 ± 250**
Ca (µg/g)	**373 ± 13***	**319 ± 6.93***
Mg (µg/g)	**237 ± 14**	**202 ± 23**
% Sand	**14 ± 1**	**15 ± 2**
% Silt	**58 ± 1**	**57 ± 2**
% Clay	**29 ± 1**	**28 ± 1**
CEC meq/100g	**28 ± 1**	**23 ± 3**

*** Indicate significant differences (alpha = 0.05)**