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Conversion of grassland to coniferous woodland has limited effects on soil nitrogen cycle processes

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ABSTRACT

In the last century, conversion of native North American grasslands to *Juniperus virginiana* forests or woodlands has dramatically altered ecosystem structure and significantly increased ecosystem carbon (C) stocks. We compared soils under recently established *J. virginiana* forests and adjacent native C_4-dominated grassland to assess changes in potential soil nitrogen (N) transformations and plant available N. Over a 2-year period, concentrations of extractable inorganic N were measured in soils from forest and grassland sites. Potential gross N ammonification, nitrification, and consumption rates were determined using ^{15}N isotope-dilution under laboratory conditions, controlling for soil temperature and moisture content. Potential nitrification rates (V_{max}) and microbial biomass, as well as soil physical and chemical properties were also assessed. Extractable NH_4^+ concentrations were significantly greater in grassland soils across the study period ($P \leq 0.01$), but analysis by date indicated that differences in extractable inorganic N occurred more frequently in fall and winter, when grasses were senescent but *J. virginiana* was still active. Laboratory-based rates of gross N mineralization (ammonification) and nitrification were greater in grassland soils ($P \leq 0.05$), but only on one of four dates. Potential nitrification rates (V_{max}) were an order of magnitude greater than gross nitrification rates in both ecosystems, suggesting that nitrification is highly constrained by NH_4^+ availability. Differences in plant uptake of N, C inputs, and soil microclimate as forests replace grasslands may influence plant available N in the field, as evidenced by seasonal differences in soil extractable NH_4^+, and total soil C and N accumulation. However, we found few differences in potential soil N transformations under laboratory conditions, suggesting that this grassland-to-forest conversion caused little change in mineralizable organic N pools or potential microbial activity.

1. Introduction

Juniperus virginiana L. (eastern redcedar) has encroached into grasslands in the eastern Great Plains and other parts of in its range at an unprecedented rapid rate, affecting millions of hectares (Schmidt and Leatherberry, 1995; Briggs et al., 2002; Briggs et al., 2005). Factors contributing to *Juniperus* encroachment include fire suppression and/or overgrazing (Briggs et al., 2002). Once established, *J. virginiana* can form dense nearly monospecific stands in just a few decades, which modifies soil microclimate (McKinley et al., 2007), alters litter quality and decomposition rates (Norris et al., 2001b), and may influence soil N cycling and availability. Soil N availability affects plant productivity in N-limited ecosystems (Reich et al., 1997; Vitousek, 2004), thus assessing changes in soil N cycling with conversion of grasslands to woodlands or forests is essential for understanding whole ecosystem dynamics.

Juniperus virginiana encroachment substantially increases net ecosystem productivity and alters the quantity and distribution of C and N in plant and soil pools (Norris et al., 2001a; Smith and Johnson, 2004; McKinley et al., 2007; McKinley and Blair, 2008). Increased total ecosystem C and N storage in *J. virginiana* forests results from fire exclusion and increased annual aboveground net plant primary productivity (ANPP), which is as much as three times greater compared to the grasslands they replace (Norris et al., 2001a, 2007). But, *J. virginiana* generally produces poor quality litter (i.e., high C:N ratios), which may slow decomposition and increase microbial immobilization of N, resulting in reduced plant available N (Vitousek, 1982). Thus, potential productivity of encroaching *J. virginiana* forests may be increasingly constrained by reduced plant available N over time if soil N-cycling dynamics change as a result of greater microbial N demand.

We hypothesized that conversion of grasslands to *J. virginiana* forests would increase potential gross N mineralization and N immobilization rates, but reduce gross nitrification rates and nitrification potential. Greater C inputs should increase microbial N

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demand in forest soils, and decrease net rates of ammonification and substrate (NH_4^+) availability for nitrifiers. Inorganic N concentrations in field-collected soil should reflect these changes in microbial processes, resulting in lower inorganic N concentrations much of the year in forest soils, assuming similar rates of plant N uptake in both vegetation types. But, previous studies indicate that *J. virginiana* forests are able to rapidly accrue C with little apparent change in *in situ* soil net N mineralization (an index of plant available N) (McKinley and Blair, 2008). Assessing changes in potential gross soil N cycling rates may provide insights into this apparent contradiction.

Ammonification and nitrification are key processes affecting N availability in soils; both processes are highly influenced by substrate quality and accessibility, as well as abiotic factors such as soil moisture, temperature, and pH (Booth et al., 2005). Growing season soil temperatures in *J. virginiana* stands are as much as 8 °C lower than in adjacent grasslands, and soil moisture is reduced in some seasons (Smith and Johnson, 2004; McKinley et al., 2007). Despite a less favorable microclimate in forest soils, net N mineralization in the field tended to be somewhat greater than in grassland soils over a 2-year study period (McKinley and Blair, 2008). *Juniperus virginiana* encroachment generally increases soil organic matter, but little is known about potential differences in substrate quality and internal N-cycle processes relative to grassland soils (McKinley et al., 2007; McKinley and Blair, 2008).

Newly established *J. virginiana* forests accumulate large quantities of N compared to grasslands, and up to 70% of N accrual is attributable to fire exclusion (McKinley and Blair, 2008), but little is known about differences in potential for N loss or retention mediated by soil microbial processes. The nitrification pathway is important because of increased potential for gaseous N losses associated with nitrification and denitrification (Davidson et al., 1991), and leaching losses of NO_3^- in the soil solution. Gross nitrification and NO_3^- consumption estimates have illuminated the importance of nitrification in systems such as an old growth coniferous forest and other undisturbed coniferous forests (Davidson et al., 1992; Hart et al., 1994a; Stark and Hart, 1997) where nitrification previously was thought to be of little importance because of low net nitrification rates and small NO_3^- pools. Nitrification in grasslands is also important, but may be masked because of the strong potential to immobilize NO_3^- similar to some coniferous forests (Schimel et al., 1989; Davidson et al., 1990; Corre et al., 2002).

The objectives of this study were to assess changes in potential internal cycling of N and soil N availability following *J. virginiana* forest encroachment into grassland. We coupled concurrent assays of N availability in field-collected soils and laboratory-based assays of potential N fluxes across several seasons to capture dynamics of N availability and to understand internal soil N cycle processes that could influence potential N availability. We focused on potential gross rates of ammonium and nitrate production and consumption using a laboratory based ^{15}N isotope-dilution approach, and included another concurrent measure of potential nitrification to provide further insights into how substrate availability affects nitrification. We also sampled inorganic N concentrations throughout the 2-year study to characterize ammonium and nitrate availability in the field, and estimated microbial biomass as a component of labile soil N.

2. Methods and materials

2.1. Site description

This study was conducted in the Flint Hills region of NE Kansas, USA (39°05′N, 96°35′W), where the prevalent native vegetation is tallgrass prairie, dominated by perennial, warm-season C_4 grasses including big bluestem (*Andropogon gerardii* Vit.), little bluestem (*Schizachyrium scoparium* Michx.), indiangrass (*Sorghastrum nutans* Nash) and switchgrass (*Panicum virgatum* L.) (Freeman and Hulbert, 1985). These C_4 grasses contribute the majority of ANPP (Knapp et al., 1998). However, a mixture of less abundant species, including C_3 grasses, sedges and a diverse array of forbs, contributes to floristic diversity (Freeman and Hulbert, 1985). The native flora also includes a smaller number of less abundant native woody plants, such as buckbrush (*Symphoricarpos orbiculatus* Moench.), New Jersey tea (*Caenothus herbaceous* Raf.), redcedar (*Juniperus virginiana* L.), smooth sumac (*Rhus glabra* L.) and rough-leaved dogwood (*Cornus drummondii* CA May), which can be locally abundant, especially in grassland 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.

2.2. Experimental design

Four paired sites comprised of contiguous or nearly contiguous *J. virginiana* forest adjacent to native grassland were chosen in close proximity (<1 km) to the Konza Prairie Biological Station (KPBS), the primary location of the Konza Prairie Long-Term Ecological Research (LTER) program. This allowed the use of a variety of baseline data on ecological processes in native grassland. All study sites (each with both forest and grassland communities) were located in upland topographic positions, with relatively shallow mineral soils (~10 cm deep) overlying fragmented limestone layers. This is the most common topoedaphic condition for *J. virginiana* forest encroachment in this region. Three sites had silty clay loam soil; fine, mixed, active, mesic Udothentic Haplustols. The fourth site had a silt loam soil; fine, mixed, superactive, mesic Udertic Argiustolls (United States Department of Agriculture Soil Conservation Service, 1975). Soil physical and chemical attributes are presented in Table 1.

Each *J. virginiana* forest (≥0.5 ha) had dense (680–1360 trees ha^{-1}), complete canopy cover. Tree diameter at breast height (dbh) ranged from 15.2–22.1 cm (mean 18.1 cm). Historical aerial photographs since 1950 and analysis of soil organic carbon (SOC) isotopic composition were used to verify the recent replacement of native grasslands with *J. virginiana* forests and estimate the age of each

Table 1
Soil physical and chemical properties in the surface 10 cm in forest and grassland communities

Variable	Forest	Grassland
Soil physical properties		
Soil texture		
Sand (%)	14 ± 1	15 ± 2
Silt (%)	58 ± 1	57 ± 2
Clay (%)	29 ± 1	28 ± 3
Soil chemical properties		
SOM		
SOC (mg g^{-1})	40.3 ± 1.2**	32.5 ± 1.8**
SON (mg g^{-1})	3.4 ± 0.1**	2.9 ± 0.1**
C:N	11.8 ± 0.2	11.2 ± 0.2
Bulk density (M g m^{-3})	0.94 ± 0.03	1.04 ± 0.07
pH	7.3 ± 0.2	7.6 ± 0.2
Extractable base cations (μg g^{-1})		
Ca^{2+}	373 ± 13*	319 ± 7*
K^+	5,620 ± 320	5,530 ± 250
Mg^{2+}	237 ± 14	202 ± 23
Bray P (μg g^{-1})	3.0 ± 0.4	3.3 ± 0.8
CEC (meq/100 g)	28 ± 1	23 ± 3

Means ± 1SE ($n = 4$) followed by asterisks indicate significant differences between grassland and forest soils (one-way ANOVA, *$P \leq 0.05$; **$P \leq 0.01$).

forest stand (McKinley and Blair, 2008). Estimated ages of individual stands ranged from 35 to 75 years, with a mean around 45–50 years. Each forest site was paired with an adjacent native grassland site in the same topographic position and soil type. These adjacent grassland sites were not grazed in the recent past (>15 years, personal communication, Joseph McGill) and had a contemporary average fire return interval of 1–2 years, as a result of prescribed fires conducted in early spring.

At each study site (*Juniperus* forest or grassland), one 50 m transect ($n = 4$/vegetation type) was used to randomly locate plots where soil and other ecosystem measurements were made. Concentrations of extractable ammonium and nitrate were measured 15 times at six plots along each transect over a 2-year period to provide baseline information on patterns of extractable soil N. In October 2003 (fall), March 2004 (spring), July 2004 (summer), and October 2004 (fall) additional laboratory assays of gross N fluxes, potential nitrification, and microbial biomass were performed on the soil samples. Soils for each assay in each season were handled and incubated similarly with respect to time.

2.3. Extractable inorganic soil N

On each of 15 dates over a 2-year period (June 2003 and June 2005), six soil cores (5 cm diameter × 10 cm) were collected from transects at each site ($n = 4$ for both forest and grassland) to determine concentrations of extractable inorganic N. Intact cores were refrigerated, then sieved (4 mm) field-moist and extracted for inorganic N within 48 h of collection. Ammonium and nitrate were extracted from 10–12 g of field-moist soil with 50 ml of 2 M KCl for 1 h on an orbital shaker (200 rpm), and filtered through a 0.4 μm polycarbonate membrane. Analytical details are provided below.

2.4. Potential soil N-cycling rates

On four dates (October 2003, March 2004, July 2004, and October 2004), subsamples of soil were taken from the cores collected for extractable N, and used for laboratory assays of gross ammonification and nitrification. Within 48 h of collection, three randomly selected 15 g moist soil sub-samples from each transect were placed in 20 ml glass scintillation vials. Soil water content was determined gravimetrically from another sub-sample of the original cores, which was oven dried at 105 °C. Soil samples were adjusted to 30% gravimetric soil moisture (60% water-filled pore space), to later include additions of 0.5 ml of labeled nitrogen suspension, and then covered with one thin layer of polyethylene (Glad® Cling Wrap) secured with rubber bands. Soil samples were pre-incubated at 4 °C for 14 days to minimize the disturbance effects on microbial communities and soil processes (Hart et al., 1994b); previous soil incubations showed stabilization (at lower rates than disturbed soils) of soil CO_2 flux after 14 days. But, extractable N concentrations changed over the pre-incubation period compared to soils freshly extracted from the field, and thus were not representative of field conditions. After the pre-incubation period, soils were labeled with $(^{15}NH_4)_2SO_4$, or with $K^{15}NO_3$ (both highly enriched 98-99% ^{15}N). Additions of labeled solutions were made by eight injections with a micro-syringe so that ^{15}N additions generally did not exceed 10% of ambient inorganic N pools. Duplicate samples were extracted after 15 min to provide initial (T_0) values, and the remaining (T_1) samples were extracted after incubation in the dark in an environmentally controlled chamber at 25 °C (optimal for soil microbial activity) for 24 h.

Ammonium and NO_3^- from all soil samples was extracted with 2 M KCl using standard methods. Inorganic nitrogen (NH_4^+-N and $NO_3^- + NO_2^-$-N) concentrations were determined colorimetrically with an Alpkem® Flow Solution autoanalyzer (Wilsonville, OR) using the indophenol blue method for NH_4^+-N and cadmium reduction followed by diazotization with sulfanilamide for NO_2^-/NO_3^--N. Preparation of soil extracts for ^{15}N isotopic analyses was done by diffusion (Davidson et al., 1992). All ^{15}N analyses were done at the Kansas State University stable isotope lab, with a Thermo Finnigan Delta Plus mass spectrometer (samples combusted with a CE Elemental Analyzer with ConfloII). Gross rates of mineralization (ammonification) and nitrification, as well as consumption were calculated using standard published formulae (Hart et al., 1994a). However, a portion of the apparent NH_4^+ consumption could also be due to abiotic NH_4^+ fixation (Barrett et al., 2002), which was not addressed in this study.

2.5. Potential nitrification

Nitrification potentials (V_{max}) were determined by a shaken soil-slurry procedure (Hart et al., 1994b). The procedure yields maximum nitrification rates in the absence of substrate limitations. Replicate controls were treated with acetylene to block autotrophic nitrification to determine the rate of NO_3^- consumption due to denitrification, and to assess the relative importance of autotrophic and heterotrophic nitrification. The soil nitrification potential assay was performed using sieved soils within 72 h of collection in spring, summer and fall 2004. Aliquots of each of the original six soil samples per transect were combined into one sample, for a total of eight composite samples, or four replicates from each vegetation type ($n = 4$ forest and 4 grassland samples).

2.6. Microbial biomass

Soil microbial biomass C and N was determined at the peak of the growing season, summer 2004 (July), and at the end of the growing season, fall 2004 (October), using a chloroform fumigation incubation technique (Jenkinson and Powlson, 1976) initiated approximately 96 h after collection from the field. Microbial biomass C was determined by difference in the amount of CO_2-C respired from fumigated and non-fumigated samples, measured with a Shimadzu GC-8A gas chromatograph. Microbial biomass N was determined by difference in the amount extractable inorganic N from fumigated and non-fumigated samples. Inorganic nitrogen (NH_4^+-N and $NO_3^- + NO_2^-$-N) concentrations were determined colorimetrically as previously described. Conversion factors (K_C and K_N) of 0.41 and 0.54 were used to estimate biomass C and N from the fumigation incubations (Horwarth and Paul, 1994).

2.7. Soil properties

Soil texture was determined by a wet sieving method. Bulk soils were analyzed for C and N content using dry combustion/gas chromatography with a Carlo Erba model NA1500 C/N analyzer. Soil pH was measured using 1:1 slurry with deionized water. Base cations were extracted with 1 M ammonium acetate, pH 7, and analyzed by flame atomic absorption or ICP spectrometry. The Bray-1-P test was used for extractable phosphorus, which utilized HCl–ammonium fluoride extractant, coupled with a colorimetric analyses (Olsen and Sommers, 1982). Cation exchange capacity was determined by displacement with ammonium acetate (Bache, 1976).

2.8. Statistical methods

Each paired site included both forest and grassland transects in a randomized complete block design; values derived from individual plots along a transect were pooled to derive a single mean value ($n = 4$ per vegetation type). Means for each transect (replicate) were derived from three plots for gross mineralization and nitrification assays, while means of microbial biomass, field-based extractable N, and soil properties were based on six plots.

Nitrification potential (V_{max}) was based on a single composite sample from each transect ($n = 4$). Means obtained from gross mineralization (ammonification), gross nitrification, potential nitrification, microbial biomass, and associated calculations were examined with one- or two-way analysis of variance (ANOVA) to test for significant differences between ecosystem type and season, if applicable ($P = 0.05$). Tukey's honest significant difference was used for post-hoc comparisons when appropriate. Concentrations of extractable N were analyzed with one-way ANOVAs for each sampling period to assess differences by date, and with repeated-measures ANOVA to assess trends over the entire sampling period.

3. Results

3.1. Soil properties and extractable inorganic soil N

Soil physical and chemical properties were very similar in forest and grassland sites after approximately 45–50 years of forest encroachment, with the exception of significantly greater SOC, SON, and exchangeable calcium in forest soil (Table 1).

Total extractable inorganic N concentrations were not significantly different in grassland and forests sites across the 2-year study ($P = 0.144$), although analyses by date indicated significantly greater inorganic soil N concentrations in grasslands during the fall and early spring of the first year of study (Fig. 1a). Extractable soil NH_4^+ was significantly greater in grassland soil compared to forest soil across the study period, and on 8 of 15 sample dates ($P \leq 0.001$) (Fig. 1b). In contrast, extractable NO_3^- concentrations were not significantly different between grassland and forest ecosystems on most dates ($P = 0.850$) (Fig. 1c).

3.2. Potential soil N-cycling rates

Laboratory-based gross ammonification rates were significantly greater in grassland soil compared to forest soil during spring 2004 ($P = 0.019$, Fig. 2a). Ammonium consumption was also significantly greater in grassland soil compared to forest soil in spring 2004 samples ($P = 0.049$, Fig. 2b). Neither gross ammonification nor consumption differed significantly between forest and grassland at any other time ($P > 0.05$).

Mean residence times (MRTs), a metric of the dynamics of inorganic N pools, indicated no differences ($P > 0.05$) in the turnover rates of NH_4^+ in forest and grassland soil pools in any season. The MRTs of NH_4^+ in both forest and grassland soils were very short (<30 h), indicating rapid uptake and strong microbial demand for NH_4^+. Most of the NH_4^+ produced was immobilized (gross NH_4^+ consumption − gross nitrification) by microbial uptake in both forest (79.7 ± 12.6%) and grassland soils (72.4 ± 14.3%), across all seasons measured. Despite potential differences in microbial communities and substrate quality and quantity, these data suggest that the dynamics of forest and grassland NH_4^+ pools were similar across all measurement dates.

Gross nitrification rates measured in samples collected in fall 2003 were significantly greater in grassland compared to forest soil ($P = 0.049$, Fig. 3a). NO_3^- consumption (Fig. 3b) was also significantly greater in grassland compared to forest soil during fall 2003 ($P = 0.014$). In fall 2004 gross nitrification ($P = 0.059$) and consumption ($P = 0.062$) rates were both greater in forest soils, although these differences were only marginally significant. Neither gross nitrification nor consumption differed between forests and grasslands at other times. Nitrate consumption rates were greater than gross nitrification rates (negative net rates) for both forest and grassland soils in all seasons.

Potential nitrification (V_{max}) was not significantly different in forest and grassland on any date ($P > 0.05$). Potential nitrification (V_{max}) rates in both forest and grassland soils were much greater than potential gross nitrification rates obtained from ^{15}N pool-dilution.

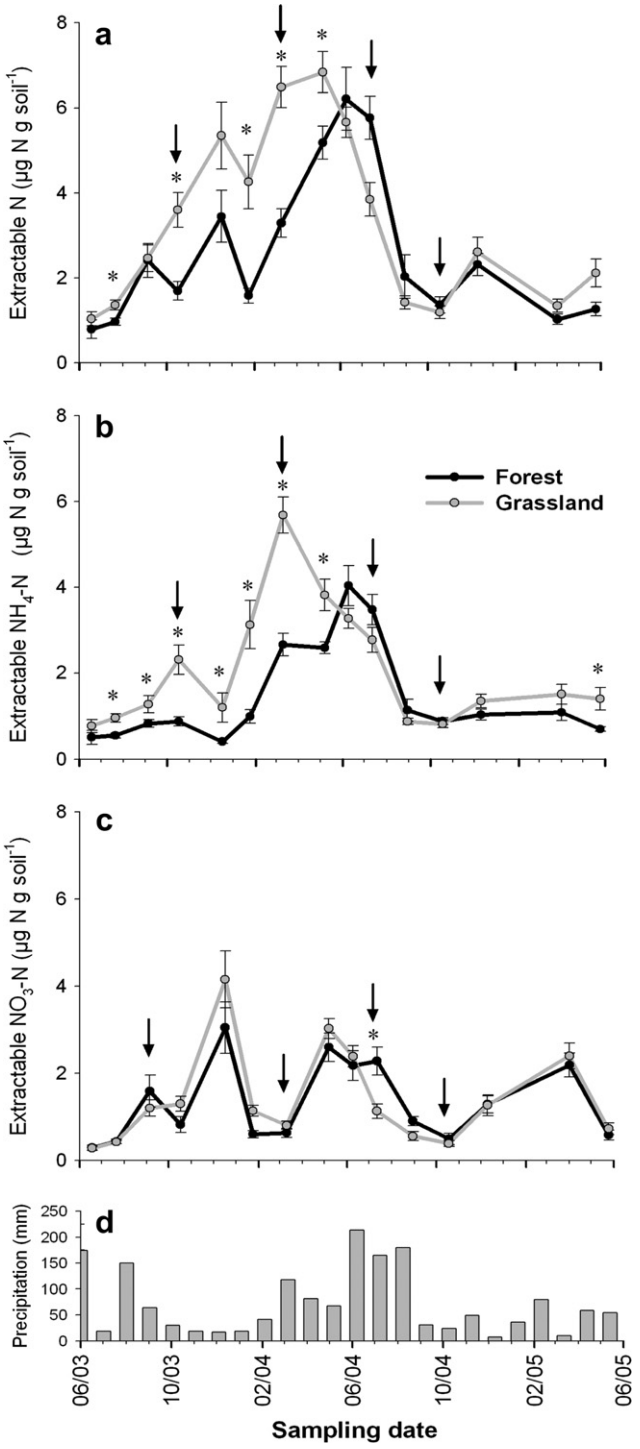

Fig. 1. Total soil extractable inorganic nitrogen (a), extractable NH_4^+ (b), extractable NO_3^- (c), and monthly precipitation (d) over a 2-year period in forest and grassland soils. Means ± 1SE ($n = 4$) with an asterisk indicate significant differences between grassland and forest soils (one-way ANOVA, $P \leq 0.05$). Only NH_4^+ concentrations were significantly greater over the entire sampling period (repeated-measures ANOVA, $P \leq 0.01$). Arrows represent times when soils were collected and used for laboratory-based assays of soil N cycle processes and estimates of microbial biomass.

There were no detectable changes in NO_3^- concentrations in acetylene-amended (control) samples, indicating no significant loss of NO_3^- from denitrification and that autotrophic nitrification was responsible for almost all of the nitrification in both grassland and

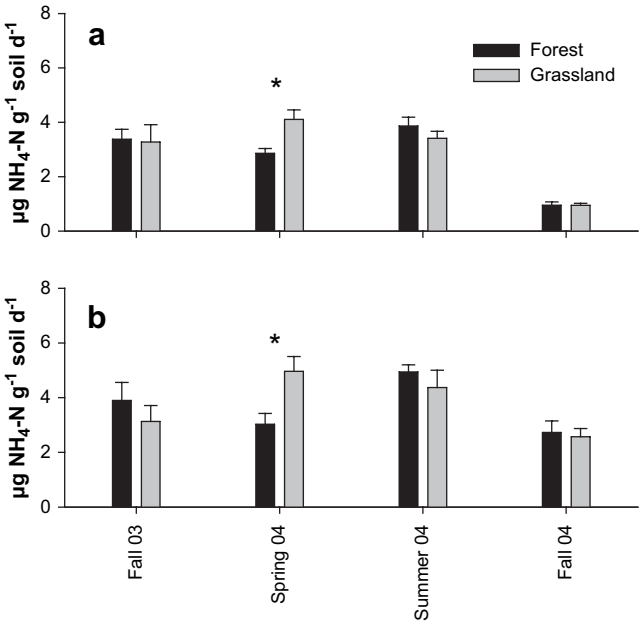

Fig. 2. Potential gross ammonification (a) and NH_4^+ consumption (b) in forest and grassland soils. Means ± 1SE ($n = 4$) with an asterisk indicate significant differences (i.e. spring 2004) between grassland and forest soils (one-way ANOVA, $P \leq 0.05$). Neither gross ammonification nor consumption was significantly different between vegetation types at any other time ($P > 0.05$).

forest soils (Fig. 4). Potential nitrification rates in fall 2004 were significantly greater than in the spring and summer for both vegetation types ($P < 0.001$, Tukey's HSD, Fig. 4).

3.3. Microbial biomass

Microbial biomass C and N pools, which compose a small, yet active, portion (3% C and 5–6% N) of the soil organic pools, were not

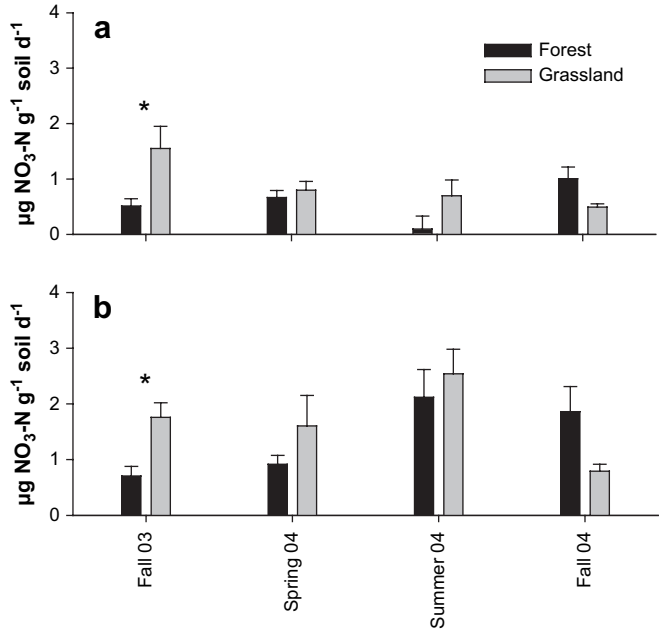

Fig. 3. Potential gross nitrification (a) and NO_3^- consumption (b) in forest and grassland soils. Means ± 1SE ($n = 4$) with asterisks indicate significant differences (i.e. fall 2003) between grassland and forest soils (one-way ANOVA, $P \leq 0.05$). Gross nitrification ($P = 0.059$) and consumption ($P = 0.062$) in fall 2004 were marginally significantly different in forest soils; no significant differences were detected at any other measured time ($P > 0.05$).

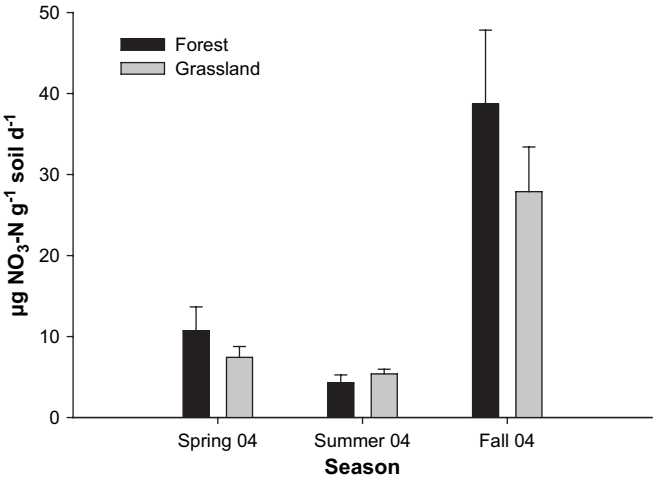

Fig. 4. Potential nitrification (V_{max}) in forest and grassland soils. Means ± 1SE ($n = 4$) were not significantly different between forest and grassland soils at any time ($P > 0.05$).

different ($P > 0.05$) between forest and grassland soils in either growing or non-growing seasons. Microbial biomass C (μg C g^{-1} soil) was 1,367 ± 128 in forest soils compared to 1,128 ± 53 in grassland soils across seasons. Microbial biomass N (μg N g^{-1} soil) was 175 ± 5 in forest soils and was 164 ± 10 in grasslands across seasons.

4. Discussion

Given the major change in vegetation type, from C_4 grass-dominated grassland to conifer-dominated forest, and the length of time over which this change had taken place (45–50 years on average), we expected substantial changes in soil N pools, as well N transformations. Similarities in soil physical and chemical properties other than SOM and Ca^{2+}, confirm that edaphic characteristics were probably similar before conversion to forest. There were some differences in concentrations of extractable soil inorganic N, particularly NH_4^+, across the 2-year study, but these differences occurred mainly in year one (Fig. 1b). Contrary to expectations, potential rates of key soil N transformations under these divergent vegetation types were surprisingly similar given observed differences in long-term environmental conditions in the field, in quantity and quality of plant litter inputs, and SOM content (Norris et al., 2001b; McKinley et al., 2007; McKinley and Blair, 2008), all of which could affect mineralizable C and N fractions and the soil microbial community, and consequently, potential N transformation rates assessed under controlled laboratory conditions.

By controlling soil temperature and moisture content in laboratory assays we were able to assess potential differences in N transformation rates due to differences in substrate quantity/quality or microbial community composition. We expected greater potential gross N mineralization in forest soils compared to grassland soils, owing to significantly greater concentrations of SOM, including SON (Table 1). Gross N mineralization correlates well with total SOM across a broad range of soils (Booth et al., 2005). In contrast, when significant differences existed between vegetation types, grassland soils had higher rates of potential gross ammonification and nitrification. Although differences in gross N fluxes in grassland and forest soils were occasionally large (3-fold), this may not reflect differences in plant available N, since consumption of NH_4^+ and NO_3^- was commensurate with production (resulting in similar potential net N ammonification and nitrification rates).

These findings are consistent with concurrent monthly field measurements of *in situ* net N mineralization and nitrification rates (McKinley and Blair, 2008) which were similar in both ecosystems. In the present study, we speculate that the differences in gross N transformations on specific sample dates may have been due to differences in availability of labile N substrates, and perhaps the residual effects of different field environmental conditions on soil microbial communities and potential function.

Juniperus virginiana, is an evergreen, able to photosynthesize year round (Knapp et al., 2007), which may extend plant uptake of inorganic soil N throughout the year. In contrast, the dominant grasses and forbs are dormant during fall and winter. Plant uptake of N by *J. virginiana* may have contributed to observed differences in extractable NH_4^+ in fall through early spring in the first year of study (Fig. 1b). The ability of these forests to utilize available NH_4^+ year round may also exert greater substrate limitation on nitrification than in grassland. A trend for somewhat greater *in situ* net N mineralization in forest sites during the same 2-year period (McKinley and Blair, 2008) suggests that differences in extractable N pools are due to greater uptake by forest vegetation rather than reduced N supply or increased microbial N immobilization. Relatively large amounts of growing season precipitation in the second year of the study (Fig. 1d) appeared to correspond with decreased extractable N (Fig. 1a). Plant growth stimulated by increased growing season precipitation could have altered soil N cycling and N availability by increasing inorganic N uptake, as well as altering soil C inputs and microbial demand for N.

Booth et al. (2005) found that grassland SOM generally produced more NH_4^+ than SOM from coniferous forests, owing to differences in the C:N ratios of SOM. We did not see a significant change in the soil C:N ratio despite significant accrual of SOC in forested sites (Table 1), which is consistent with the limited differences in soil N transformations reported here, although there was also considerable inter-annual and seasonal variability in the magnitude and direction in N flux rate measurements. In spring 2004 (Fig. 2a), we found significantly greater gross N mineralization rates in grassland soils compared to forest soils. Previous studies have also indicated highest concentrations of extractable N early in the growing season in these grasslands (Blair, 1997) and extractable N concentrations increased dramatically (Fig. 1a) in spring 2004 in the present study. This may reflect an increase in labile N in grassland soils in the spring (as suggested by greater potential gross mineralization rates), or decreased plant N demand before grasses "green up" that could allow greater accumulation of inorganic soil N.

The magnitude of gross nitrification, about one-third of the total N mineralized, and high potential nitrification (V_{max}) in both forest and grassland soils indicate that nitrification is not trivial. Gross nitrification:V_{max} ratios demonstrate that gross nitrification in both ecosystem types is strongly limited by substrate (NH_4^+) availability since these soils only reach, on average, approximately 7% and 10% of their potential nitrification (V_{max}) rates across all seasons for forest and grassland soils, respectively (see Figs. 3a and 4). Low ratios of gross nitrification:V_{max} suggest that the maintenance energy requirements of existing nitrifier populations may not be met (Davidson et al., 1990). Significantly lower gross nitrification rates in forest were found in fall 2003, but the direction of differences was not consistent between ecosystem types, with measurements in fall 2004 showing the opposite trend ($P = 0.059$) (Fig. 3a). Lower concentrations of NH_4^+ (Fig. 1b) were observed in forest soils in fall 2003, but it is unclear if differences in potential gross nitrification rates in fall 2003 (Fig. 3a) were due to differences in NH_4^+ availability or nitrifier communities. That potential nitrification rates (V_{max}) remained very high at all times in both ecosystems, suggests that the supply of NH_4^+ in both ecosystems is an important control of nitrification in these soils.

The importance of nitrification has been demonstrated in other grassland ecosystems (Schimel et al., 1989; Davidson et al., 1990; Corre et al., 2002), but could have been overlooked in these forest and grassland soils, due to low extractable NO_3^- concentrations (Fig. 1c) and net nitrification rates (McKinley and Blair, 2008). Dell and Rice (2005) found that NO_3^- was rapidly immobilized in burned grassland soils (located near our study site) and after 6 days, about 75% of the immobilized NO_3^- was found in SON and nearly all of remaining portion in plant biomass. Forest soils also have the potential for rapid NO_3^- immobilization and retention (Woodmansee et al., 1981; Davidson et al., 1992; Berntson and Aber, 2000; Corre et al., 2007). The large magnitude of microbial NO_3^- immobilization in both forest and grassland may be an important N retention mechanism, which would be particularly important in grassland soil in the non-growing season or immediately after a fire, when plant uptake is minimal or absent.

Based on our initial predictions, we expected that microbial immobilization of inorganic N would be greater in *J. virginiana* forest soils. However, a metric of microbial N immobilization suggests much less total N immobilization in forest soils, at least under controlled conditions and standardized for SOC content (potential yield). Using estimates of gross mineralization minus immobilization processes (NH_4^+ immobilization (gross NH_4^+ consumption − gross nitrification) + NO_3^- immobilization) we found that microbes in forest soils immobilize less N (gross) per gram of organic soil C (102.3 ± 10.0 μg N g^{-1} soil-C day^{-1}) than grassland soils (126.7 ± 12.1 μg N g^{-1} soil-C day^{-1}) when averaged across all seasons ($P = 0.056$). But, decreased microbial N immobilization in forest soils would not necessarily result in greater plant available N or net N mineralization, because gross ammonification (production) (μg N g^{-1} soil-C d^{-1}) was also significantly less ($P = 0.013$) per gram of organic soil C. Lower yield of inorganic N in forest soils suggests lower quality SON compared to grasslands. But, greater SOM in forest soils appears to offset differences in potential inorganic N yield, resulting in similar gross production/consumption rates in bulk soils. Therefore, differences in potential plant available N between forest and grassland soils resulting from competing microbial processes appear to be minimized.

5. Conclusions

The conversion of native grasslands to *J. virginiana* forests had relatively minor effects on potential soil N transformations and plant available N, at least after 45–50 years of forest establishment. In general, potential soil nitrogen transformation rates, microbial biomass (labile N pools), and potential activity of nitrifiers in *J. virginiana* forests were surprisingly similar to native grasslands, especially considering differences in SOM and environmental conditions in the field. Both *J. virginiana* forest and grassland had minimal potential N losses via nitrification, thus, the additional soil N accrual in forest is more likely due to exclusion of fire than greater conservation of soil NO_3^-. Greater plant N demand in *J. virginiana* forests and different microclimatic conditions could have greater influence on these N processes in the field than we detected in our controlled laboratory assays, as evidenced by differences in extractable N concentrations in field-collected soils. But, previous studies suggest only minor differences in *in situ* net N mineralization rates (McKinley and Blair, 2008). Significant N accrual in *Juniperus* ecosystems (McKinley and Blair, 2008) may be paramount for producing SOM with similar C:N ratios compared to grasslands, thereby maintaining similar rates of microbial production and consumption of inorganic N. Maintenance of internal N transformations in these new forests despite large changes in many other ecosystem properties (i.e., increased plant productivity and biomass, accumulating SOC stocks) may contribute to sustained forest productivity during this ecosystem transition.

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