Changes in soil nitrogen storage and δ^{15}N with woody plant encroachment in a subtropical savanna parkland landscape

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[1] Subtropical woodlands dominated by N-fixing tree legumes have largely replaced grasslands in the Rio Grande Plains, southwestern United States, during the past century. To evaluate the impact of this vegetation change on the N cycle, we measured the mass and isotopic composition (δ^{15}N) of N in the soil system of remnant grasslands and woody plant stands ranging in age from 10 to 130 years. Nitrogen accumulated at linear rates following woody encroachment in the litter (0.10–0.14 g N m^{-2} yr^{-1}), roots (0.63–0.98 g N m^{-2} yr^{-1}), and soils (0.75–3.50 g N m^{-2} yr^{-1}), resulting in a 50%–150% increase in N storage in the soil system (0–30 cm) in woody stands older than 60 years. Simultaneous decreases in soil δ^{15}N of up to 2‰ in the upper 30 cm of the profile are consistent with a scenario in which N inputs have exceeded losses following woody encroachment and suggest N accrual was derived from symbiotic N fixation by tree legumes and/or differential atmospheric N deposition to wooded areas. Vertical uplift and lateral transfer of N by the more deeply and intensively rooted woody plants may have contributed to N accumulation in wooded areas, but soil δ^{15}N values are inconsistent with this explanation. N accumulation following woody encroachment may alter soil N availability, species interactions and successional dynamics, flux rates of key trace gases such as NO$_X$ and N$_2$O and ecosystem C sequestration. Given the geographic dimensions of woody encroachment, these results may have implications for atmospheric composition and the climate system.

1. Introduction

[2] Woody plant encroachment into grass-dominated systems has been among the most important global land cover changes over the past 100–200 years [*Van Auken*, 2000, 2009; *Archer et al.*, 2001; *Asner et al.*, 2004; *Tape et al.*, 2006; *Maestre et al.*, 2009]. This widespread shift in land cover is generally attributed to human land use activities (particularly livestock grazing and fire suppression) but could also be influenced by changes in climate, atmospheric composition, and atmospheric deposition [*Bond and Midgley*, 2000; *Archer et al.*, 2001; *Tape et al.*, 2006; *Wigley et al.*, 2010]. In the Rio Grande Plains of the southwestern United States, subtropical thorn woodlands dominated by N-fixing tree legumes have largely replaced grassland over the last century [*Archer et al.*, 1988; *Boutton et al.*, 1998]. Four of the most dominant woody species in this region (*Prosopis glandulosa* [Torr.] var. *glandulosa*, *Acacia rigidula* Benth., *Acacia farnesiana* (L.) Willd., and *Acacia berlandieri* Benth.) are known to be capable of symbiotic N fixation [*Johnson and*

Mayeux, 1990; *Zitzer et al.*, 1996]. Because many arid/semiarid plant communities generally exhibit low productivity due to the constraints of water stress and N limitation [*Rundel et al.*, 1982; *Hooper and Johnson*, 1999; *Bustamante et al.*, 2006], the establishment of these N-fixing tree species in grasslands has strong potential for altering the N cycle, primary production, and other key ecosystem processes [*Bush and Van Auken*, 1986; *Vitousek and Walker*, 1989; *Schlesinger et al.*, 1990; *Stock et al.*, 1995; *Pugnaire et al.*, 1996].

[3] Plant species influence ecosystem N cycling via alterations in N use efficiency and by changing N inputs and losses [*Knops et al.*, 2002]. Changes in plant species composition, particularly woody plant invasions, have been shown to increase the storage, turnover, and availability of N in many ecosystems [*Liao et al.*, 2008; *Rout and Callaway*, 2009]. In the Rio Grande Plains, grassland-to-woodland conversion has resulted in increases in aboveground and belowground productivity in woodlands relative to adjacent remnant grasslands [*Archer et al.*, 2001, 2004; *Hibbard et al.*, 2001]. The increased productivity resulting from grassland-to-woodland conversion in the Rio Grande Plains region is accompanied by changes in the quality and quantity of litter and root inputs to the soil [*Filley et al.*, 2008], the storage of N in plant biomass and soils [*Archer et al.*, 2004; *Northup et al.*, 2005], the size and activity of the soil microbial biomass pool [*Liao and*

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Boutton, 2008], and rates of N mineralization and nitrification [*Hibbard et al.*, 2001; *McCulley et al.*, 2004]. Despite the significance of these changes in ecosystem properties and processes, little is known regarding rates and mechanisms by which N stores increase following woody plant encroachment.

[4] The natural abundance of soil ^{15}N is an integrator of N cycling processes and is affected by both the biotic and abiotic environment [*Robinson*, 2001; *Dawson et al.*, 2002; *Evans*, 2007]. However, a complete understanding of the controls over patterns of δ^{15}N in the plant-soil system is still lacking. Globally, patterns of plant and soil δ^{15}N values are related to precipitation and temperature with δ^{15}N values decreasing with increasing mean annual precipitation and decreasing mean annual temperature [*Handley et al.*, 1999; *Amundson et al.*, 2003; *Craine et al.*, 2009]. Thus, arid and semiarid regions are predicted to have the highest soil δ^{15}N, with values ranging from about 6.2‰ to 10.3‰ [*Amundson et al.*, 2003]. Locally, soil δ^{15}N values are generally more enriched than plant and litter δ^{15}N and tend to increase with depth in the soil profile; these trends reflect the cumulative effects of N isotope fractionation during decomposition and humification [*Shearer et al.*, 1978; *Nadelhoffer et al.*, 1996; *Koba et al.*, 1998]. However, soil δ^{15}N values are also influenced by a number of factors such as quantity and quality of litter inputs, soil N sources, and isotopic fractionation resulting from N transformations [*Nadelhoffer and Fry*, 1988; *Piccolo et al.*, 1994; *Evans*, 2007].

[5] Although multiple factors influence soil δ^{15}N values, alterations to the soil total N pool via a change in the balance of N inputs versus losses should be apparent in the natural abundance of ^{15}N in the plant-soil system, particularly in arid and semiarid environments where soil δ^{15}N values are relatively high. First, N inputs from the atmosphere (N fixation, wet and dry deposition) add N with low δ^{15}N values of approximately −3‰ to +3‰ [*Handley et al.*, 1999; *Amundson et al.*, 2003] and second, nearly all N transformations lose ^{14}N enriched N, leaving the product depleted and the residual N substrate relatively enriched in ^{15}N [*Peoples et al.*, 1991; *Hopkins et al.*, 1998; *Robinson*, 2001]. Hence, changes in the δ^{15}N of soil total N should reflect the net result of input processes that generally deliver ^{15}N depleted N to the system and loss processes that generally leave the residual N pool enriched in ^{15}N.

[6] The purpose of this study is to investigate the consequences of grassland invasion by N-fixing tree legumes on the N cycle of a subtropical savanna parkland landscape using a chronosequence approach. More specifically, this study (1) quantified rates of N accumulation in litter, root biomass, and soil in woody plant stands differing in age and (2) utilized δ^{15}N values of litter, roots, and soil to provide qualitative insights into changes in N cycling processes following grassland-to-woodland transitions.

2. Methods and Materials

2.1. Study Area

[7] Field sampling was conducted in December 2001 at the Texas AgriLife LaCopita Research Area (27°40′N, 98°12′W) located 65 km west of Corpus Christi, TX, USA in the eastern Rio Grande Plains. Climate is subtropical, with a mean annual temperature of 22.4°C. Mean annual

precipitation is 715 mm and is bimodally distributed with peaks in May–June and September. Topography consists of nearly level uplands, which grade (1%–3%) into lower-lying drainage woodlands. Elevations range from 75 to 90 m. This site has been grazed by domestic livestock over the past century.

[8] Uplands have sandy loam soils (Typic and Pachic Argiustolls) with a laterally continuous subsurface argillic horizon and are dominated by C_4 grasslands interspersed with small, discrete clusters of woody plants. Grasses of the genera *Paspalum*, *Bouteloua*, *Chloris*, and *Eragrostis* dominate the grasslands. The dominant plant in all wooded landscape elements is *Prosopis glandulosa* [Torr.] var. *glandulosa* (honey mesquite), an N-fixing tree legume [*Johnson and Mayeux*, 1990, *Zitzer et al.*, 1996]. Discrete woody clusters are characterized by a *Prosopis* overstory with species of *Condalia hookeri* (M.C. Johnst.), *Berberis trifoliolata* (Moric.), and *Zanthoxylum fagara* (L.) dominating the understory. Clusters expand laterally and fuse to form larger groves of vegetation where the argillic horizon is absent. Soils in lower-lying portions of the landscape are finer-textured loamy sands and clay loams (Pachic Argiustolls) and support closed canopy woodlands. The vegetation composition of these lower-lying drainage woodlands is similar to that in upland clusters and groves. Evidence from present vegetation patterns, sequential aerial photography, tree ring analyses, models of vegetation dynamics, and the isotopic composition of soils all indicate that this region was once relatively open grassland and that woody plants have encroached into these grasslands over the past 150 years [*Archer et al.*, 1988; *Boutton et al.*, 1998; *Archer et al.*, 2001, 2004]. Additional details on plant communities and soils have been presented elsewhere [*Scifres and Koerth*, 1987; *Archer et al.*, 1988; *Boutton et al.*, 1998].

2.2. Chronosequence Approach

[9] A space-for-time, chronosequence approach was used to quantify N pool sizes, their isotopic composition, and their rates of change in litter, roots, and soils following woody plant encroachment into areas that were previously grassland. Ten sites were sampled within each of the three major upland landscape elements (remnant grasslands, clusters, and groves); 11 sites were sampled within the lower-lying drainage woodlands. All sites were located within a 2 km^2 area. Remnant grasslands were sampled to characterize N pool sizes at time 0 (i.e., prior to woody encroachment), whereas clusters, groves, and drainage woodlands of known age were sampled to evaluate changes in N pools at different points in time after woody invasion.

[10] Ages of clusters, groves, and drainage woodlands were based on the fact that the formation of these wooded landscape elements is initiated only after the establishment of *P. glandulosa* in grassland [*Archer et al.*, 1988]. Thus, the age of a woody plant stand corresponds to the age of the largest *P. glandulosa* tree in that stand. The ages of *P. glandulosa* trees were determined by measuring their basal diameters and then using those values to predict tree ages from regression equations specific to each landscape element [*Stoker*, 1997]. Woody plant stands sampled in this study were selected to encompass the full range of *P. glandulosa* basal diameters at this site, corresponding to tree ages ranging in age from approximately 10 to 130 years.

Table 1. Soil Physical and Chemical Characteristics of Grassland and Wooded Landscape Elements at LaCopita Research Area[a]

	0–15 cm				15–30 cm			
	Grassland	Cluster	Grove	Drainage	Grassland	Cluster	Grove	Drainage
pH	6.5 (0.1)[a]	6.4 (0.6)[a]	6.3 (0.1)[a]	6.2 (0.1)[a]	6.9 (0.6)[a]	6.4 (0.6)[b]	6.7 (0.5)[a]	6.3 (0.1)[b]
Texture	Loamy Sand	Loamy Sand	Loamy Sand	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Loam	Sandy Clay Loam
Sand (%)	80.5 (0.4)[a]	81.3 (0.5)[a]	81.4 (0.6)[a]	66.0 (3.0)[b]	74.1 (1.5)[a]	78.8 (0.9)[a]	78.0 (0.8)[a]	56.1 (4.1)[b]
Silt (%)	10.5 (2.0)[ab]	9.6 (1.6)[b]	12.0 (1.3)[ab]	15.4 (2.7)[a]	16.9 (1.9)[ab]	12.5 (2.2)[ab]	8.9 (2.2)[c]	21.0 (2.1)[a]
Clay (%)	9.0 (2.0)[b]	9.1 (1.6)[b]	6.6 (1.3)[b]	18.6 (1.9)[a]	9.1 (2.3)[b]	8.7 (2.0)[b]	13.1 (1.8)[b]	22.9 (2.8)[a]
Bulk Density (g cm^{-3})	1.2 (0.02)[a]	1.0 (0.03)[b]	1.0 (0.02)[b]	1.0 (0.04)[b]	1.3 (0.03)[a]	1.3 (0.02)[ab]	1.2 (0.02)[ab]	1.2 (0.02)[b]
SOC (g C kg^{-1} soil)	6.2 (0.3)[c]	11.2 (1.3)[bc]	13.5 (1.6)[b]	21.1 (3.1)[a]	5.0 (0.4)[b]	4.5 (0.3)[b]	5.7 (0.3)[b]	11.9 (1.3)[a]
C/N	11.3 (0.2)[ab]	10.8 (0.2)[b]	11.3 (0.3)[ab]	11.6 (0.2)[a]	11.1 (0.4)[b]	10.4 (0.2)[c]	10.4 (0.2)[c]	13.3 (0.4)[a]

[a]Fisher's LSD was used to separate differences between landscape elements within a depth interval following ANOVA. Different letters represent significant differences between means within a row for each depth interval. Standard errors of the mean are in parentheses.

2.3. Collection of Soil, Root, and Litter Samples

[11] Surface litter was collected from a 0.25-m^2 quadrat at each site. In wooded landscape elements, the quadrat was located within 0.5 m of the bole of the largest *P. glandulosa* tree. Litter samples were washed with water over a 2 mm sieve to remove adhering soil particles, dried at 60°C to constant weight, weighed, pulverized in a centrifugal mill (Angstrom, Inc., Belleville, MI), and saved for elemental and isotopic analyses.

[12] Four soil cores (5 cm diameter × 30 cm length) were taken within 0.5 m of the bole of the largest *P. glandulosa* at each wooded site, one in each cardinal direction from the bole. Sampling was identical in each remnant grassland site, but instead, centered around the base of a large C$_4$ grass plant. Soil surface litter was removed gently to expose mineral soil prior to taking each soil core. All soil cores were sectioned into 0–15 and 15–30 cm increments and stored at 4°C. In the laboratory, each soil sample was mixed thoroughly, and a subsample was dried at 105°C to determine bulk density. Then, the four cores from each site were pooled by depth increment and mixed. Sampling was confined to the upper 30 cm of the soil profile, because this interval encompasses the A horizon and because the greatest impacts of woody encroachment on root biomass and soil organic matter occur within this depth interval at this site [*Boutton et al.*, 1998, 1999].

[13] Prior to further treatment, an aliquot of each soil sample was set aside for determination of root biomass. Another aliquot was passed through a 2 mm sieve to remove large organic fragments and used for physical, chemical, and isotopic analyses. Soil pH was determined on a 1:2 (soil/water) mixture using a glass electrode [*McLean*, 1982]. Soil texture was determined by the pipet method [*Sheldrick and Wang*, 1993]. The remainder of the sieved aliquot was dried at 60°C, pulverized in a centrifugal mill, and used for elemental and isotopic analyses.

[14] Root biomass was quantified on well-mixed aliquots (100 g) from each pooled soil sample using a hydropneumatic elutriation system [*Smucker et al.*, 1982] (Gillison's Variety Fabrication, Inc., Benzonia, MI) equipped with a 410 μm screen. Roots were then dried at 60°C, weighed, pulverized with a mortar and pestle, and saved for elemental and isotopic analyses.

2.4. Elemental and Isotopic Analyses

[15] Litter, root, and soil samples were analyzed for C and N concentrations and for δ^{15}N values using a Carlo Erba EA-1108 (CE Elantech, Lakewood, NJ) interfaced with a Delta Plus (ThermoFinnigan, San Jose, CA) isotope ratio mass spectrometer operating in continuous flow mode. Nitrogen isotope ratios are presented in δ notation:

$$\delta^{15}N(‰) = [(R_{SAMPLE} - R_{STD})/R_{STD}] \times 10^3, \quad (1)$$

where R_{SAMPLE} is the ^{15}N/^{14}N ratio of the sample and R_{STD} is the ^{15}N/^{14}N ratio of the atmospheric N standard [*Mariotti*, 1983]. Precision was <0.15‰.

2.5. Statistical Analyses

[16] ANOVA was used to test for differences in soil physical and chemical characteristics and N stocks and their δ^{15}N values (in litter, roots, and soils) due to landscape element, soil depth, and their interaction. Fisher's LSD method was used to identify significant differences among measurements due to the main effect of landscape element within each depth increment following ANOVA. Regression analyses were performed using Sigma Plot 4.0 [*SPSS, Inc.*, 1997] to determine the significance of relationships between woody plant stand age and N pool sizes in litter, roots, and soil.

3. Results

3.1. Soil Characterization

[17] Soil pH was 6.5 in the upper 15 cm of the profile in remnant grasslands and was not altered following woody plant encroachment (Table 1). Bulk density of the 0–15 cm depth interval decreased from 1.2 g cm^{-3} in grasslands to 1.0 g cm^{-3} in wooded landscape elements (clusters, grove, drainage woodlands); at 15–30 cm, bulk densities ranged from 1.2 to 1.3 g cm^{-3} but differed only between grassland and drainage woodland. Upland soils beneath grasslands, clusters, and groves were loamy sands, with a particle size distribution of 80% sand, 10% silt, and 10% clay. In contrast, soils in the lower-lying drainage woodlands were sandy loams composed of 60% sand, 20% silt, and 20% clay. Soil organic C at 0–15 cm increased from 6 g C kg^{-1} soil in remnant grasslands to 11–21 g C kg^{-1} soil in wooded landscape elements; at 15–30 cm, soil organic C was 5 g C kg^{-1} soil in grasslands and 5–15 g C kg^{-1} soil in wooded areas (Table 1). Soil C/N ratios ranged from 10 to 13 and were largely unaffected by woody encroachment.

3.2. N Storage in Litter, Roots, and Soils Following Grassland-to-Woodland Conversion

[18] On average, N stocks in surface litter in the three wooded areas (6–14 g N m^{-2}) were 200%–600% greater
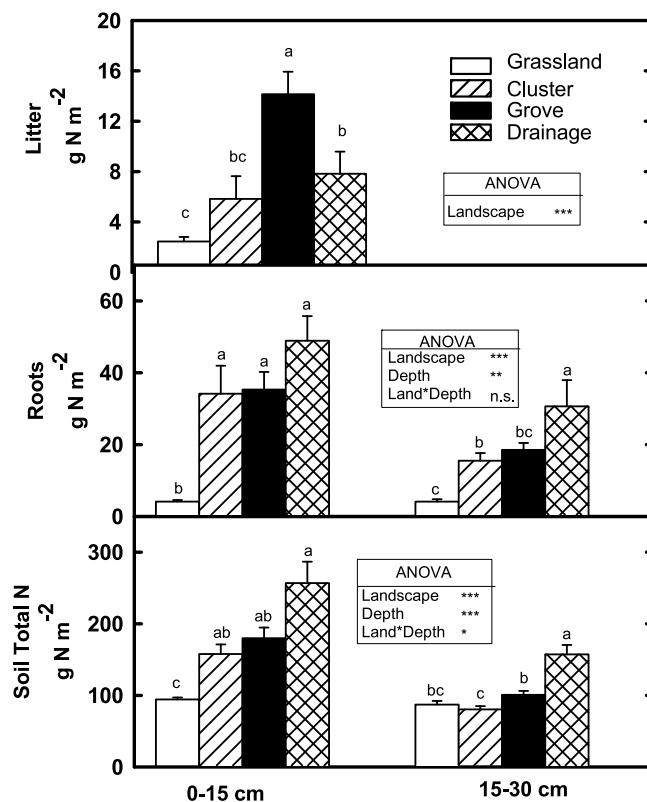
Figure 1. Nitrogen densities (g N m^{-2}) in litter, roots, and soil in different landscape elements (grassland, cluster, grove, drainage woodland) in a subtropical savanna parkland at LaCopita Research Area by soil depth (0–15 and 15–30 cm) ($n = 10$ for grasslands, clusters, and groves; $n = 11$ for drainage woodlands). Error bars are standard errors of the mean. Asterisks denote level of significance from ANOVA (single asterisk, $p < 0.05$; double asterisk, $p < 0.01$; triple asterisk, $p < 0.001$; n.s., not significant at $p < 0.05$). Different letters above bars indicate differences between means within a depth from Fisher's LSD.

than those in grassland litter (2 g N m^{-2}) (Figure 1). Nitrogen stocks in root biomass at 0–15 cm in wooded areas (35–50 g N m^{-2}) were significantly greater (600%–900%) than N in grassland root biomass (5 g N m^{-2}). At 15–30 cm, N stocks in root biomass in wooded areas (15–30 g N m^{-2}) were 200%–500 % greater than N stocks in grassland roots (5 g N m^{-2}). Soil total N in the 0–15 cm depth increment was 50%–150% greater in soils from wooded areas (150–250 g N m^{-2} soil) than in soil from remnant grasslands (100 g N m^{-2} soil at 0–15 cm); differences in soil N between grasslands and wooded areas were less pronounced at 15–30 cm (Figure 1).

[19] Pool sizes of N (g N m^{-2}) in litter, roots, and soil increased linearly over the past 100–130 years on those portions of the landscape where woody plants have encroached into grassland (Figure 2). Woody plant stand age accounted for a significant proportion of the variation (approximately 30%–80%) in the N stocks in litter and in both roots and soils in the upper 15 cm of the profile. Accumulation rates for N in litter, roots, and soil were derived from the slopes of the linear regression fit to the data

in Figure 2 (Table 2). Nitrogen accumulation rates in whole soil to a depth of 30 cm ranged from 0.75 to 3.50 g N m^{-2} yr^{-1}. Most of the accumulation (75%–83%) was in the upper 15 cm of the soil profile. Nitrogen accumulation in litter accounted for only 2%–6% of N accumulation rates in the soil system. In contrast, to litter, accumulation rates of N in roots comprised 20%–40% of the N accumulation rates in whole soil (0–30 cm). Overall accumulation rates for the entire soil system (litter + roots + soil) ranged from 1.58 g N m^{-2} yr^{-1} in upland clusters to 4.64 g N m^{-2} yr^{-1} in lower-lying drainage woodlands.

3.3. Changes in Soil δ^{15}N Following Grassland-to-Woodland Conversion

[20] On average, δ^{15}N values of surface litter were not significantly different among landscape elements, ranging from 2.0‰ to 2.5‰ (Figure 3). However, δ^{15}N values of roots in surface soils (0–15 cm) of wooded areas (2.0–2.4‰) were significantly lower than those in grasslands (3.2‰). In the 15–30 cm depth increment, δ^{15}N values of roots in both grasslands (2.1‰) and woodlands (0.6‰) were more depleted than those in surface soils. The δ^{15}N values of soil total N at 0–15 cm were significantly higher in grasslands (7.5‰) than in all three wooded areas (6.0–6.4‰). This pattern was similar at 15–30 cm, but δ^{15}N values at this depth were more enriched than surface soil values by 1–2‰.

[21] The δ^{15}N values of soil total N in the 0–15 cm depth increment decreased with time following woody encroachment from 7.5‰ in remnant grasslands to 6‰ in clusters, groves, and drainage woodlands (Figure 4). This 1.5‰ decrease occurred largely within the first 60 years of woodland development, and δ^{15}N values remained relatively constant from 60 to 120 years following woody plant encroachment. At 15–30 cm, whole-soil δ^{15}N values decreased over time following woody encroachment from 8.9‰ in remnant grasslands to 8‰ in clusters and to 7‰ in groves and drainage woodlands. The δ^{15}N values of litter and roots changed little over time following woody plant establishment, and their relationships with woody plant stand age were not significant.

4. Discussion

4.1. Storage and Accumulation of N in Litter, Roots, and Soils

[22] Nitrogen storage in litter, roots, and soil has increased linearly over the past 130 years following woody plant invasion of grassland in the Rio Grande Plains of southern Texas. Soil total N increased 30%–130% in the upper 30 cm of the soil profile following grassland-to-woodland conversion (Figure 1). These increases in soil total N storage are similar in magnitude to those reported in other dryland ecosystems experiencing woody plant encroachment [*Tiedemann and Klemmedson*, 1973; *East and Felker*, 1993; *Zavaleta and Kettley*, 2006; *Wheeler et al.*, 2007; *McKinley and Blair*, 2008; *Springsteen et al.*, 2009]. Woodland soils (0–30 cm) in these areas in southern Texas are accumulating soil N at an average rate of 1.9 g N m^{-2} yr^{-1}. This rate is comparable to those estimated for woodland soils in the upper 20 cm of the profile by simulation modeling using CENTURY (0.6–1.2 g N m^{-2} yr^{-1}) and by previous field studies (1.90–4.64 g N m^{-2} yr^{-1}) at this same site [*Archer et al.*, 2001, 2004]. The average N accumulation rates for woodland soils in this study is also com-
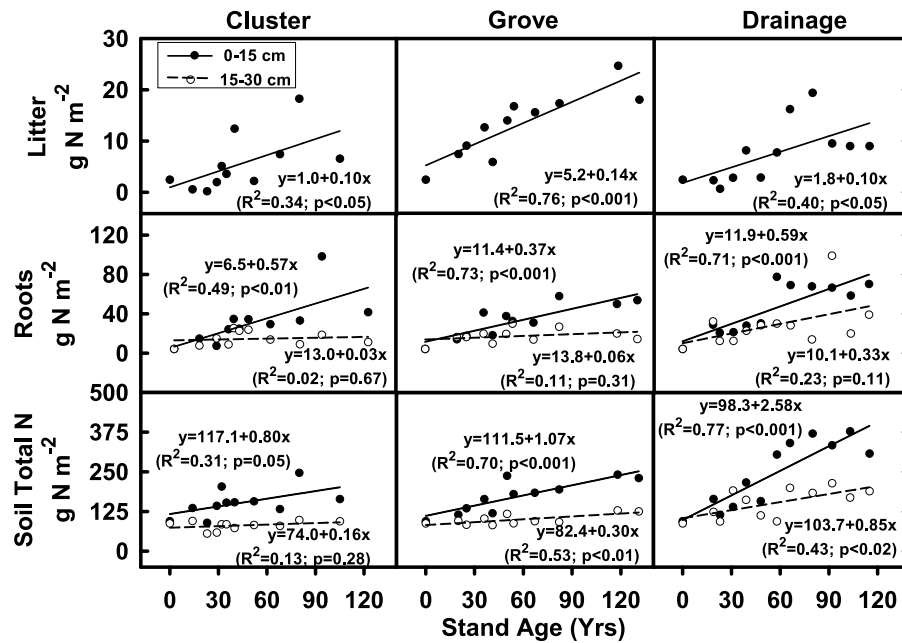
Figure 2. Nitrogen densities (g N m^{-2}) in litter, roots, and soil relative to woody plant stand age in different landscape elements (cluster, grove, drainage woodland) in a subtropical savanna parkland. In each frame, time 0 represents values for remnant grasslands.

parable to estimated N accumulation rates of 1–2 g N m^{-2} yr^{-1} for soils in other dryland ecosystems experiencing woody plant encroachment [*Rundel et al.*, 1982; *Geesing et al.*, 2000; *Neff et al.*, 2009; *Springsteen et al.*, 2009].

[23] Patterns of soil N storage and accumulation varied across the landscape, reflecting spatial variation in soil texture and soil moisture. Highest soil N accumulations occurred in the drainage woodlands. The more favorable soil moisture regimes in these lower-lying portions of the landscape [*Bai et al.*, 2008a] may enable higher rates of aboveground and belowground primary production and N fixation, and the higher silt and clay content there may effectively stabilize these organic matter inputs and protect them from decay [*Boutton et al.*, 2009b]. In contrast, lower N accumulations in clusters and groves are likely due to the coarser soil textures in uplands, which afford less physical protection for organic matter. Similarly, in a Sonoran Desert ecosystem, highest N accumulations occurred in soils beneath mesquite on lower elevational sites with high clay content while lower accumulations of soil N were observed beneath mesquite on sandy dunes [*Virginia and Jarrell*, 1983].

[24] It should be emphasized that N storage and rates of N accrual documented here (and most other studies of woody encroachment) are based on soil samples acquired from only a single location within a woody patch. In this study, all samples were taken near the bases of *P. glandulosa* trees, which are known to initiate and facilitate woody patch development at our site, and therefore represent the oldest portions of the woody patches [*Archer et al.*, 1988]. However, there can be significant spatial variation in soil C and N within woody patches, with pool sizes decreasing rapidly from the centers of woody patches (usually the oldest portion) out toward the woody patch/grassland edge [*Millard et al.*, 2008; *Throop and Archer*, 2008]. Thus, our estimates of N storage and accrual are likely to be maximal values for clusters, groves, and drainage woodlands.

[25] The significant increases in soil total N in this ecosystem indicate that N inputs have exceeded outputs following woody invasion of grassland during the past 100 years. What are the mechanisms responsible for N accrual in this landscape? We suggest that N accumulation in wooded areas may be due to (1) symbiotic N fixation by tree legumes that dominate the woody plant assemblages,

Table 2. Accumulation Rates of N in Litter, Roots, Soil, and the Total Soil System[a]

		Roots			Soil			
	Litter	0–15 cm	15–30 cm	0–30 cm[c]	0–15 cm	15–30 cm	0–30 cm[c]	Total
Cluster	0.10[d]	0.57[d]	0.03	0.63[d]	0.80[d]	0.16	0.75[d]	1.58[d]
Grove	0.14[d]	0.37[d]	0.06	0.61[d]	1.07[d]	0.30[d]	1.43[d]	2.15[d]
Drainage	0.10[d]	0.59[d]	0.33	0.98[d]	2.58[d]	0.85[d]	3.50[d]	4.64[d]

Accumulation Rates (g N m^{2} yr^{-1})[b]

[a]Litter + roots + soil.
[b]N accumulation rates are derived from the slopes of the regression lines in Figure 2.
[c]Rates from 0 to 30 cm for the total soil system were obtained by performing separate regressions on summed (0–15 cm + 15–30 cm) data.
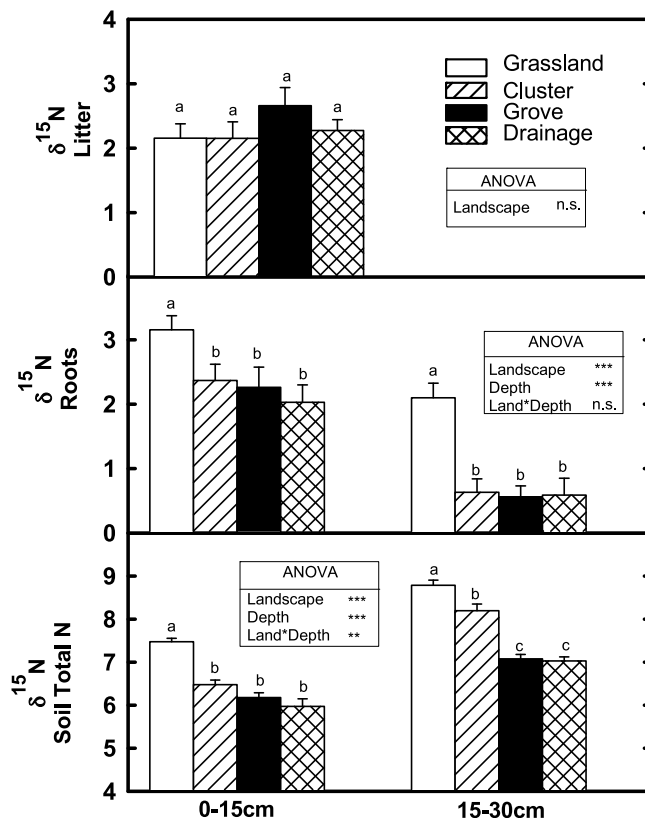[d]Linear regression significant at $p < 0.05$.

Figure 3. $\delta^{15}N$ values of litter, roots, and soil total N in different landscape elements (grassland, cluster, grove, drainage woodland) in a subtropical savanna parkland. Error bars are standard errors of the mean. Asterisks denote level of significance from ANOVA (single asterisk, $p < 0.05$; double asterisk, $p < 0.01$; triple asterisk, $p < 0.001$; n.s., not significant at $p < 0.05$). Different letters above bars represent significant differences among means within a depth from Fisher's LSD.

(2) differential atmospheric N deposition to woody plant canopies compared to adjacent remnant grasslands, and/or (3) concentration of N in surface soils through vertical uplift and lateral transfer of N by the more deeply and intensively rooted woody plants.

4.2. Role of Symbiotic N Fixation by Tree Legumes

[26] Temporal decreases in soil $\delta^{15}N$ values at both 0–15 and 15–30 cm depths in clusters, groves, and drainage woodlands (Figure 4) are consistent with a situation where N inputs have exceeded N losses following woody plant encroachment. Because ecosystem N inputs via atmospheric deposition ($\delta^{15}N = -3‰$ to $+3‰$) and N_2 fixation ($\delta^{15}N = -2‰$ to $+1‰$) are ^{15}N depleted relative to soil total N in our study area (6‰–9‰) and because gaseous N losses via microbial transformations (NH_3, NO_x, N_2O, N_2) are also ^{15}N depleted ($\delta^{15}N = -40‰$ to $0‰$), changes in inputs and/or loss rates can alter the $\delta^{15}N$ of the soil total N pool [*Hogberg*, 1997; *Handley et al.*, 1999; *Robinson*, 2001; *Evans*, 2007]. When N inputs exceed losses at this site, the soil total N pool should become ^{15}N depleted (i.e., lower $\delta^{15}N$); conversely, when losses exceed inputs, the soil total N pool should become ^{15}N enriched (i.e., higher $\delta^{15}N$) due to loss of ^{15}N

depleted gaseous N. Soil total N cannot become depleted or enriched in ^{15}N without the transfer of N into or out of the soil [*Handley and Raven*, 1992].

[27] One of the most likely mechanisms that can account for this temporal decrease in soil $\delta^{15}N$ in wooded landscape elements is symbiotic N fixation, a known source of isotopically light N inputs ($\delta^{15}N = -2‰$ to $1‰$) to ecosystems. *P. glandulosa* (the dominant woody plant in clusters, groves, and drainage woodlands) is well known for its ability to fix atmospheric N throughout its biogeographic range [*Johnson and Mayeux*, 1990], and has been shown to develop active root nodules when grown in soils from this study area [*Zitzer et al.*, 1996]. The $\delta^{15}N$ values of *P. glandulosa* foliage are significantly lower ($\delta^{15}N = 1.0‰$) than other woody species ($\delta^{15}N = 2‰$–4‰) in this landscape [*Bai et al.*, 2008b], as would be expected for a plant deriving some portion of its N from the atmosphere. In addition, roots in wooded landscape elements ($\delta^{15}N = 2‰$) are isotopically more depleted than roots in remnant grasslands ($\delta^{15}N = 3‰$) (Figure 3). Since *P. glandulosa* roots comprise 50%–80% of total root biomass in wooded landscape elements at this site [*Watts*, 1993], these low root $\delta^{15}N$ values may also indicate that *P. glandulosa* is acquiring some of its N from fixation and contributing to the temporal decline in soil $\delta^{15}N$ values. Perhaps due to these relatively depleted organic matter inputs, soil $\delta^{15}N$ values decreased by approximately 1.5‰–2.0‰ within 40 years following woody encroachment in both 0–15 and 15–30 cm depth increments (Figure 4).
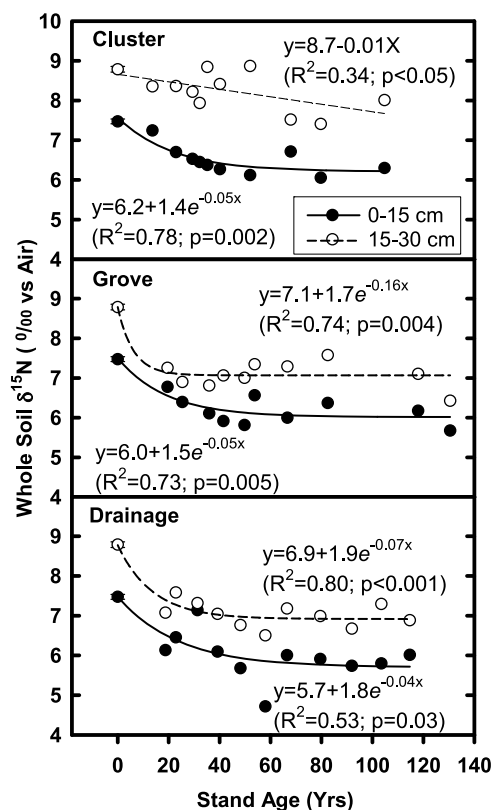

Figure 4. Changes in $\delta^{15}N$ values of soil total nitrogen with respect to soil depth and woody plant stand age in a subtropical savanna parkland. Time 0 in each frame is the $\delta^{15}N$ value of soil total nitrogen in remnant grasslands.

Others have reported similar dilutions of soil $\delta^{15}N$ values by N derived from N-fixing tree species [*Shearer and Kohl*, 1989; *Peoples et al.*, 1991; *Stock et al.*, 1995]. Estimates of N derived from fixation in mesquite-dominated woodlands in the southwestern United States range from approximately 4 to 15 g N m^{-2} yr^{-1} [*Rundel et al.*, 1982; *Jarrell and Virginia*, 1990; *Johnson and Mayeux*, 1990]. These estimates are more than adequate to account for the observed N accumulations in the soil system (litter + roots + soil) in wooded portions of this landscape. Estimated inputs of N from nonsymbiotic N fixation by free-living cyanobacteria in similar ecosystems range from 0.1 to 0.5 g N m^{-2} yr^{-1} [*Boring et al.*, 1988; *Cleveland et al.*, 1999], but it is unknown if rates of this process differ between grasslands versus wooded landscape elements.

[28] We emphasize that rates of N accumulation following woody plant encroachment comparable to those quantified in this study (1.58–4.64 g N m^{-2} yr^{-1}) have been documented in other regions where woody encroachment has occurred but where none of the encroaching species are known to be capable of symbiotic N fixation. For example, invasion by the shrub *Baccharis pilularis* into coastal California grassland resulted in a 20% increase in soil N storage and N accrual rates of 3.5 g N m^{-2} yr^{-1} in the upper 30 cm of the soil profile [*Zavaleta and Kettley*, 2006]. Pinyon pine encroachment into semiarid shrub steppe in the southwestern United States increased soil N storage by 169% and caused N accumulation rates of 1 g N m^{-2} yr^{-1} in the 0–30 cm depth increment [*Neff et al.*, 2009]. Invasion of non-N-fixing shrub communities into mixed grasslands of the northern Great Plains caused soil total N to increase by 30% at a rate of 1.7 g N m^{-2} yr^{-1} in the 0–15 cm depth increment [*Springsteen et al.*, 2009]. And, a recent meta-analysis showed that N storage in roots and soils generally increases by 112% and 19%, respectively, following woody plant invasions [*Liao et al.*, 2008]. Hence, mechanisms other than N fixation by the encroaching woody plant species are clearly capable of accounting for rates of N accrual comparable to those documented in this savanna landscape.

4.3. Differential Atmospheric N Deposition to Woody Plant Canopies

[29] Another potential mechanism for increased N inputs in woodland soils is more efficient entrapment of atmospheric deposition by woody plant canopies. The greater canopy height and leaf area in woodlands versus grasslands [*Asner et al.*, 1998] may provide more surface area that can capture bulk atmospheric N deposition, resulting in greater accumulation of this source in wooded areas [*Pugnaire et al.*, 1996; *Weathers et al.*, 2001]. $\delta^{15}N$ values of atmospheric deposition are generally depleted [*Heaton*, 1986; *Evans*, 2007]. Hence, if bulk atmospheric deposition accumulated more rapidly in wooded landscape elements than grasslands, this could contribute to lower soil $\delta^{15}N$ values over time following woody encroachment. Neither atmospheric deposition nor its $\delta^{15}N$ was measured in this study. However, the Clean Air Status and Trends Network [*CASTNET*, 2008] reports total N deposition (wet + dry) of 0.19–0.53 g N m^{-2} yr^{-1} near our study site and $\delta^{15}N$ values of NO_3^- in precipitation range from −3.5 to −5.5‰ in this region [*Kendall et al.*, 2007]. Thus, more efficient capture of atmospheric deposition by wooded landscape elements could potentially account for a portion of

the soil N accrual rates and perhaps some of the reduction in soil $\delta^{15}N$ values documented in this study; however, this mechanism alone is probably of insufficient magnitude to account for observed changes in N cycle characteristics at this site.

4.4. Vertical Uplift and Lateral Translocation of N by Woody Plant Roots

[30] Since these woody plant communities are more deeply rooted and have more root biomass at all soil depths compared to grasslands [*Boutton et al.*, 1999, 2009b; *Hibbard et al.*, 2001], they should have greater potential for acquiring N from throughout the soil profile, incorporating it into their tissue, and then concentrating it near the soil surface via litterfall and root turnover. Thus, N stores in the upper soil profile could increase due to more intensive exploration of the soil by roots. Nutrient uplift from deep soil layers is a well-documented mechanism by which deep-rooted species can concentrate nutrients in the surface soil [*Jobbagy and Jackson*, 2001, 2004], and redistribution of soil N from deeper to shallower soil depths can be an important consequence when deep-rooted plants replace shallow-rooted plants during succession [*Knops and Tilman*, 2000]. Although it has also been suggested that wooded patches might concentrate nutrients by proliferation of lateral roots into the surrounding grassland areas [*Schlesinger et al.*, 1996; *Scholes and Archer*, 1997], root systems of woody plants in clusters and groves in this study area attenuate rapidly at the grassland/woody patch boundary [*Watts*, 1993] so that lateral mining of surrounding grasslands is unlikely to be a significant contribution to N enrichment in these wooded landscape elements.

[31] Soil $\delta^{15}N$ values cast doubt on the potential role of both vertical uplift and lateral translocation of N by roots as a major mechanisms of soil N accrual at this site. First of all, soil $\delta^{15}N$ values are significantly higher in the grassland than in the woodland, so that lateral translocation of grassland N would tend to enrich soil ^{15}N in the wooded landscape elements over time. Second, soil $\delta^{15}N$ values generally increase significantly with depth in the soil profile [*Nadelhoffer and Fry*, 1988; *Hobbie and Ouimette*, 2009], and vertical uplift of deeper soil N would also cause ^{15}N enrichment of the surface soil. However, soil $\delta^{15}N$ values have actually decreased over time in the surface soil of wooded areas (Figure 4), suggesting that neither vertical nor horizontal translocation of N is a major mechanism of N accumulation or that these processes are masked by other more dominant processes that affect soil $\delta^{15}N$.

4.5. Biogeochemical and Ecological Implications of Increased N Storage in the Soil System

[32] Nitrogen is a limiting nutrient for plants [*Vitousek and Howarth*, 1991; *LeBauer and Treseder*, 2008] and microbes [*Kaye and Hart*, 1997; *Schimel and Weintraub*, 2003] in most terrestrial ecosystems, so the increased N storage in the soil system should have strong impacts on the structure and function of this savanna parkland landscape. In particular, pool sizes of soil total N are strongly correlated with the size of the soil microbial biomass and with N mineralization and nitrification rates [*Booth et al.*, 2005]. Our prior studies at this site indicate that the N accrual documented in this study is accompanied by a larger soil microbial

biomass pool, higher rates of mineralization and nitrification, and larger pool sizes of NH_4^+ and NO_3^- [*Hibbard et al.*, 2001, *McCulley et al.*, 2004; *Liao and Boutton*, 2008]. This increased availability of N coupled with higher rates of net primary productivity [*Archer et al.*, 2001] may represent a positive feedback between woody encroachment and C and N cycles in this savanna parkland and in other ecosystems where N-fixing woody plants have invaded [*Liao et al.*, 2008].

[33] Larger pools of N in wooded areas could also result in higher N trace gas emissions resulting from more rapid rates of nitrification and denitrification. For example, NO_X emissions were significantly higher from soils in wooded areas than in remnant grasslands in this study area [*Archer et al.*, 2001] and were correlated with *P. glandulosa* biomass in a temperate savanna in north central Texas [*Martin et al.*, 2003]. NO_X is a major reactant in the formation of tropospheric O_3, an important air pollutant and a climatically active trace gas. Similarly, fluxes of the greenhouse gas N_2O have been shown to be higher under *Prosopis* canopies in other arid and semiarid plant communities in the southwestern United States [*Virginia and Jarrell*, 1982; *McLain and Martens*, 2006].

[34] In addition to altering the availability of N, it is possible that the availabilities of other important elements could be affected by the encroachment of N-fixing woody plants. For example, *Houlton et al.* [2008] showed that phosphatase enzyme production rates were 3 times higher in soils beneath N-fixing plants than beneath non-N-fixing plants in tropical savannas and lowland forests, resulting in increased P availability in those soils. In our study area, both phophatase enzyme activity and plant-available P are significantly greater beneath *Prosopis* canopies than in grasslands [*Boutton et al.*, 2009a]. Thus, both N and P availability are enhanced following woody encroachment, potentially altering plant-microbe and plant-plant interactions and successional dynamics in this ecosystem. Furthermore, increased availability of N and P could also stimulate primary production and C sequestration in vegetation in soils. In fact, during the past 150 years, average rates of C sequestration in litter, roots, and soils in wooded portions of this landscape have ranged from 21 to 62 g C m^{-2} yr^{-1} [*Boutton et al.*, 2009b].

[35] Woody encroachment into grassland is one of the most geographically extensive land cover changes occurring around the world today [*Archer et al.*, 2001; *Asner et al.*, 2004; *Van Auken*, 2009]. Thus, if our observations on N accumulation are representative of other ecosystems around the world where woody encroachment has occurred, then woody encroachment into grasslands is a significant phenomenon that can alter C and N gas fluxes at the ecosystem scale that then have the potential to influence atmospheric chemistry and the climate system at regional and perhaps even global scales [*Schlesinger et al.*, 1990; *Asner et al.*, 2004].

5. Conclusions

[36] Woody plant invasion of grassland has increased N storage in the soil system (litter, roots, and soils) of a subtropical savanna parkland landscape in the Rio Grande Plains of southern Texas. Rates of N accumulation in the upper 30 cm of the soil system have been linear over the past 100 years and show no signs of departing from linearity at present. Soil texture and moisture may have influenced patterns of N storage across the landscape, with higher N accumulations associated with lower-lying drainage woodland soils with higher silt and clay content and greater water availability. Soil $\delta^{15}N$ decreased with time following woody encroachment, consistent with a scenario in which N inputs have exceeded N outputs following woody encroachment. Evidence from $\delta^{15}N$ coupled with the known N-fixing potential of dominant tree legumes in this ecosystem suggests N fixation could be an important N source in these woodlands. However, differential atmospheric N deposition to woody plant canopies relative to grasslands could also contribute to both N accrual and observed decreases in soil $\delta^{15}N$ following woody encroachment. Significant accumulations of N following woody encroachment may alter soil N availability, thereby altering plant-microbe and plant-plant interactions, and potentially influencing competitive interactions and successional dynamics in this ecosystem. In addition, larger pools of soil N may accelerate rates of soil N transformations, increase flux rates of key trace gases such as NO_X and N_2O, and increase ecosystem C storage. Given the geographic dimensions of woody encroachment, these results may have implications for atmospheric composition and the climate system.

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