

## $^{15}\text{N}$ isoscapes in a subtropical savanna parkland: spatial-temporal perspectives

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**Abstract.** Spatial patterns of soil  $\delta^{15}\text{N}$  reflect variation in rates of N-cycling processes across landscapes. However, the manner in which soil  $\delta^{15}\text{N}$  is affected by vegetation and topographic properties under non-steady state conditions is understood poorly. Here we propose and evaluate a conceptual model that explains how soil  $\delta^{15}\text{N}$  values will respond to changes in disturbance regimes (intensification of grazing and removal of fire) and the resultant invasion of a subtropical grassland by woody vegetation dominated by *Prosopis glandulosa* (honey mesquite), a N-fixing tree legume. Spatially-specific sampling along a catena (hill-slope) gradient where woody plants are known to have displaced grasses over the past 100 years revealed a positive relationship between soil  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ , and a negative relationship between NDVI and soil  $\delta^{15}\text{N}$  on upland portions of the landscape, indicating that plant cover is a critical determinant of  $\delta^{15}\text{N}$  spatial patterns. Because the dominant woody invader is a N-fixer, its invasion has increased N input and reduced soil  $\delta^{15}\text{N}$ . However, while honey mesquite also invaded and came to dominate lowland portions of the landscape, soil  $\delta^{15}\text{N}$  values in woodlands of intermittent drainages were significantly elevated relative to those in uplands. This is likely attributable to higher soil moisture, clay content, and total N in the lower portions of the catena gradient, which create conditions favoring more rapid N-transformation rates, higher preferential  $^{14}\text{N}$  losses (e.g., gaseous), and thus enrichment of  $^{15}\text{N}$ . Thus, while spatial and temporal variation of soil  $\delta^{15}\text{N}$  has the potential to be an indicator of disturbance-induced changes in the net N balance, its sensitivity is compromised in topographic settings where rates of N-transformation are high. Continued improvements in our understanding of controls over the spatial variability of soil  $\delta^{15}\text{N}$  at the landscape-scale will enhance our ability to use  $\delta^{15}\text{N}$  as a diagnostic tool for inferring N dynamics under both steady-state and disturbed conditions.

**Key words:** disturbance;  $^{15}\text{N}$ ; nitrogen isotope ratio; soil nitrogen; soil texture; Special Feature: Isoscapes; vegetation change; woody plant encroachment.

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## INTRODUCTION

Spatial variation in soil nitrogen (N) isotope ratios ( $\delta^{15}\text{N}$ ) provide integrative assessments of ecosystem N-cycling processes at landscape, regional and global scales (Hogberg 1997, Evans 2001, Robinson 2001, Houlton et al. 2006, Craine et al. 2009b, Pardo and Nadelhoffer 2010, Rascher et al. 2012). Under steady-state conditions, soil  $\delta^{15}\text{N}$  is primarily controlled by input (deposition and fixation) and output (N leaching and gaseous loss)  $\delta^{15}\text{N}$ , but is not affected by internal N cycling processes such as plant uptake or microbial N-transformations that result in no net N loss (Brenner et al. 2001, Houlton et al. 2007, Bai and Houlton 2009, Houlton and Bai 2009, Koba et al. 2012). Although N isotope fractionation may occur during plant N uptake (e.g., due to mycorrhizal associations), plants and their associated root symbionts return N to the soil with the same weighted  $\delta^{15}\text{N}$  as that of N uptake. Thus, bulk soil  $\delta^{15}\text{N}$  is not changed by plants or their root symbionts as long as that N remains in the soil. When ecosystems undergo disturbances that modify vegetation composition and primary production, soil  $\delta^{15}\text{N}$  is likely to be altered to reflect the net outcome of changes in the rates of processes mediated by plants (e.g., N-uptake, litterfall, root turnover) and soils (e.g., N-transformations and losses). However, our incomplete understanding of how plant and soil processes interact to influence soil  $\delta^{15}\text{N}$  across topographically heterogeneous landscapes currently limits the utility of this metric as a diagnostic tool.

Many studies have demonstrated a positive relationship between soil  $\delta^{15}\text{N}$  and soil total N (TN) concentration (Garten and Van Miegroet 1994, Amundson et al. 2003, Nardoto et al. 2008, Bai et al. 2009, Craine et al. 2009a, Posada and Schuur 2011). The likely mechanism is that rates of N-transformations (ammonification, nitrification, denitrification) and N-losses increase with increasing soil TN, resulting in higher preferential losses of  $^{14}\text{N}$  and hence enrichment of soil  $\delta^{15}\text{N}$  (Hogberg 1997, Houlton et al. 2006). However, other studies have shown an inverse relationship between soil TN and soil  $\delta^{15}\text{N}$  (Perakis and Kellogg 2007, Wheeler et al. 2007, Cheng et al. 2009, Fang et al. 2011). These contrasting perspectives suggest the underlying determinant of soil  $\delta^{15}\text{N}$  is not the pool size of

soil TN, but rather the balance between N inputs and outputs. If N input is equal to output, areas with higher soil TN typically have higher soil  $\delta^{15}\text{N}$  because more N is lost through high-fractionation pathways (e.g., gaseous losses) (Brenner et al. 2001, Houlton et al. 2006). When disturbance causes an increase in N input without a concomitant increase in output fluxes, soil  $\delta^{15}\text{N}$  will likely decrease even if soil TN is increased (Compton et al. 2007, Perakis and Kellogg 2007). Conversely, if soil N losses exceed inputs following disturbance, soil  $\delta^{15}\text{N}$  will likely increase as soil TN declines (Bekele and Hudnall 2005, Billings and Richter 2006).

These contrasting perspectives on changes in soil  $\delta^{15}\text{N}$  in response to disturbance are integrated in the conceptual model in Fig. 1. At steady-state, plant input and output are equal and soil  $\delta^{15}\text{N}$  is not influenced by vegetation. Ecosystem input (I) is also equal to output (O) at steady-state and

$$\delta^{15}\text{N}_{\text{soil}} = \delta^{15}\text{N}_{\text{input}} + \varepsilon_{\text{output}} \quad (1)$$

where  $\delta^{15}\text{N}_{\text{soil}}$  is the  $\delta^{15}\text{N}$  value of soil TN,  $\delta^{15}\text{N}_{\text{input}}$  is the weighted mean  $\delta^{15}\text{N}$  of N-deposition and fixation inputs, and  $\varepsilon_{\text{output}}$  is the weighted mean isotope discrimination factor associated with soil N losses. Following disturbance, while an ecosystem is under non-steady state conditions, soil  $\delta^{15}\text{N}$  could increase (if  $I < O$ ) or decrease (if  $I > O$ ). This reflects the fact that  $\delta^{15}\text{N}_{\text{input}}$  from N-fixation or atmospheric N-deposition typically ranges from  $-3\text{‰}$  to  $+3\text{‰}$  (Freyer et al. 1996, Buzek et al. 1998, Handley et al. 1999, Houlton et al. 2006), and is usually lower than  $\delta^{15}\text{N}_{\text{soil}}$ . Thus, if N inputs increase without corresponding increases of output, more  $^{14}\text{N}$  enters the system and  $\delta^{15}\text{N}_{\text{soil}}$  declines. In contrast, if N inputs decline relative to losses, preferential losses of  $^{14}\text{N}$  will increase  $\delta^{15}\text{N}_{\text{soil}}$ . Although not illustrated in Fig. 1,  $\delta^{15}\text{N}_{\text{soil}}$  should eventually reach a new steady-state condition, given sufficient time and the absence of further perturbations; and, the degree to which  $\delta^{15}\text{N}_{\text{soil}}$  might return to its prior steady state level may depend on the time-frame and whether or not a regime shift (e.g., Folke et al. 2004) has occurred. It should be noted that although nearly all soils around the world have positive  $\delta^{15}\text{N}$  values, there are some limited geographic regions at high northern latitudes where they are negative

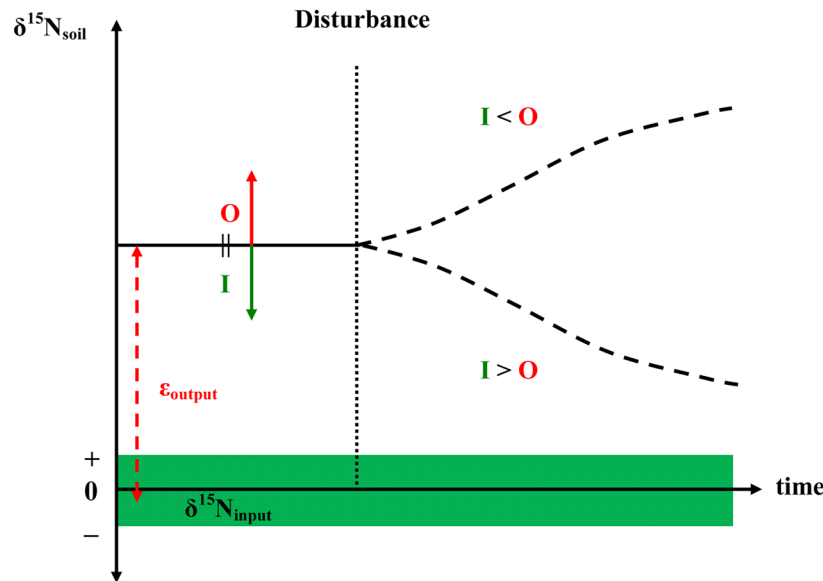


Fig. 1. Conceptual model of potential changes in soil  $\delta^{15}\text{N}$  before and after disturbance. At steady-state (solid line), soil  $\delta^{15}\text{N}$  is not affected by plants (because plant input is equal to output). Ecosystem input (I) is equal to output (O) and soil  $\delta^{15}\text{N} = \delta^{15}\text{N}_{\text{input}} + \epsilon_{\text{output}}$ . In this equation,  $\delta^{15}\text{N}_{\text{input}}$  is the weighted mean  $\delta^{15}\text{N}$  of N-deposition and fixation inputs (range of likely values shown in green zone); and  $\epsilon_{\text{output}}$  is the weighted mean of a soil output N discrimination factor and (red dashed line).  $\delta^{15}\text{N}_{\text{input}}$  is usually within  $-3\%$  to  $+3\%$  and is less than soil  $\delta^{15}\text{N}$ . After disturbance the ecosystem is under non-steady state conditions and soil  $\delta^{15}\text{N}$  could increase (if  $I < O$ ) or decrease (if  $I > O$ ) (dashed lines) with time.

(Amundson et al. 2003). In those areas, the logic outlined above would be different.

At steady-state ( $\delta^{15}\text{N}_{\text{soil}} = \delta^{15}\text{N}_{\text{input}} + \epsilon_{\text{output}}$ ), higher  $\epsilon_{\text{output}}$  means more N is lost through high-fractionation pathways (e.g., gaseous losses) than low-fractionation pathways (e.g., leaching losses) (Amundson et al. 2003, Bai and Houlton 2009, Houlton and Bai 2009). Fractionation by leaching is caused mainly by isotope discrimination during microbial processes that produce mobile,  $^{15}\text{N}$ -depleted forms of N (e.g., production of  $\text{NO}_3^-$  during nitrification), causing residual soil N to become  $^{15}\text{N}$ -enriched when those products are leached. A variety of factors can influence soil  $\delta^{15}\text{N}$  by affecting  $\delta^{15}\text{N}_{\text{input}}$  and  $\epsilon_{\text{output}}$ . These include climate (Handley et al. 1999, Evans 2001, Amundson et al. 2003, Sah et al. 2006), topography (Sutherland et al. 1993, Garten and Van Miegroet 1994), soil nutrients (notably N and P; McKee et al. 2002, Craine et al. 2009b, Boutton and Liao 2010), mycorrhizal fungi (Hobbie et al. 2000, Pardo and Nadelhoffer 2010), and soil texture (Ledgard et al. 1984, Baisden et al. 2002, Quideau et al. 2003, Hobbie and Ouimette 2009).

These factors would all interact to determine soil  $\delta^{15}\text{N}$  response to disturbance and to influence spatial patterns of soil  $\delta^{15}\text{N}$ . It is important to recognize that most ecosystem N inputs are in the range of  $-3\%$  to  $+3\%$  (Freyer et al. 1996, Buzek et al. 1998, Handley et al. 1999, Houlton et al. 2006), which is slightly lower than  $\delta^{15}\text{N}$  of most soils ( $0$ – $10\%$ ; Amundson et al. 2003). In contrast,  $\delta^{15}\text{N}$  values of gaseous N lost via nitrification, denitrification, and other N cycle processes are generally highly  $^{15}\text{N}$ -depleted ( $-14$  to  $-50\%$ ; Li and Wang 2008, Koba et al. 2012) relative to soil total N. Therefore, N-inputs would tend to have less impact on soil  $\delta^{15}\text{N}$  values than gaseous N-losses of an identical magnitude.

This conceptual model can be evaluated by quantifying spatial-temporal changes in soil  $\delta^{15}\text{N}$  following disturbance or changes in disturbance regimes. We therefore examined spatial-temporal variations in soil  $\delta^{15}\text{N}$  following a shift in vegetation life form composition along a hill-slope (catena) gradient on a landscape in the Rio Grande Plains of southern Texas, USA. Previous studies at this site have documented how

changes in a disturbance regime (intensification of livestock grazing and elimination of fire) have caused vegetation to change from domination by herbaceous vegetation (grasses) to woody plants (shrubs and arborescents) over the past 100+ years. On upland portions of the landscape, tree/shrub patches are distributed throughout a remnant grassland matrix, whereas lower-lying landscape positions are currently dominated by closed-canopy woodlands similar in composition to upland woody patches (Archer et al. 1988, Boutton et al. 1998). This shift from grass to woody plant dominance was initiated and facilitated by the establishment of honey mesquite (*Prosopis glandulosa*) (Archer et al. 1988), a N-fixing tree legume (Johnson and Mayeux 1990, Zitzer et al. 1996). Following woody encroachment in both uplands and lowlands, N accumulates in the soil system (0–30 cm) at linear rates (1.6–4.6 g N m<sup>-2</sup> yr<sup>-1</sup>), resulting in a 50–150% increase in N storage in woody stands (Archer et al. 2004, Boutton and Liao 2010). These changes in soil TN pools are accompanied by significant increases in rates of ammonification, nitrification, and N trace gas fluxes in woody plant stands compared to remnant grasslands (Archer et al. 2001, Hibbard et al. 2001, McCulley et al. 2004, Sparks et al. 2011). Thus, both N pools and fluxes have been highly modified in response to the displacement of grasses by woody plants.

The purpose of this study was to relate landscape-scale spatial patterns of soil  $\delta^{15}\text{N}$  values to known changes in vegetation life form composition, N storage, and N transformation rates. We hypothesized that: (1) within a given topographic setting, soil  $\delta^{15}\text{N}$  in woody plant communities would be lower than those occurring in the grasslands they have displaced, owing to increased N inputs; and (2) soil  $\delta^{15}\text{N}$  in lowlands would differ from those in the uplands due to elevation-related differences in factors influencing N losses via gaseous and hydrologic forms (e.g., soil texture, soil moisture, and woody plant cover).

## MATERIALS AND METHODS

### Study area

Research was conducted at the Texas AgriLife La Copita Research Area (27°40' N; 98°12' W; elevation 80 m) in the eastern Rio Grande Plains

of the Tamaulipan Biotic Province (mean annual temperature 22.4°C; mean annual precipitation 680 mm). Landscapes at this site typically grade (1–3% slopes) from convex sandy loam uplands (Typic Argiustolls) to concave clay loam lowlands (Pachic Argiustolls). Woody encroachment has occurred in the study area over the past 75–100 years as a consequence of interactions between heavy livestock grazing and reduced fire frequency (Archer 1995). On upland portions of the landscape, discrete woody clusters (3–10 m diameter; each comprised of a single mesquite tree with up to 15 understory tree/shrub species) and larger groves (10 to >30 m diameter; comprised of several woody clusters that have fused together [Bai et al. 2012]) are embedded within a matrix of grassland vegetation. The grasslands are dominated by C<sub>4</sub> grasses and C<sub>3</sub> forbs. Shrub clusters and groves are dominated by *P. glandulosa* and *Zanthoxylum fagara*. Mesquite and several other leguminous trees and shrubs present in wooded areas are capable of symbiotic N<sub>2</sub>-fixation (Johnson and Mayeux 1990, Zitzer et al. 1996). Lowland portions of the landscape consist of closed-canopy woodlands similar in plant species composition to upland clusters and groves. The  $\delta^{15}\text{N}$  of mesquite foliage is generally near 1‰, while the foliage of other non-N-fixing woody species is generally 2–5‰ (Bai et al. 2009). Other detailed biological characteristics of the woody species that dominate upland and lowland plant communities have been presented elsewhere (Bai et al. 2008). Woody plant basal area in upland and lowland communities has been reported in Liu et al. (2010). The study area was a working ranch that was heavily and continuously grazed by livestock until its acquisition as a research area in 1981 (Scifres and Koerth 1987). Since that time it has been grazed rotationally and at greatly reduced stocking rates. Fire history of the site is not formally known, but there are no indications of fire in ranch records dating back to the 1950s; and heavy, continuous grazing prior to that time would likely have kept fine fuel mass low, and hence the probability of fire low.

### Experimental design

A 100 × 160 m plot consisting of 10 × 10 m grid cells was established on an upland portion of the landscape which included woody clusters and

groves within the grassland matrix. Soil samples were collected randomly at two points within each cell, yielding a total of 320 sample points within the grid.

In addition, six discrete shrub clusters and three groves were selected randomly in the upland near the grid. Three transects radiating from the center to the perimeter of each cluster and into the adjoining grassland matrix were established in random directions at approximately 120° apart. A total of seven points were sampled along each transect, four within the shrub canopy and three beyond the shrub canopy, resulting a total of 126 sample points. Distances between sample points under and away from the shrub canopy were standardized relative to the bole-to-dripline distance. Three groves were sampled using tree-to-tree transects originating at a central mesquite tree and progressing from one mesquite tree to the next in a direction towards the grove perimeter and into the grasslands. This approach yielded 45, 19, and 29 sample points for groves one, two and three, respectively. These transect points were supplemented with an additional 11 random points within the groves. Points along the grove transects were classified into one of four categories based on their position relative to mesquite trees: (1) base: next to the bole of a mesquite tree; (2) mid: half-way between the canopy edge and a bole; (3) edge: within 15 cm of a mesquite canopy edge; and (4) grass: in the grassland matrix beyond shrub canopies.

Three 6 × 10 m grassland plots located at least 5 m from any woody patch were also established in the upland. Each plot was subdivided into a grid of 2 × 2 m cells. Four sample points were randomly designated in each cell, resulting in a total of 180 samples. In the drainage woodland, a 50 × 30 m plot consisting of 5 × 5 m grid cells was established. Two sample points were selected randomly within each grid cell. Two tree-to-tree transects at the two parallel edges of the grid, each about 70 m in length, were established. Transect 1 generally followed the topographic low of the intermittent drainage; and Transect 2 was upslope about half-way between the topographic low and the upland/lowland interface defined by the woodland margin. These transects zigzagged from one mesquite tree to the next in a generally north-south orientation along a contour

line. Transect points were categorized to three classes: “base” (points adjacent to mesquite boles); “edge” (points at the dripline of mesquite canopies; and “mid” (points halfway between the canopy dripline and the bole). Coordinates of all sample points ( $\pm 0.1$  m) were determined using a global positioning system (Trimble GPS Pathfinder Pro XRS, Trimble Navigation Limited, Sunnyvale, CA).

#### *Aerial photography*

Color-infrared aerial imagery encompassing the upland and drainage woodland grids and transects were obtained in 2003 in order to relate spatially explicit soil  $\delta^{15}\text{N}$  values and other soil properties to present vegetation cover. Images were scanned and georeferenced in a UTM projection (1 m resolution). A Normalized Difference Vegetation Index (NDVI) (Rouse et al. 1973) was calculated as  $(\text{NIR} - \text{RED})/(\text{NIR} + \text{RED})$ , in which RED and NIR represent the spectral reflectance measurements acquired in the red and near-infrared regions, respectively. Areas occupied by woody plants have typically higher NDVI values than areas characterized by grasses and bare soil area (Scanlon et al. 2002).

#### *Soil sampling and laboratory analyses*

In January 2003, three soil cores (15 cm deep × 2.24 cm in diameter) were taken within 10 cm of each other at each of the sample points described earlier. The first core was utilized to determine bulk density (core method; Soil Survey Staff 1996), gravimetric water content (GWC; Hillel 1982), and soil particle size distribution (pipette method; Gee and Bauder 1986). All samples were collected on the same day and no rainfall occurred during the collection process. Although we have only a single time point for soil moisture, we use it here as a qualitative index to show how soil moisture might vary with topography and soil texture.

The second core was used to determine root biomass after washing soils through a hydro-pneumatic elutriation system equipped with 410  $\mu\text{m}$  mesh filters (Gillison's Variety Fabrications, Benzonia, MI, USA; Smucker et al. 1982). Roots were dried for at least 72 h at 60°C to determine dry weight, and then ashed at 400°C in a muffle furnace to obtain ash-free root biomass. The third core was utilized to determine pH and the

Table 1. Chemical and physical properties (mean and standard deviation) of soils (0–15 cm) associated with four plant communities occurring on upland and lowland landscape elements in the study area. Different letters associated with means within a column indicate statistically significant differences ( $p < 0.05$ ).

Community type	Grassland (n = 378)		Cluster (n = 182)		Grove (n = 170)		Woodland (n = 208)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
$\delta^{15}\text{N}$ (‰)	7.1 <sup>a</sup>	0.5	6.8 <sup>b</sup>	0.8	6.4 <sup>c</sup>	0.7	7.2 <sup>d</sup>	0.5
$\delta^{13}\text{C}$ (‰)	-18.9 <sup>a</sup>	1	-21.0 <sup>b</sup>	2	-21.4 <sup>c</sup>	1.8	-19.4 <sup>d</sup>	1.5
Organic carbon (g/m <sup>2</sup> )	1273 <sup>a</sup>	185	1560 <sup>b</sup>	511	1823 <sup>c</sup>	477	3131 <sup>d</sup>	786
Total nitrogen (g/m <sup>2</sup> )	126 <sup>a</sup>	18	150 <sup>b</sup>	44	185 <sup>c</sup>	51	269 <sup>d</sup>	78
Bulk density (g/cm <sup>3</sup> )	1.36 <sup>a</sup>	0.07	1.27 <sup>b</sup>	0.15	1.24 <sup>ac</sup>	0.14	1.11 <sup>d</sup>	0.14
Root biomass (g/m <sup>2</sup> )	291 <sup>a</sup>	438	591 <sup>b</sup>	664	905 <sup>c</sup>	671	936 <sup>c</sup>	946
Gravimetric water content (%)	2.8 <sup>a</sup>	0.7	2.4 <sup>a</sup>	0.6	3.0 <sup>a</sup>	0.9	9.8 <sup>b</sup>	4
pH	6.6 <sup>a</sup>	0.4	6.6 <sup>a</sup>	0.6	6.8 <sup>a</sup>	0.8	6.2 <sup>b</sup>	0.8
NDVI	0.11 <sup>a</sup>	0.07	0.19 <sup>b</sup>	0.1	0.30 <sup>c</sup>	0.12	0.37 <sup>d</sup>	0.08
Sand (%)	78 <sup>a</sup>	2	78 <sup>a</sup>	3	76 <sup>a</sup>	4	56 <sup>b</sup>	7
Silt (%)	11 <sup>a</sup>	1	11 <sup>ab</sup>	1	12 <sup>b</sup>	2	21 <sup>c</sup>	3
Clay (%)	11 <sup>a</sup>	2	12 <sup>a</sup>	2	12 <sup>a</sup>	2	23 <sup>b</sup>	5

elemental concentration and isotopic composition of soil C and N. Soils from these cores were passed through a 2 mm sieve to remove coarse organic fragments and gravel, and then mixed thoroughly. Soil pH was determined on 12 g soil in 0.01 M CaCl<sub>2</sub> solution using an Accumet Basic pH meter (Fisher Scientific, Pittsburgh, PA). The remainder of the soil was dried at 60°C for 48 h and pulverized in a centrifugal mill (Angstrom, Inc., Belleville, MI) for elemental (C and N concentrations) and isotopic ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) analyses using a Delta Plus/Carlo Erba EA-1108 continuous flow isotope ratio mass spectrometer (ThermoFinnigan, San Jose, CA) in the Stable Isotopes for Biosphere Science Laboratory at Texas A&M University.

C and N isotope ratios are presented in delta notation:

$$\delta = [(R_{\text{SAMPLE}} - R_{\text{STD}})/R_{\text{STD}}] \times 10^3$$

where  $R_{\text{SAMPLE}}$  is the  $^{15}\text{N}/^{14}\text{N}$  ratio (or  $^{13}\text{C}/^{12}\text{C}$  ratio for carbon isotopes) of the sample and  $R_{\text{STD}}$  is the  $^{15}\text{N}/^{14}\text{N}$  ratio (or  $^{13}\text{C}/^{12}\text{C}$  ratio for carbon isotopes) of the atmospheric dinitrogen standard (or V-PDB standard for carbon isotopes) (Mariotti 1983, Coplen 1996). Precision of duplicate measurements was  $<0.2\text{‰}$  for  $\delta^{15}\text{N}$ , and  $<0.1\text{‰}$  for  $\delta^{13}\text{C}$ .

#### Statistical analyses and modeling

Descriptive statistics on each variable were obtained using SPSS for Windows, version 11.5 (SPSS Inc. 2002). Correlations between soil  $\delta^{15}\text{N}$ , root biomass, soil organic carbon (SOC), TN and

soil particle size distribution were assessed using Pearson's correlation coefficients and modified T-tests for correlation. The latter adjusts the degrees of freedom based on the extent of spatial autocorrelation in the data, and therefore compensates for spatial autocorrelation (Clifford et al. 1989, Dutilleul et al. 1993). The software used for Pearson's correlation and modified t-test was PASSAGE version 1 (Rosenberg 2001). Ordinary kriging was used as a spatial interpolation tool for predicting values at unsampled locations based on sample data and its spatial structure derived from variogram analysis. The latter were conducted using VARIOWIN (Pannatier 1996). GIS analyses were conducted using ArcView GIS Spatial Analyst (ESRI 1998).

## RESULTS

### Soil chemical and physical properties

The physical and chemical properties of soils associated with the four landscape elements are summarized in Table 1. SOC, TN, and root biomass all increased significantly in the rank order: upland grassland < upland cluster < upland grove < drainage woodland. In contrast, bulk density increased in the reverse order. Soil pH was significantly lower in the drainage woodland (6.2) compared to the upland landscape elements (6.6–6.8). NDVI increased from upland grassland to woody cluster and grove and was highest in the drainage woodland. Soil texture was similar among upland landscape elements, but was significantly different between uplands (more sand) vs. drainage woodland (more clay).

Table 2. Pearson's correlation coefficients (r) showing relationships between soil  $\delta^{15}\text{N}$ , total nitrogen (TN), root biomass (Root), soil particle size classes, and soil  $\delta^{13}\text{C}$  in the upper 15 cm of the soil profile. A modified T-test for correlation was used to test for significance.

Characteristic	NDVI	TN ( $\text{g N m}^{-2}$ )	Root ( $\text{g m}^{-2}$ )	Sand (%)	Silt (%)	Clay(%)	$\delta^{13}\text{C}(\text{‰})$
<b>Upland grassland</b>							
$\delta^{15}\text{N}(\text{‰})$	-0.11	-0.21*	-0.13*	0.21	-0.14	-0.20	0.37*
NDVI		0.32*	0.11	-0.27	0.30	0.20	-0.23*
TN			0.09	-0.55*	0.48*	0.47*	-0.34*
Root				-0.06	0.10	0.03	-0.13*
Sand					-0.77*	-0.92*	0.01
Silt						0.45*	-0.07
Clay							0.02
<b>Upland cluster</b>							
$\delta^{15}\text{N}(\text{‰})$	-0.36*	-0.47*	-0.26*	0.08	-0.07	-0.07	0.56*
NDVI		0.26*	0.20*	0.21	-0.21	-0.18	-0.50*
TN			0.39*	-0.62*	0.53*	0.57*	-0.70*
Root				-0.03	-0.04	0.07	-0.50*
Sand					-0.84*	-0.95*	-0.16
Silt						0.61	0.04
Clay							0.21
<b>Upland grove</b>							
$\delta^{15}\text{N}(\text{‰})$	-0.22*	-0.26*	-0.22*	0.18	-0.16	-0.18	0.42*
NDVI		0.34	0.15	0.16	-0.19	-0.12	-0.52
TN			0.40	-0.45	0.43	0.44	-0.59*
Root				-0.15	0.16	0.14	-0.48*
Sand					-0.96*	-0.97*	-0.14
Silt						0.86*	0.05
Clay							0.19
<b>Drainage woodland</b>							
$\delta^{15}\text{N}(\text{‰})$	0.09	-0.37*	-0.16	-0.48*	0.20	0.51*	0.57*
NDVI		0.13	-0.01	-0.20	-0.01	0.26	0.05
TN			0.41*	-0.10	0.12	0.06	-0.70*
Root				0.04	0.01	-0.06	-0.48*
Sand					0.68*	-0.90*	-0.41*
Silt						0.29*	0.22
Clay							0.41*
<b>Upland all points</b>							
$\delta^{15}\text{N}(\text{‰})$	-0.40*	-0.45*	-0.32*	0.27	-0.28	-0.21	0.53*
NDVI		0.55*	0.37*	-0.23	0.31	0.12	-0.62*
TN			0.47*	-0.57*	0.60*	0.44	-0.69*
Root				-0.20	0.28*	0.10	-0.51*
Sand					-0.84*	-0.92*	0.10
Silt						0.55*	0.22
Clay							0.02

\* $p < 0.05$ .

Accordingly, there were no differences in the single point-in-time soil gravimetric water content among the three sandy loam upland landscape communities (2.4–3.0%); however, this parameter was significantly higher in the finer-textured soils of drainage woodlands (9.9%).

#### *Soil $\delta^{15}\text{N}$ spatial patterns and their relationships with vegetation, topography, and soil properties*

Soil  $\delta^{15}\text{N}$  differed significantly in each of the communities, ranging from a mean of 6.4‰ in groves to a mean of 7.2‰ in drainage woodlands (Table 1). In upland grassland, soil  $\delta^{15}\text{N}$  was significantly negatively correlated with TN and root biomass and positively correlated with soil

$\delta^{13}\text{C}$  (Table 2). In upland clusters and groves, soil  $\delta^{15}\text{N}$  was negatively correlated with NDVI, TN, and root biomass, and positively correlated with soil  $\delta^{13}\text{C}$ . Soil particle size did not correlate significantly with soil  $\delta^{15}\text{N}$  in any of the upland communities. When data were pooled for the three upland communities, soil  $\delta^{15}\text{N}$  was negatively correlated with NDVI, TN, and root biomass, and positively correlated with soil  $\delta^{13}\text{C}$  (Table 2). In the drainage woodland community, soil  $\delta^{15}\text{N}$  was negatively correlated with TN and sand content, and positively correlated with soil  $\delta^{13}\text{C}$  and clay content.

Within cluster communities, soil  $\delta^{15}\text{N}$  values were lowest in the center near the base of

mesquite trees (6.3‰) and steadily increased along transects extending to the cluster dripline and into the adjacent grasslands which had nearly constant values (7.4‰) (Fig. 2A). Soil TN along this same gradient declined about 50%, from 230 g N m<sup>-2</sup> near the shrub cluster center to 120 g N m<sup>-2</sup> in the adjacent grassland (Fig. 2B).

Transects through the groves yielded similar spatial patterns, with lowest  $\delta^{15}\text{N}$  values occurring near the base of mesquite trees (6.1‰) and increasing to approximately 7.1‰ in the adjacent grasslands (Fig. 3A). Soil TN declined from 200 g N m<sup>-2</sup> near the mesquite base and mid-canopy locations to 120 g N m<sup>-2</sup> in the surrounding grassland (Fig. 3B). In contrast to upland clusters and groves, transects within the drainage wood-

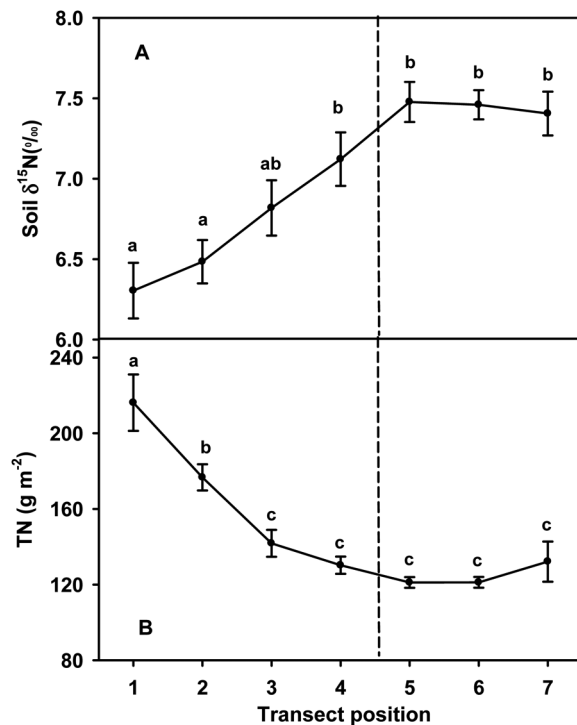


Fig. 2. Mean ( $\pm$ SE) soil  $\delta^{15}\text{N}$  (‰) (A) and soil total nitrogen (TN) (B) along transects originating at the bole of the mesquite tree near the center of discrete shrub clusters (Transect position 1) and extending into the surrounding grasslands (Transect positions 5–7). All values are for the 0–15 cm soil depth. The dashed vertical line represents the location of the shrub cluster-grassland boundary. Different lower case letters indicate differences between means (Fisher's LSD;  $p < 0.05$ ).

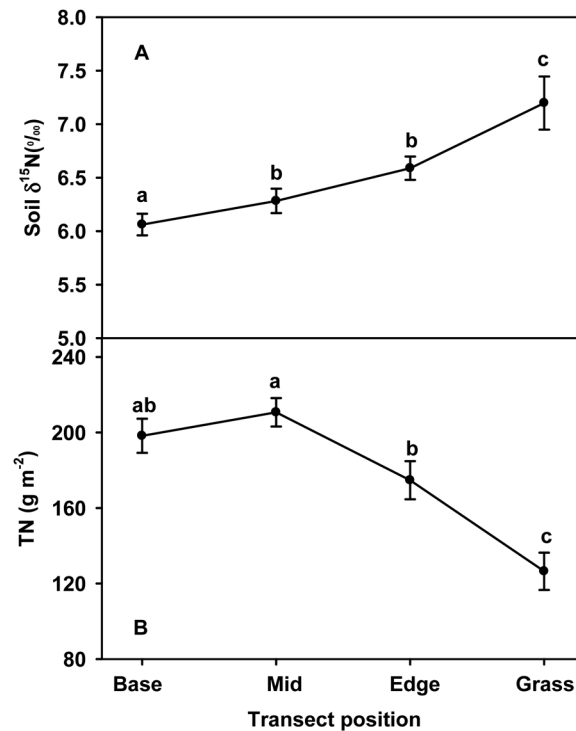


Fig. 3. Mean ( $\pm$ SE) soil  $\delta^{15}\text{N}$  (A) and soil total nitrogen (TN) (B) along tree-to-tree transects within upland groves. All values are for the 0–15 cm soil depth. Transect positions within groves are defined as: (1) “base,” adjacent to the bole of the mesquite trees; (2) “mid,” midway between the bole and the edge of the mesquite canopy; (3) “edge,” at the mesquite canopy drip line; and (4) “grass,” soils of the adjoining grassland community. Different letters indicate differences between means (Fisher's LSD;  $p < 0.05$ ).

land revealed little influence of mesquite trees on soil  $\delta^{15}\text{N}$  or TN (Fig. 4A, B).

From a spatial perspective based on individual sample points, upland soil  $\delta^{15}\text{N}$  values ranged from 4.1 to 8.1‰, with patterns that were strongly related to the distribution of grassland vs. woody plant cover, as seen by visual comparison with the aerial photo of the site (Fig. 5A, B). Areas with lower soil  $\delta^{15}\text{N}$  values corresponded to areas with well-developed woody cover, whereas soils with higher  $\delta^{15}\text{N}$  were mainly associated with remnant grassland. This relationship between plant cover type and soil  $\delta^{15}\text{N}$  is confirmed by both the significant negative correlation between NDVI and soil  $\delta^{15}\text{N}$ , and the significant positive correlation



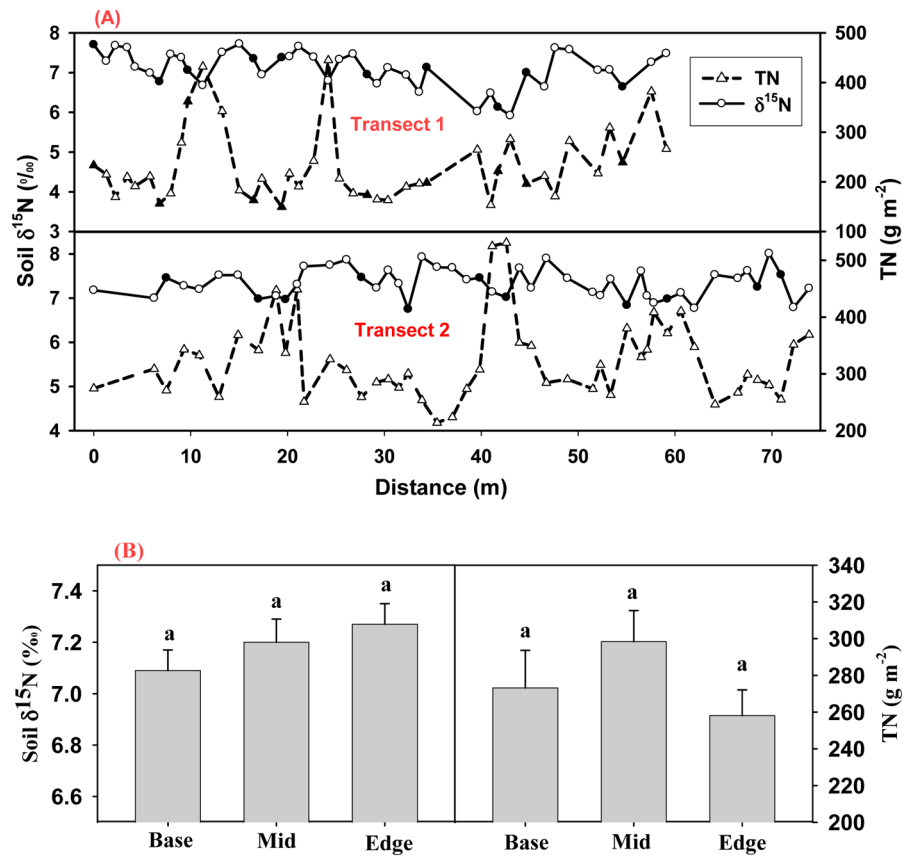


Fig. 4. Soil  $\delta^{15}\text{N}$  (‰) soil total nitrogen (TN,  $\text{g m}^{-2}$ ) along two tree-to-tree transects within drainage woodland (A) and the mean soil  $\delta^{15}\text{N}$  at different positions relative to mesquite trees (B). All values are for the 0–15 cm soil depth. Circles and solid line represent soil  $\delta^{15}\text{N}$ ; triangles and dashed line represent TN. Solid black symbols indicate points adjacent to mesquite boles. Transect 1 (T1) generally followed the topographic low of the intermittent drainage; and Transect 2 (T2) was upslope about half-way between the topographic low and the upland/lowland interface defined by the woodland margin. “Base” indicates points adjacent to mesquite tree trunks; “edge” indicates points on the dripline of the tree canopy; and “mid” indicates points between the canopy edge and the base. Different letters represent significant difference between the means tested by ANOVA ( $p = 0.05$ ).

between soil  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  across the entire upland landscape (Table 2).

Within drainage woodlands, which were characterized by a high and nearly continuous cover by woody plants, there was no obvious visual correspondence between vegetation cover and soil  $\delta^{15}\text{N}$  (Fig. 6A, B) and correlations with NDVI were low and non-significant (Table 2). Instead, areas with higher soil  $\delta^{15}\text{N}$  values were concentrated in topographic low spots where the density of mesquite trees was locally reduced compared to the other areas in the drainage woodland.

## DISCUSSION

### *Spatial patterns of soil $\delta^{15}\text{N}$ in uplands*

Soil  $\delta^{13}\text{C}$  values obtained here and in prior studies confirm that woody plants have encroached into areas that were originally grassland (Boutton et al. 2009, Bai et al. 2012). Consistent with Hypothesis 1, this encroachment by woody plants into areas that were once grassland decreased soil  $\delta^{15}\text{N}$  significantly (Tables 1 and 2, Figs. 2, 3 and 5). The most likely mechanism responsible for this change is that the dominant encroaching woody plant (mesquite) is

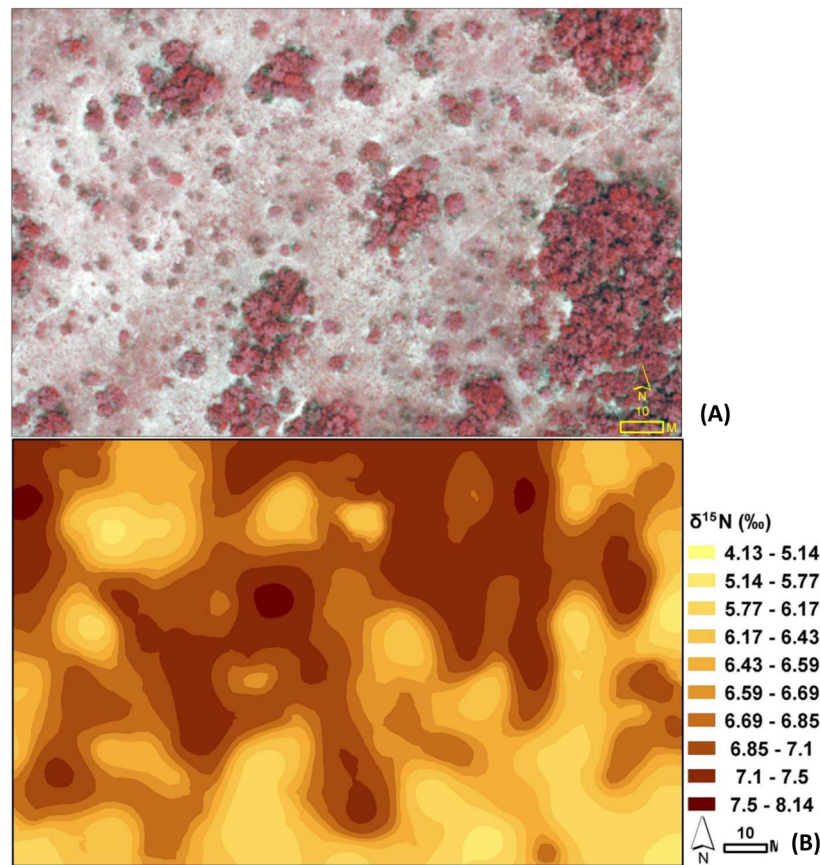


Fig. 5. Color-infrared aerial photo of upland sampling grid (A) and kriged map of soil  $\delta^{15}\text{N}$  in the upper 15 cm of the profile (B). Lighter areas in the aerial photo are bare ground (white) and herbaceous vegetation; darker areas are woody vegetation. The area represented in (A) is identical to that shown in (B).

a tree legume known to fix nitrogen (Johnson and Mayeux 1990, Zitzer et al. 1996). The  $\delta^{15}\text{N}$  of biologically fixed N typically approximates the 0‰ value for atmospheric  $\text{N}_2$  (Freyer et al. 1996, Buzek et al. 1998, Handley et al. 1999, Houlton et al. 2006), which is significantly and substantially lower than soil  $\delta^{15}\text{N}$  in our study area (Table 1). Increased N input via fixation by this proliferating shrub would therefore decrease soil  $\delta^{15}\text{N}$  (Fig. 1). Accordingly, as the rate of N-fixation rises following mesquite encroachment, ecosystem N inputs exceed N outputs, thereby lowering soil  $\delta^{15}\text{N}$  and increasing soil TN pool sizes (Fig. 1). The fact that soil TN has increased where woody plants have displaced grasses (Table 1, Figs. 2 and 3) coupled with the positive correlation between soil  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  (Table 2) substantiates the interpretation that the decrease

of soil  $\delta^{15}\text{N}$  is linked to woody encroachment. Furthermore, shrub cover had a strong effect on NDVI; and soil TN in uplands was positively correlated with NDVI (Tables 1 and 2, Fig. 5). This constitutes another line of evidence that larger soil TN pool sizes are associated with areas with well-developed woody plant canopies. The preponderance of evidence thus indicates that woody plant encroachment initiated and dominated by mesquite has increased ecosystem N inputs. This is consistent with previous studies in this same study area (Liao et al. 2006a, Bai et al. 2009, Boutton and Liao 2010) and in other geographic areas where N-fixing woody plants have encroached (Vitousek and Walker 1989, Liao et al. 2008, Throop and Archer 2008).

Spatial patterns along transects originating from the centers of woody clusters and groves

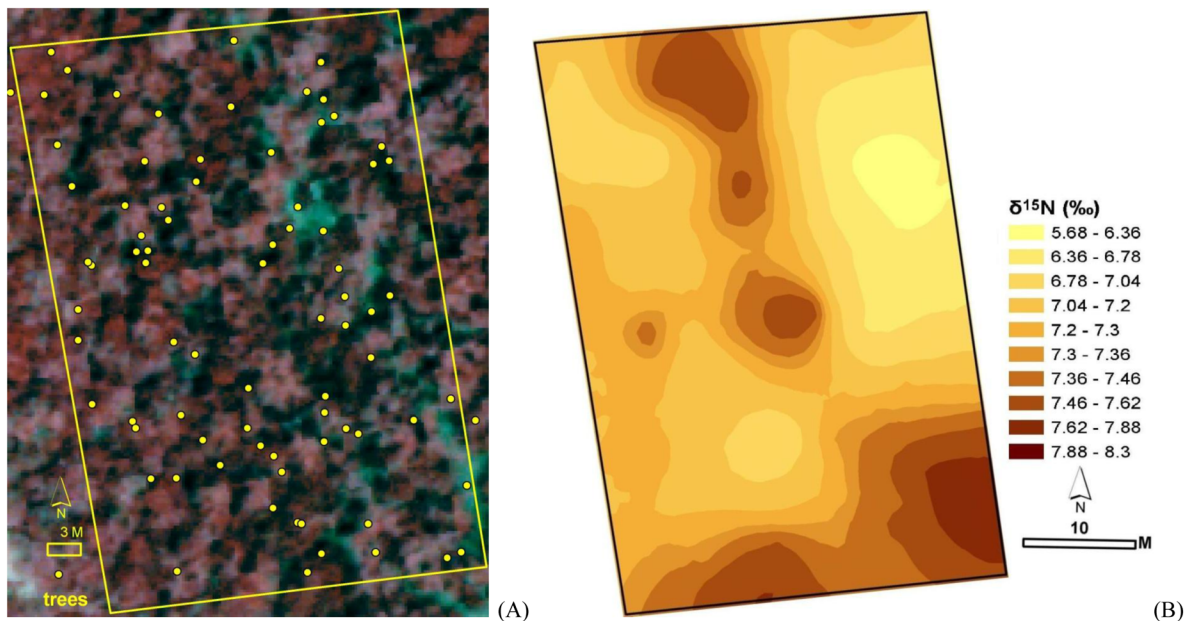


Fig. 6. Aerial photo of drainage woodland sampling grid (A) and kriged map of soil  $\delta^{15}\text{N}$  (0–15 cm) (B). Darker areas in the aerial photo represent denser woody cover. The area represented in (A) corresponds to the area outlined in yellow in (B). Yellow dots in (A) represent the locations of mesquite tree boles. The topographic low runs approximately north-south through center of both figures, with elevation gradually increasing to the east and west

and extending into the surrounding grassland matrix are also indicative of woody plant encroachment impacts on soil  $\delta^{15}\text{N}$ . Changes in values along these transects represent changes associated with time of woody plant occupation (e.g., Throop and Archer 2008), whereby locations nearest the bole of mesquite plants are areas of longest occupation and greatest influence; and points at shrub canopy drip lines represent locales of recent, but limited influence (relative to adjoining remnant grasslands experiencing no woody plant influence). The fact that soil  $\delta^{15}\text{N}$  increased along bole-to-dripline-to-grassland transects (Figs. 2 and 3) is clear evidence that a disturbance-induced change in vegetation, in this case displacement of grasses by N-fixing woody plants, can alter N inputs relative to outputs to systematically affect soil  $\delta^{15}\text{N}$ .

In summary, these results indicate that the development of woody communities dominated by N-fixing trees in upland grasslands has had a dramatic impact on the spatial pattern of soil  $\delta^{15}\text{N}$ . These results are consistent with findings in other studies. For example, proliferation of

*Prosopis velutina* in semi-desert grasslands in Arizona increased TN and decreased soil  $\delta^{15}\text{N}$  (Wheeler et al. 2007). Conversely, decreased N-fixation associated with intensification of land use increased soil  $\delta^{15}\text{N}$  in southern African ecosystems (Aranibar et al. 2008). However, some studies showed a different direction of change in soil  $\delta^{15}\text{N}$  after woody proliferation. For example, Bekele and Hudnall (2005) found that encroachment of the non-N-fixing evergreen arborescent red cedar (*Juniperus virginiana*) into a relict calcareous prairie caused soil  $\delta^{15}\text{N}$  enrichment, ostensibly due to changes in rates of decomposition processes. Similarly, soil  $\delta^{15}\text{N}$  increased during 40 years of forest development in southeastern USA (Billings and Richter 2006). In this case, increased abundance of ectomycorrhizal (ECM) fungi accompanying increases in non-N-fixing woody plant abundance were thought to have promoted N losses from the system. Therefore, the direction of soil  $\delta^{15}\text{N}$  change in response to vegetation change is not always consistent, but generally appears to be regulated by the balance between soil N inputs

and outputs (Fig. 1), as influenced by the functional properties of the dominant plant cover.

#### *Spatial patterns of soil $\delta^{15}\text{N}$ in lowlands*

Our results also supported Hypothesis 2: spatial patterns of soil  $\delta^{15}\text{N}$  on lowland portions of the landscape differed from those in the uplands. These differences were most likely a function of elevation-related differences in soil texture, soil moisture, and woody plant cover. Although woody plant basal area was substantially greater in the drainage woodland located in the topographically lower portion of the landscape (Liu et al. 2010), soil  $\delta^{15}\text{N}$  values there were significantly higher than those in upland areas (Table 1). Here, local topography had a much more pronounced influence on soil  $\delta^{15}\text{N}$  than did mesquite plants (Fig. 4). In the drainage woodland, both soil water content and soil TN were significantly higher than in the uplands (Table 1). Because soil water and TN are major determinants of decomposition and N-transformation rates (Matson and Vitousek 1981, Booth et al. 2005, Robertson and Groffman 2007), drainage woodlands should have accelerated rates of N-cycling processes, and higher preferential  $^{14}\text{N}$  losses (e.g., gaseous N loss) and enrichment of soil  $\delta^{15}\text{N}$  (Amundson et al. 2003, Houlton and Bai 2009). Consistent with these predictions, potential mineralization and nitrification rates and soil pool sizes of  $\text{NH}_4^+\text{-N}$  and  $\text{NO}_3^-\text{-N}$  are known to increase in the rank order clusters < groves < drainage woodlands (McCulley et al. 2004); and, soil  $\text{NO}_x$  gas losses from drainage woodland communities are significantly higher than those from upland landscape elements under both wet and dry soil moisture conditions (Cole et al. 1996, Sparks et al. 2011). Higher N transformation rates and larger pool sizes of inorganic N in the drainage woodlands would create circumstances favoring soil  $^{15}\text{N}$  enrichment via N gas losses. Our results are consistent with other assessments of landscape-scale variation in soil  $\delta^{15}\text{N}$ , which found positive correlations between soil water availability and  $\delta^{15}\text{N}$  (Garten and Van Miegroet 1994, Farrell et al. 1996, Dijkstra et al. 2003), and higher nitrification/denitrification rates and soil  $\delta^{15}\text{N}$  in lower-lying landscape settings (Sutherland et al. 1993). The higher soil moisture content of drainage woodlands is a consequence of

receipt of runoff from uplands (e.g., Wu and Archer 2005) which is then well-retained by the clay loam and clay soils that dominate the lowlands.

There was a significant positive correlation between clay content and  $\delta^{15}\text{N}$  in drainage woodland soils ( $r = 0.52$ ) (Table 2) and the highest  $\delta^{15}\text{N}$  values in drainages were typically associated with the topographic low-points where fine soil particle size fractions accumulate (Fig. 6B). This is consistent with prior studies showing that silt and clay fractions have significantly higher  $\delta^{15}\text{N}$  values than other soil physical fractions (Liao et al. 2006b), and that soils with more clay-sized particles generally have higher soil  $\delta^{15}\text{N}$  than more coarsely-textured soils (Ledgard et al. 1984, Silver et al. 2000, Baisden et al. 2002, Quideau et al. 2003, Hobbie and Ouimette 2009). The most likely mechanism for this is that silt- and clay-associated organic matter is often the oldest organic matter present in the soil, and therefore has a long history of decay and microbial processing which results in  $^{15}\text{N}$ -enrichment of the residual substrate. The lack of a relationship between these two variables in the uplands was probably because soil clay content there is half that of the lowlands, thereby minimizing the potential influence of this factor on whole soil  $\delta^{15}\text{N}$ .

#### *Alternate mechanisms affecting soil $\delta^{15}\text{N}$ spatial patterns*

The above discussion rests on the assumption that *Prosopis glandulosa*, a known N-fixing tree legume (Johnson and Mayeux 1990), is actually fixing atmospheric  $\text{N}_2$  at the study site and is therefore the primary driver causing lower soil  $\delta^{15}\text{N}$  values in woody plant dominated portions of the landscape. Although we have demonstrated directly that *P. glandulosa* grown from seeds produced by trees in our study area will readily nodulate and actively fix N when inoculated with soils from our study area (Zitzer et al. 1996), mechanisms other than N-fixation could potentially be responsible for these landscape scale patterns in soil  $\delta^{15}\text{N}$ . These include the transfer of soil N into plant tissue as woody stands accumulate biomass, atmospheric N-deposition, and the root-mediated transfer of N from deep to shallow soil depths. Are there other mechanisms at play?

Plant N accumulation was ruled out as a potential basis for the spatial patterns of soil  $\delta^{15}\text{N}$  we observed. Plant  $\delta^{15}\text{N}$  values are generally more negative than the soils in which they grow (Amundson et al. 2003), and net N accumulation in plant biomass (plant uptake > litter return) would cause enrichment of the residual soil  $\delta^{15}\text{N}$ . Therefore, the lower soil  $\delta^{15}\text{N}$  values following woody plant encroachment could not be caused by N accumulation in an expanding plant biomass pool.

Atmospheric N-deposition also seems to be an unlikely explanation for the observed patterns. The  $\delta^{15}\text{N}$  of atmospheric N deposited in this region ranges from  $-3.5$  to  $-5.5\text{‰}$  (Kendall et al. 2007). This is significantly lower than that of soils at our study site and could therefore lower soil  $\delta^{15}\text{N}$  if deposition has increased in recent decades. The taller canopies and more extensive leaf area of the wooded landscape elements may enable them to capture atmospheric deposition more efficiently than grasslands, perhaps accounting for some of the reduction in soil  $\delta^{15}\text{N}$  observed in wooded areas. However, total wet + dry atmospheric N-deposition is only  $0.19\text{--}0.53 \text{ g N m}^{-2} \text{ yr}^{-1}$  (CASTNET 2008), which is far less than rates of N accumulation quantified previously in soils beneath upland and lowland woodlands ( $1.6\text{--}4.6 \text{ g N m}^{-2} \text{ yr}^{-1}$ ) at our site (Archer et al. 2004, Boutton and Liao 2010). Thus, while atmospheric deposition may have contributed to some spatial-temporal variation of soil  $\delta^{15}\text{N}$  across this landscape, it is unlikely to be the principal mechanism.

Another alternative explanation is that deep-rooted encroaching woody species acquire soil N from depths not accessed by the herbaceous plants that dominate the grasslands, and that they transfer this N to surface soils via litterfall and shallow root turnover, thereby influencing soil  $\delta^{15}\text{N}$ . Indeed, our previous studies show that wooded communities in both uplands and lowlands have root systems that extend far deeper into the soil profile than those in herbaceous grassland communities (Boutton et al. 1999). Furthermore, nutrient translocation from deep soil layers is a well-documented pathway by which deep-rooted plants can concentrate nutrients in the upper portion of the soil profile (Jobbagy and Jackson 2001, Jobbagy and Jackson 2004). Redistribution of soil

N from deeper to shallower soil depths could thus be expected to occur when deep-rooted plants replace shallow-rooted plants during succession (Knops and Tilman 2000). However, soil  $\delta^{15}\text{N}$  generally increases significantly with depth in the soil profile (Nadelhoffer and Fry 1988, Hobbie and Ouimette 2009). At the La Copita study site, the  $\delta^{15}\text{N}$  of soil total N is approximately  $6\text{--}7\text{‰}$  in the  $0\text{--}15 \text{ cm}$  depth increment (Table 1), but this value increases to  $>8\text{‰}$  at  $1 \text{ m}$  below the soil surface in wooded areas (Zitzer, Boutton, Archer, *unpublished data*). Assuming that isotope fractionation during N uptake by roots and their symbionts is similar throughout the soil profile, vertical uplift of deeper soil N would likely cause  $^{15}\text{N}$  enrichment of the surface soil. Therefore, vertical uplift of deep soil N is an unlikely mechanism by which soil  $\delta^{15}\text{N}$  becomes significantly more depleted on wooded portions of this landscape.

Although we cannot definitively rule out either atmospheric N-deposition or uplift of deep soil N as mechanisms by which our observed landscape-scale spatial patterns in soil  $\delta^{15}\text{N}$  evolved, they appear to be either of insufficient magnitude or incorrect isotopic composition to yield the observed patterns. Therefore, we suggest that variation in the spatial distribution of soil  $\delta^{15}\text{N}$  across the study area largely reflects the net outcome of changes in the rates of N-input from fixation and N-losses from altered rates of N-transformations.

## CONCLUSIONS

Based on current evidence regarding the factors that influence and control soil  $\delta^{15}\text{N}$  values, we propose a simple conceptual model to explain how these values will respond to disturbance (Fig. 1). The model forecasts that when N inputs to soil are higher than N losses after disturbance, soil  $\delta^{15}\text{N}$  will decrease. In contrast, when N inputs to soil are lower than N losses after disturbance, soil  $\delta^{15}\text{N}$  will increase. A test of this general conceptual model in a subtropical grassland undergoing woody plant encroachment illustrates how this model can be locally refined to incorporate topographic contrasts within a landscape.

Under steady-state grassland conditions,  $\delta^{15}\text{N}$  values for the poorly drained, fine-textured

lowland soils should be higher than those in well-drained, coarser-textured upland soils because of the predominance of N losses via high-fractionation pathways (e.g., gaseous losses, higher  $\epsilon_{\text{output}}$ ). With a change in the disturbance regime (intensification of livestock grazing and cessation of fire), encroachment by woody plants, some of which are N-fixing, occurred across the landscape. In upland woody communities dominated by N-fixing mesquite trees, the addition of fixed N ( $\delta^{15}\text{N} \approx 0\text{‰}$ ) increased N input and hence reduced soil  $\delta^{15}\text{N}$ . Although woody plant densities were much higher in the drainage woodland communities located in a topographically lower portion of the landscape, soil  $\delta^{15}\text{N}$  values there were significantly higher than those in upland woody patches. Soil water, clay content, and total N are all higher in the lower landscape positions, creating conditions that favor more rapid N-transformation rates, resulting in higher preferential  $^{14}\text{N}$  losses and enrichment of soil  $\delta^{15}\text{N}$  in these lowlands. Results indicate that temporal variation of soil  $\delta^{15}\text{N}$  has the potential to be an indicator of disturbance-induced changes in the net N balance. However, the sensitivity of this indicator was diminished in topoedaphic settings with high rates of N-transformation. Continued improvements in our understanding of controls over the spatial variability of soil  $\delta^{15}\text{N}$  at the landscape-scale will enhance our ability to use  $\delta^{15}\text{N}$  as a diagnostic tool for inferring N dynamics under both steady-state and disturbed conditions.

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