Vegetation change alters soil profile $\delta^{15}$N values at the landscape scale

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A B S T R A C T

The assessment of spatial variation in soil $\delta^{15}$N could provide integrative insights on soil N cycling processes across multiple spatial scales. However, little is known about spatial patterns of $\delta^{15}$N within soil profiles in arid and semiarid ecosystems, especially those undergoing vegetation change with a distinct shift in dominance and/or functional type. We quantified how changes from grass to woody plant dominance altered spatial patterns of $\delta^{15}$N throughout a 1.2 m soil profile by collecting 320 spatially-specific soil cores in a 160 m $\times$ 100 m subtropical savanna landscape that has undergone encroachment by Prosopis glandulosa (an N2-fixer) during the past century. Leaf $\delta^{15}$N was comparable among different plant life-forms, while fine roots from woody species had significantly lower $\delta^{15}$N than herbaceous species across this landscape. Woody encroachment significantly decreased soil $\delta^{15}$N throughout the entire soil profile, and created horizontal spatial patterns of soil $\delta^{15}$N that strongly resembled the spatial distribution of woody patches and were evident within each depth increment. The lower soil $\delta^{15}$N values that characterized areas beneath woody canopies were mostly due to the encroaching woody species, especially the N2-fixer P. glandulosa, which delivered $^{15}$N-depleted organic matter via root turnover throughout the soil profile. Soil $\delta^{15}$N increased with depth, reached maximum values at intermediate depths, and slightly decreased at greater depths. This vertical pattern may be related to the decrease of $^{15}$N-depleted organic matter inputs with depth, and to the presence of a subsurface clay-rich argillic horizon at intermediate depths across this landscape, which may favor the accumulation of $^{15}$N-enriched residues. These results indicate that succession from grassland to woodland has altered the spatial variation in soil $\delta^{15}$N across the landscape and to considerable depth, suggesting significant changes in the relative rates of N-inputs vs. N-losses in this subtropical system after vegetation change.

1. Introduction

The stable nitrogen isotope composition ($\delta^{15}$N) of bulk soil, as an integrator of the soil N cycle, reflects the long-term net difference between $\delta^{15}$N values of N inputs (e.g. fixation, deposition) and those of N outputs (e.g. gaseous losses, leaching) (Högberg, 1997; Robinson, 2001; Amundson et al., 2003; Hobbie and Ouimette, 2009; Pardo and Nadelhofer, 2010; Craine et al., 2015a,b; Denk et al., 2017). As a result, soil $\delta^{15}$N values can provide integrative insights regarding the behavior of the soil N cycle across a range of spatial and temporal scales.

Soil $\delta^{15}$N values can vary by as much as 9–10‰ within a profile (Hobbie and Ouimette, 2009), and generally increase with depth (Bundt et al., 2001; Huygens et al., 2008; Yang et al., 2015). In some cases, maximum soil $\delta^{15}$N values occur at intermediate soil depths, with lower values both near the soil surface and deeper in the profile (Bustamante et al., 2004; Hobbie and Ouimette, 2009). Several mechanisms have been proposed to explain these vertical patterns of $^{15}$N enrichment throughout the soil profile (reviewed in Hobbie and Ouimette, 2009), including: (1) accumulation of $^{15}$N-enriched microbial residues at depth as a result of transfer of $^{15}$N-depleted N to plants by mycorrhizae, especially ectomycorrhizal fungi (Högberg, 1997; Lindahl et al., 2007; Huygens et al., 2008; Hobbie and Ouimette, 2009; Hobbie and Högberg, 2012; Mayor et al., 2015; Denk et al., 2017); (2) preferential preservation of $^{15}$N-enriched compounds during organic N decomposition (Hobbie and Ouimette, 2009); (3) fractionation against $^{15}$N during N transformations (e.g. nitrification and denitrification) followed by the subsequent loss of $^{15}$N-depleted gases (e.g. NO, N2O, N2) produced during those transformations (Nadelhofer and Fry, 1988; Hobbie and Ouimette, 2009; Craine et al., 2015a,b; Denk et al., 2017).

Vegetation can directly affect soil $\delta^{15}$N through symbiotic N2-fixation and mycorrhizal associations (Högberg, 1997; Craine et al., 2015a,b; Mayor et al., 2015), or indirectly through the modification of substrate quality/quantity and/or micro-environmental conditions that
may influence rates of soil N transformations (Bai et al., 2009a, b; 2013; Wang et al., 2013). For these reasons, when ecosystems undergo disturbances that modify vegetation dominance, primary production, and/or rates of N transformations, soil $^{15}N$ is likely to be altered (Bai et al., 2013). These potential modifications of soil $^{15}N$ following vegetation changes represent another complication to the utility of soil $^{15}N$ as a diagnostic tool for inferring prevalent soil N cycling processes under disturbed conditions.

A notable example of a change in vegetation dominance is the geographically widespread phenomenon of woody plant encroachment into grass-dominated ecosystems in arid and semiarid regions, which appears to be caused by livestock overgrazing, reduced fire frequency, rising atmospheric CO$_2$ concentration, and climate change (Bond and Midgley, 2000; Eldridge et al., 2011; Archer et al., 2017; Stevens et al., 2017), all of which have the potential to favor the productivity of C$_3$ woody plants at the expense of C$_4$ grasses. Woody plant encroachment is a complex social-ecological issue that has long been of concern to land managers because it has the potential to reduce the productivity of grazing livestock (e.g., cattle, sheep, equines) whose diets are strongly grass-based (Archer et al., 2017). In addition, woody encroachment has been shown to have significant impacts on biodiversity, hydrology, and biogeochemistry at ecosystem to global scales (Hibbard et al., 2001; Huxman et al., 2005; Pacala et al., 2007; Ratajczak et al., 2012; Ge and Zou, 2013; Anadón et al., 2014; Poulter et al., 2014).

In the context of soil N cycle, woody plant encroachment has been demonstrated to increase N inputs, intensify rates of soil N cycling processes, and accelerate N losses through leaching and trace gas emissions (Hibbard et al., 2001; Martin et al., 2003; McCulley et al., 2004; Liao et al., 2006; McKinley et al., 2008; Eldridge et al., 2011; Creamer et al., 2013; Soper et al., 2016). Although many studies have found decreased soil $^{15}N$ after woody plant encroachment (Wheeler et al., 2007; Boutton and Liao, 2010; Sitters et al., 2013), others have found either no net change (Blaser et al., 2014), or even increased soil $^{15}N$ (Bekele and Hudnall, 2005; Billings and Richter, 2006). Reasons for these discrepancies remain unclear, but may be related to whether or not the encroaching woody species include plants capable of symbiotic N-fixation, and/or the degree of impact that increased woody plant abundance has on individual soil N processes that vary in the extent of fractionation against $^{15}N$. More importantly, most of these studies investigating the impact of woody encroachment on soil $^{15}N$ have focused on surface soils (mostly < 30 cm). However, recent studies have emphasized that woody encroachment can have significant impacts on deep soil biogeochemistry (Chiti et al., 2017; Zhou et al., 2017a), as encroaching woody species generally have root systems that are distributed more deeply than those of the herbaceous species (Schenk and Jackson, 2002). Therefore, there is currently a knowledge gap regarding the direction and magnitude of soil $^{15}N$ changes in deeper portions of the soil profile following disturbance and vegetation change in arid and semiarid ecosystems.

Spatial variations in soil $^{15}N$ could provide integrative insights on the soil N cycle across multiple spatial scales (Craine et al., 2009a, b; Pardo and Nadelhoff, 2010; Bai et al., 2013; Wang et al., 2013; Rascher et al., 2012; Ruiz-Navarro et al., 2016). Woody plant encroachment into grass-dominated ecosystems is generally associated with the amplification of spatial heterogeneity in soil properties (“islands of fertility”, Schlesinger et al., 1996), making it more difficult to generalize ecosystem processes based on sampling at small spatial scales and limited sample sizes (Troop and Archer, 2008; Liu et al., 2011; Zhou et al., 2017b). Previous studies have demonstrated the existence of spatial variations in soil $^{15}N$ in arid and semiarid ecosystems and identified driving factors (such as vegetation type, topographic properties, and water availability) responsible for these spatial variations (Bai et al., 2013; Wang et al., 2013; Ruiz-Navarro et al., 2016). However, these studies were largely confined to surface soils, and it remains unclear how changes in plant life forms and/or functional types in arid and semiarid ecosystems may influence spatial variation in soil $^{15}N$ values in deeper portions of the soil profile.

In this study, we investigated a well-studied subtropical savanna ecosystem that has undergone encroachment by Prosopis glandulosa (an N$_2$-fixing tree legume) and other subordinate tree/shrub species during the past century in southern Texas, USA (Archer et al., 1988; Boutton et al., 1998). Prior research in this region has documented that woody plant encroachment has altered spatial patterns of soil $^{15}N$ in surface soils (0–15 cm) (Bai et al., 2013). To further expand on this work, we collected 320 spatially specific soil cores to a depth of 1.2 m across a 160 m × 100 m landscape in this subtropical savanna to test the following two hypotheses: (1) the deep-rooting characteristics of the encroaching woody species would modify soil $^{15}N$ values to considerable depth within the soil profile; and (2) landscape-scale spatial patterns of soil $^{15}N$ in the horizontal plane would be evident throughout the soil profile and correlated with the distribution patterns of the encroaching woody vegetation.

2. Methods and materials

2.1. Study site

Research was conducted at the Texas A&M AgriLife La Copita Research Area (27°40' N, 98°12' W; elevation 75–90 m a.s.l.) in Jim Wells County, Texas, USA (Fig. S1). The climate is subtropical, with mean annual temperature and precipitation of 22.4 °C and 680 mm, respectively. Landscapes consist of well-drained uplands that grade gently (1–3% slopes) to lower-lying drainage woodlands. Soils on upland portions of the landscape are sandy loams (Typic and Pachic Argiuvoisols) with a laterally extensive but discontinuous clay-rich argillic horizon (B$_{s}$) which begins 30–50 cm below the surface (Archer, 1995; Zhou et al., 2017b).

Multiple lines of evidence (i.e. historical accounts, tree-ring analyses, and coupled $^{3}^{13}C$-$^{14}C$ analyses of soil organic matter) have indicated that upland vegetation was once almost exclusively dominated by C$_4$ grasses, and woody encroachment into C$_4$ dominated grasslands has occurred during the past century due to overgrazing and reduced fire frequency (Archer et al., 1988, 2001; Archer, 1995; Boutton et al., 1998). Current upland vegetation is comprised of discrete woody patches distributed within a remnant C$_4$ grassland matrix (Archer et al., 1988; Archer, 1995). Woody patches consist of small shrub clusters (generally < 100 m$^2$) and large groves (generally > 100 m$^2$). The formation of woody patches is initiated by the colonization of Prosopis glandulosa, an N$_2$-fixing tree legume (Zitter et al., 1996; Soper et al., 2015). Established P. glandulosa trees then serve as nurse plants, facilitating the recruitment of other trees/shrubs underneath their canopies to form discrete clusters (Archer et al., 1988). The spatial distribution of clusters across this landscape is random and not related to the spatial heterogeneity in subsurface soil texture (Zhou et al., 2017b). However, if clusters occur on non-argilic inclusions (i.e. coarse-textured soils), they expand laterally and coalesce to form groves (Archer, 1995; Zhou et al., 2017b). In the process of occupying the non-argilic inclusions, groves will often merge with clusters that develop on the argillic soils. Therefore, the peripheral areas of groves often occupy soils where the argillic horizon is present. At present, the remnant grassland matrix is dominated by C$_4$ grasses, but also includes C$_3$ forbs and a small portion of crassulaceous acid metabolism (CAM) species. Groves are dominated by P. glandulosa trees with up to 15–20 other tree/shrub species in the understory. Clusters consist of the same woody species as groves, but P. glandulosa in clusters are significantly smaller and younger than those in groves (Boutton et al., 1998). Grasses and other herbaceous species are extremely rare underneath clusters and groves. Species composition can be found in Table S1. Based on the plant and soil properties and processes that characterize grasslands, clusters, and groves, each are unique ecosystems that comprise the upland landscape, and we refer to them as landscape elements (Turner et al., 2001).
2.2. Field sampling and lab analyses

On an upland portion of this study site, a 160 m × 100 m landscape consisting of 10 m × 10 m grid cells was established in January 2002 (Liu et al., 2011) (Fig. S1). Each corner of each grid cell was georeferenced using a GPS unit, assigning X, Y coordinates (UTM WGS84 zone 14N). In each grid cell, two random sampling points were selected in July 2014, yielding 320 sampling points across this landscape (Fig. S1). Distances from each sampling point to two georeferenced cell corners were recorded for calculating coordinates of each point. The vegetation of each sampling point was categorized as grassland, cluster or grove based on vegetation type and canopy size of woody patches. At each sampling point, two adjacent soil cores (120 cm deep x 2.8 cm diameter) were collected (PN150 JMC Environmentalist’s Subsoil Probe, Clements Associates Inc., Newton, IA, USA) and subdivided into six depth increments (i.e. 0–5, 5–15, 15–30, 30–50, 50–80, and 80–120 cm). A color-infrared aerial photograph of this landscape was acquired in July 2015 and the normalized difference vegetation index (NDVI) of each sampling point was calculated (Zhou et al., 2017a).

Leaf and fine root (<2 mm) tissues of each plant species occurring on this landscape were collected in September 2016. For each woody species, approximately equal amounts of leaves from three individuals were collected to make a composite sample; then, fine roots were excavated carefully from surface soils (0–15 cm) after confirming their linkages to the selected three individuals and mixed to make a composite sample. The same sampling method was applied to forbs, grasses, and cacti (CAM species), but more than three individuals/species were sampled in order to meet the mass requirements for elemental and isotopic analyses. Composite leaf and fine root samples were washed carefully, dried, and pulverized in a Mixer Mill MM 400 (Retsch GmbH, Haan, Germany).

Soils within each depth increment from one of the two soil cores were oven-dried at 105 °C to determine soil bulk density (no gravel was present in any of the soil samples), and these soils were subsequently used to estimate fine and coarse (>2 mm) root biomass by washing through sieves. No attempt was made to distinguish between live or dead roots. Retrieved roots were cleaned of soil particles, dried at 65 °C, and weighed. In order to analyze δ15N of fine roots throughout the soil profile, 10 cores were selected from each landscape element (i.e. grassland, cluster, and grove). Fine roots within the selected 10 cores were combined to make a composite fine root sample for each depth increment. This process was repeated three times and three composite fine root samples were made for each landscape element. Composite fine root samples were pulverized in a Mixer Mill MM 400.

Soils within each depth increment from the other soil core were air-dried and then passed through a 2 mm sieve to remove coarse organic matter. An aliquot of sieved soil was used to determine soil texture using the hydrometer method. Another aliquot of sieved soil was dried at 65 °C for 48 h and then pulverized in a centrifugal mill (Angstrom Inc., Belleville, MI, USA). Total N concentrations and δ15N values of pulverized soils, fine roots extracted from soil cores, and composite leaf and root tissues sampled from individual plant species were determined using a Costech ECS 4010 elemental analyzer (Costech Analytical Technologies Inc., Valencia, CA, USA) interfaced via a ConFlo IV with a Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany) at the Stable Isotopes for Biosphere Science Laboratory, Department of Ecosystem Science and Management, Texas A&M University. Organic C concentrations of pulverized soil samples were determined in the same way but were treated with HCl vapor in a desiccator to remove carbonates prior to analysis (Harris et al., 2001). Stable N isotopic compositions are reported in the conventional form according to equation (1):

$$\delta^{15}N (\%o) = \left( \frac{{^{15}N/^{14}N}_{\text{sample}}}{{^{15}N/^{14}N}_{\text{standard}}} - 1 \right) \times 1000$$

where ${{^{15}N/^{14}N}_{\text{sample}}}$ and ${{^{15}N/^{14}N}_{\text{standard}}}$ are the stable N isotopic ratio of the sample and standard, respectively. The standard is atmospheric N2 (Mariotti, 1983). Precision of duplicate measurements was 0.1‰.

2.3. Data analyses

All data sets were tested for normality before performing statistical analyses and log10-transformed to improve normality when necessary. Mixed models, which consider spatial autocorrelation as a spatial covariation for adjustment (Littell et al., 2006), were used to compare means of measured variables (i.e. NDVI, root biomass, soil total N and δ15N) for different landscape elements within each depth increment. Across this 160 m × 100 m landscape, groves occur almost exclusively on non-argillic inclusions; however, some groves expand laterally beyond the non-argillic inclusion onto soils where the subsurface argillic horizon is present. In addition, some non-argillic inclusions within the grassland matrix are still not occupied by groves (Zhou et al., 2017b). To address the potential effect of the subsurface argillic horizon on soil δ15N, we subdivided soil cores from both grasslands and groves into those taken where the argillic horizon is present vs. those taken where the argillic horizon is absent using soil diagnostics for the higher categories as outlined in USDA Soil Taxonomy (Soil Survey Staff, 1999). More details can be found in Zhou et al. (2018). Seventeen out of 200 soil cores from grasslands were taken within non-argillic inclusions, whereas 24 out of 79 soil cores from groves were taken within non-argillic inclusions. All 41 soil cores from clusters were taken where the argillic horizon was present. One-way ANOVA was performed to compare δ15N values of composited fine root samples and of soil samples from grasslands and groves occurring on non-argillic vs. argillic soils within each depth increment. Post-hoc comparisons of these variables in different landscape elements were conducted with Tukey's test. A cutoff of p < 0.05 was used to indicate significant difference. These analyses were performed using JMP Pro 12.0 (SAS Institute Inc., Cary, NC, USA).

Varioiagram analyses were used to determine the spatial structure of soil total N and δ15N based on 320 random sampling points across this landscape and throughout the soil profile. A variogram is a plot of a series of semivariance values (γ) against the corresponding lag distances (h). The semivariance γ at each h is calculated according to equation (2):

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} (Z(X_i) - Z(X_{i+h}))^2$$

where $Z(X_i)$ and $Z(X_{i+h})$ are the values of soil variables at spatial location $X_i$ and $X_{i+h}$ for each depth increment, $N(h)$ is the number of sample pairs with lag distance h. The spherical model has been selected, as it has been shown to provide an adequate representation of the spatial variability of soil data in dryland ecosystems (Schlesinger et al., 1996; Bai et al., 2009b). Nugget (C0), range (A), structure variance (C), sill (C0 + C), and the ratio of structure variance to sill variance (C/C0 + C) were used to interpret spatial structure (Figs. S2 and S3). Nugget, the variance at lag distance zero, is due to measurement error and reflects the variability at scales finer than the sampling unit. Range indicates the distance of spatial autocorrelation between data pairs, beyond the range the test variable can be considered spatially independent. The ratio of structure variance to sill variance, representing the proportion of the total variance that is spatially structured, reveals the structure strength. Varioiagram analyses were conducted using R software (R Development Core Team, 2014). Ordinary kriging was used...
for spatial interpolation of soil total N and δ^{15}N values at unsampled locations based on data from 320 sampling points and their spatial structure determined by variogram analysis. Kriged maps of soil total N and δ^{15}N for each depth increment were generated using ArcMap 10.2.2 (ESRI Inc., Redlands, CA, USA).

A classified vegetation map was delineated from the aerial photograph using ArcMap 10.2.2. To more clearly evaluate variation in soil total N and δ^{15}N within woody patches vs. grasslands, the distance from each sampling point to the nearest woody patch edge was calculated and correlated with soil total N and δ^{15}N. In this calculation, sampling points located within the grassland matrix were assigned negative distance values. Thus, more negative values indicated that sampling points farther away from the nearest woody patch edges. In contrast, sampling points located within woody patches were assigned positive distance values such that larger values indicated sampling points farther away from woody patch edges.

Lacunarity, a scale-dependent measure of spatial heterogeneity or the "gappiness" of a landscape structure (Plotnick et al., 1996), was used to quantify the spatial heterogeneity of soil δ^{15}N across this landscape and throughout the soil profile. Lacunarity analysis was performed based on kriged maps of soil δ^{15}N using R software. Briefly, a gliding box of a given size (side length the box, r) was first placed at one corner of the kriged map, and the box mass (S(r)), the sum of soil δ^{15}N value of each pixel within the box, was determined. The box was then systematically moved through the kriged map one pixel at a time and the box mass was determined at each location. The lacunarity for box size r is calculated according to equation (3):

\[ \Lambda(r) = \frac{\text{var}(S(r))}{E(S(r)^2)} + 1 \]  

where var(S(r)) is the variance and E(S(r)) is the mean of the box mass (S(r)) for a given box size (r). The lacunarity curve, a log-log plot of lacunarity \( \Lambda(r) \) against box size r, was then plotted to quantify spatial heterogeneity of soil δ^{15}N at different spatial scales for each depth increment, with a higher value of lacunarity indicating a more heterogeneous distribution pattern across the landscape.

The spatial correlations of all measured parameters were assessed using Pearson’s correlation coefficients and a modified t-test, which corrects the degrees of freedom based on the extent of spatial autocorrelation in the data (Dutilleul et al., 1993). Descriptive statistics for soil parameters (e.g. soil bulk density, soil C: N ratio, and soil texture) across this landscape and throughout the soil profile have been presented elsewhere (Zhou et al., 2017a, b). Datasets were log_{10}-transformed prior to the analysis of correlations using PASaGE version 2 (Rosenberg and Anderson, 2011).

3. Results

3.1. Vegetation and soil attributes across this landscape

Grasslands, clusters, and groves covered 62.4%, 10.3%, and 27.3% of this 160 m × 100 m landscape, respectively (Table 1). The spatial patterns of the Normalized Difference Vegetation Index (NDVI) across this landscape corresponded closely to the spatial distribution of woody patches (Fig. S4); clusters (0.36 ± 0.02) and groves (0.31 ± 0.02) had significantly higher NDVI values than grasslands (0.20 ± 0.003) (Table 1).

Woody patches (both clusters and groves, hereafter) had significantly higher fine and total root biomass than grasslands through the soil profile (Fig. S5). Summed over the entire 1.2 m soil profile, woody patches had 2 times more fine root biomass and 5 times more total root biomass than grasslands (Table 1).

Mean δ^{15}N values of leaf tissues ranged from 3.1 to 4.0‰ and were comparable among different plant life-forms (Fig. 1, Table S1). In contrast, mean δ^{15}N values of fine root tissues ranged from 0.5 to 2.3‰ and were significantly lower for woody species (both N₂-fixers and non-N₂-fixers) than other plant life-forms (Fig. 1, Table S1). Throughout the entire 1.2 m sampling depth, composite fine root samples from groves had significantly lower δ^{15}N than fine roots in clusters and grasslands.

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**Table 1**

Vegetation attributes across a 160 m × 100 m landscape in a subtropical savanna. Root biomass data is presented for fine and total root biomass throughout the full 120 cm soil profile. Detailed depth distribution of root biomass can be found in Fig. S5. Significant differences (p < 0.05) between means for landscape elements are indicated with different superscript letters. Values for root biomass and NDVI (normalized difference vegetation index) are mean ± SE. Number of samples: grassland = 200, cluster = 41, and grove = 79.

<table>
<thead>
<tr>
<th>Landscape element</th>
<th>Vegetation cover</th>
<th>NDVI</th>
<th>Root biomass (Mg ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>m² (%)</td>
<td></td>
<td>Fine roots Total roots</td>
</tr>
<tr>
<td>Grassland</td>
<td>9976 62</td>
<td>0.20 ± 0.00^a</td>
<td>10.8 ± 0.19^a 12.5 ± 0.40^b</td>
</tr>
<tr>
<td>Cluster</td>
<td>1649 10</td>
<td>0.36 ± 0.02^b</td>
<td>22.9 ± 0.97^c 60.0 ± 7.63^d</td>
</tr>
<tr>
<td>Grove</td>
<td>4375 27</td>
<td>0.31 ± 0.02^c</td>
<td>25.6 ± 0.74^c 62.1 ± 5.97^a</td>
</tr>
</tbody>
</table>

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**Fig. 1.** δ^{15}N values (%o) of leaf (a) and fine root (b) tissues for different plant life-forms occurring on this 160 m × 100 m landscape. The box plots summarize the distribution of points for each plant life-form. The central box shows the inter-quartile range, median (horizontal solid line in the box), and mean (horizontal dotted line in the box). Lower and upper error bars indicate 10th and 90th percentiles, and points above and below the error bars are individuals above the 90th or below the 10th percentiles. CAM, crassulacean acid metabolism species, n = 3; F, forbs, n = 9; G, grasses, n = 13; NNFW, non-N₂-fixing woody species, n = 13; NFW, N₂-fixing woody species, n = 2. More details see Table S1.
and δ15N values of composite fine root samples from clusters were significantly lower than those from grasslands except in the 50–80 cm depth increment (Fig. 2a).

Soil total N decreased continuously with depth throughout the soil profile in all landscape elements (Fig. 2b). Soils underneath woody patches had significantly higher total N than those underneath grasslands in upper (0–30 cm) and lower (80–120 cm) soil depth increments but not in the 30–50 and 50–80 cm depth increment (Fig. 2b). Spatial patterns of soil total N strongly resembled the spatial distribution of woody patches in the 0–5 and 5–15 cm depth increments; however, the strength of this spatial relationship faded and became more obscure at depths >15 cm (Fig. 3a and b). This visual assessment was supported by strong correlations between soil total N and NDVI, and also between soil total N and distance from each sampling point to the nearest woody patch edge in the 0–5 and 5–15 cm depth increments but weak to no correlations at depths >15 cm (Table S3 and Fig. S6).

 Soil δ15N values of grasslands and clusters increased by approximately 2–3% from the soil surface down to approximately 50 cm, and then decreased slightly in the 80–120 cm increment (Fig. 2c). Soils from woody patches had significantly lower δ15N than those from grasslands in the 0–5 cm depth increment; groves had significantly lower soil δ15N than both clusters and grasslands below 5 cm of the soil profile; clusters had significantly lower soil δ15N than grasslands in the 5–15, 15–30, 30–50, and 50–80 cm depth increments (Fig. 2c). Soil δ15N values did not differ between grasslands on argillic soils vs. those on non-argillic inclusions throughout the soil profile (Fig. 2d). In contrast, soil δ15N values under groves on non-argillic inclusions were significantly lower than those where the argillic horizon was present at depths >30 cm where the argillic horizon begins to occur (Fig. 2d).

3.2. Spatial variation of soil δ15N across the landscape and the soil profile

Both means and medians of soil δ15N increased with depth, reached highest values in the 30–50 cm depth increment and decreased in the last two depth increments across this landscape (Table S2). The coefficients of variation for soil δ15N were higher in the 0–5 cm, 15–30 cm, and 30–50 cm depth increments than in other depth increments (Table S2). Variogram analyses indicated that, with spherical models, there were clear distances for spatial autocorrelation in soil δ15N across this landscape, with ranges from 19.8 m at 80–120 cm to 27.3 m at 0–5 cm in the soil profile (Table 2 and Fig. S3). The strength of spatial structure (C/(C0 + C)) in soil δ15N also increased with depth, reached the maximum in the 30–50 cm depth increment, and decreased in the two deepest increments (Table 2).

Maps of soil δ15N throughout the soil profile derived from ordinary kriging and variogram analyses displayed a strong resemblance to the spatial distribution of woody patches (Fig. 3a and c). This is consistent with significant correlations between soil δ15N and NDVI throughout the soil profile (Table 3). Soil δ15N values were lowest near the central portions of woody patches, increased towards the edges, and reached the highest values in the remnant grassland matrix (Fig. 3a and c). This spatial trend observed visually in the kriged maps was statistically supported by significantly negative correlations between soil δ15N and distance from each sampling point to the nearest woody patch edges throughout the soil profile (Fig. 4). Although these spatial patterns were strong in every soil depth increment, they were especially evident at intermediate depths (e.g. 30–50 cm). Lacunarity analyses indicated that soil δ15N in the 0–5, 5–15, and 30–50 cm depth increment was more heterogeneous than in other depth increments (Fig. 5), corresponding well with results from coefficients of variation (Table S2).

Soil δ15N was significantly and negatively correlated with soil root biomass but positively with soil bulk density across this landscape throughout the entire soil profile (Table 3). Soil δ15N was significantly and negatively correlated with soil C:N ratio throughout the soil profile except in the 15–30 cm depth increment (Table 3). Soil δ15N was significantly and negatively correlated with total soil N throughout the soil profile except in the 30–50 and 50–80 cm depth increments where soil δ15N was significantly and positively correlated with soil clay content (Table 3).

4. Discussion

Globally widespread woody encroachment has dramatically altered the structure and function of grassland and savanna ecosystems (Eldridge et al., 2011), with the potential to profoundly influence soil biogeochemical cycling (Hibbard et al., 2001). The spatially explicit approach of our study enabled us to assess the impact of the encroachment of P. glandulosa and other woody species into this subtropical savanna on spatial variation of soil δ15N in both horizontal and vertical planes. Consistent with our first hypothesis, we found that woody encroachment into areas that were once grassland decreased soil δ15N significantly throughout the entire 1.2 m soil profile (Fig. 2c). In addition, we found that spatial patterns of soil δ15N displayed strong resemblance to the spatial distribution of woody patches, and this spatial relationship was evident throughout the entire soil profile (Fig. 3a and c), supporting our second hypothesis.

4.1. Spatial variation of soil δ15N in the horizontal plane

Overall, the vegetation shift from grass to woody plant dominance,
Fig. 3. The classified vegetation map for this 160 m × 100 m landscape (a) and kriged maps of soil total N (kg N m$^{-3}$) (b) and soil $\delta^{15}$N values (‰) (c) throughout the soil profile based on 320 randomly located sampling points in a subtropical savanna.
which altered the amount of root biomass that delivers 15N-depleted organic matter to soils through root turnover (Table 1, Figs. 1, 2a, and Fig. S5), is likely to be the mechanism responsible for the observed spatial variation in soil δ15N in the horizontal plane, as spatial patterns of soil δ15N throughout the soil profile resembled the spatial distribution of woody patches (Fig. 3a and c) and soil δ15N was significantly and positively correlated with NDVI but negatively with fine root biomass (Table 3). In arid and semiarid ecosystems, trees/shrubs tend to have lower 15N in leaf and/or root tissues than the plants in the herbaceous communities they were invading. This suggests that the δ15N values of the organic matter inputs are not the sole determinants of soil δ15N following woody encroachment, and that other biotic or abiotic factors affected by encroachment may be modifying the rates and magnitudes of key soil N transformations that fractionate N isotopes and that could therefore offset or obscure the δ15N values of N inputs from litterfall and root turnover.

Apart from the addition of 15N-depleted organic matter derived from woody species, spatial patterns of soil δ15N are also affected by the net outcome of spatial variation in the extent of fractionation against 15N during soil N cycling processes across the landscape. For example, although soil δ15N in wooded areas was significantly lower than in herbaceous areas in the 0–5 cm depth increment (Fig. 2a), visual assessment of the classified vegetation map (Fig. 3a) and the kriged map of soil δ15N in the 0–5 cm depth increment (Fig. 3c) reveals other subtleties, such as (1) soils under some woody patches were more enriched in 15N than under others; (2) soils under some woody patches had δ15N similar to those within the grassland matrix; and (3) there were 15N-enriched hotspots inside some woody patches. Previous studies at this site have demonstrated that woody plant encroachment has significantly magnified the pool size of soil microbial N (McCulley et al., 2004; Liao and Boutton, 2008), soil enzyme activities for organic N decomposition (Cramer et al., 2013), and accelerated the rates of other soil N transformations (e.g., ammonification, nitrification) (Hibbard et al., 2001; McCulley et al., 2004) that can lead to N losses from surface soils. Most soil N transformation processes fractionate strongly against 15N (Hobbie and Ouimette, 2009; Denk et al., 2017). For example, δ15N values of gaseous N lost from nitrification, denitrification, and ammonia volatilization are highly 15N-depleted (ranging from -64.0‰ to −10.0‰, reviewed in Denk et al., 2017). Thus, woody plant encroachment may exert different degrees of control on these soil N transformation processes for a variety of reasons, such as woody patch sizes, microclimatic conditions, substrate concentrations, topographic properties, and bioturbation (Liao et al., 2006; Liu et al., 2011; Bai et al., 2013), leading to these discrepancies in surface soil δ15N between and inside woody patches across this landscape (Fig. 3a and c).

### 4.2. Spatial variation of soil δ15N in the vertical plane

For all landscape elements, soil δ15N values were relatively low in the uppermost portions of the profile, and then increased continuously to approximately 50 cm (Figs. 2c and 3c, and Table S2). This is consistent with other studies in a variety of ecosystems (e.g. Nadelhofer and Fry, 1988; Högberg, 1997; Bustamante et al., 2004; Hobbie and Ouimette, 2009). Low δ15N values in the upper profile results from the fact that δ15N values of leaves (3–4‰) and fine roots (2–4‰) are lower than those of soils (Figs. 1, 2a and 2c); thus, deposition of relatively 15N-depleted litter and roots to soils would decrease δ15N values of the soil N pool. In addition, organic matter inputs from litterfall and root turnover are largely concentrated in the uppermost portion of the soil profile, and decrease exponentially with soil depth (Jackson et al., 1996). Consequently, the influence of 15N-depleted litterfall and root

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**Table 2**

Parameters for spherical models fitted to semivariograms of soil δ15N throughout the soil profile based on 320 random sampling points across a 160 m × 100 m landscape in a subtropical savanna.

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>Range (m)</th>
<th>Nugget (Cg)</th>
<th>Sill (Cg + C)</th>
<th>Sill-Nugget/Sill (C/Cg (C %))</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
<td>27.3</td>
<td>0.21</td>
<td>0.55</td>
<td>61.8</td>
<td>0.46</td>
</tr>
<tr>
<td>5–15</td>
<td>22.5</td>
<td>0.09</td>
<td>0.51</td>
<td>82.4</td>
<td>0.49</td>
</tr>
<tr>
<td>15–30</td>
<td>25.6</td>
<td>0.12</td>
<td>0.82</td>
<td>85.4</td>
<td>0.62</td>
</tr>
<tr>
<td>30–50</td>
<td>25.6</td>
<td>0.08</td>
<td>1.02</td>
<td>92.2</td>
<td>0.71</td>
</tr>
<tr>
<td>50–80</td>
<td>22.8</td>
<td>0.11</td>
<td>0.65</td>
<td>83.1</td>
<td>0.62</td>
</tr>
<tr>
<td>80–120</td>
<td>19.8</td>
<td>0.14</td>
<td>0.44</td>
<td>68.2</td>
<td>0.45</td>
</tr>
</tbody>
</table>

### Table 3

Correlations between soil δ15N (%), and vegetation/soil attributes across a 160 m × 100 m landscape and throughout the soil profile. Vegetation/soil attributes include NDVI, fine root biomass (FRB) (g m⁻²), total N (TN) (kg N m⁻²), C:N ratio, soil bulk density (SBD) (g cm⁻³), and soil clay content (%). The correlations were calculated using a modified t-test which adjusts the degrees of freedom based on the extent of spatial autocorrelation in the data (Batsel et al., 1993). A full table of correlations among these variables can be found in Table S3.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>NDVI</th>
<th>FRB</th>
<th>TN</th>
<th>C:N</th>
<th>SBD</th>
<th>Clay</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
<td>0.33*</td>
<td>0.53***</td>
<td>0.63***</td>
<td>0.44***</td>
<td>0.59***</td>
<td>0.30</td>
</tr>
<tr>
<td>5–15</td>
<td>0.34***</td>
<td>0.61***</td>
<td>0.64***</td>
<td>0.56***</td>
<td>0.25*</td>
<td>0.08</td>
</tr>
<tr>
<td>15–30</td>
<td>0.34***</td>
<td>0.56***</td>
<td>0.44***</td>
<td>0.10</td>
<td>0.28***</td>
<td>0.02</td>
</tr>
<tr>
<td>30–50</td>
<td>0.34***</td>
<td>0.43***</td>
<td>0.11</td>
<td>0.43***</td>
<td>0.24***</td>
<td>0.37***</td>
</tr>
<tr>
<td>50–80</td>
<td>0.25***</td>
<td>0.52***</td>
<td>0.15</td>
<td>0.53***</td>
<td>0.44***</td>
<td>0.31***</td>
</tr>
<tr>
<td>80–120</td>
<td>0.14***</td>
<td>0.35***</td>
<td>0.30***</td>
<td>0.51***</td>
<td>0.38***</td>
<td>0.03</td>
</tr>
</tbody>
</table>

* p < 0.05; ** p < 0.01; *** p < 0.001.
turnover on soil $\delta^{15}$N values decreases with depth.

Soil $\delta^{15}$N reached maximum values between 50 and 80 cm in all landscape elements, and then declined slightly at depths $\geq$ 80 cm (Figs. 3c and 4 and Table S2). This vertical pattern was especially evident in clusters and grasslands, but was less evident in groves (Fig. 3c). These patterns resulted in the highest spatial heterogeneity of soil $\delta^{15}$N in the 30–50 cm depth compared to all other depth increments, as revealed by lacunarity analyses (Fig. 5).

What mechanism(s) might account for the different $\delta^{15}$N depth distributions between groves vs. grasslands and clusters? Hobbie and Ouimette (2009) proposed that production of $^{15}$N-depleted N$_2$O and N$_2$ during denitrification at intermediate depths could be a plausible mechanism causing $^{15}$N enrichment at intermediate depths relative to shallower and greater depths. Since all soil cores from ($n = 41$) clusters and the majority of soil cores (183 out of 200 cores) from grasslands included an argillic horizon beginning around the 30–50 cm depth, it is possible that the presence/absence of this clay-rich horizon is somehow related to the vertical patterns of soil $\delta^{15}$N values in this landscape. Higher clay content in the argillic horizon beneath grasslands and clusters would be conducive to greater moisture retention and lower oxygen availability following rainfall events (Maag and Vinther, 1996; Bouwman et al., 2002; Van der Salm et al., 2007; Zhu et al., 2013), thereby favoring the loss of $^{15}$N-depleted N$_2$O and N$_2$ via denitrification, and the accumulation of $^{15}$N-enriched residual soil N in this portion of the soil profile.

In contrast, the absence of the clay-rich argillic horizon in groves would likely be conducive to more rapid drainage of water through the soil profile, minimizing the potential for the development of anaerobic conditions and N-losses via denitrification, thereby resulting in the lower soil $\delta^{15}$N values that we observe in groves vs. clusters and grasslands. Interestingly, where groves have expanded beyond the boundaries of the non-argillic inclusions onto areas where the argillic horizon is present, their $\delta^{15}$N depth distribution patterns becomes more similar to those of clusters and grasslands (Fig. 2d), suggesting further that the presence/absence of the argillic horizon exerts an effect on N-cycling processes that influence soil $\delta^{15}$N.

However, this argument is unable to account for the fact that grasslands occurring on non-argillic inclusions that have not yet been colonized by woody plants have soil $\delta^{15}$N depth distributions patterns that are not significantly different from grasslands growing on soils where the argillic horizon is present (Fig. 2d). This suggests that there may be important plant attributes (e.g., plant tissue chemistry, root distribution patterns) that interact with the presence/absence of the argillic horizon to influence depth distribution patterns of soil $\delta^{15}$N values. Further studies are needed to explore how changes in soil texture throughout the soil profile interact with vegetation change to affect
5. Conclusion

Our results indicate that the establishment of woody patches dominated by N2-fixing trees in areas that were once grassland has had a dramatic impact on landscape-scale spatial patterns of soil $^{15}$N throughout the upper 1.2 m of the soil profile. An increasing amount of $^{15}$N-depleted inputs derived from encroaching woody species differentiated soil $^{15}$N in wooded areas from that in herbaceous areas, creating spatial patterns of $^{15}$N throughout the soil profile that resembled the spatial distribution of woody patches. While soil $^{15}$N in grasslands and clusters increased with depth to maximum values at 30–50 cm, and then decreased slightly in the 80–120 cm increment, the soil $^{15}$N values in groves increased with depth throughout the entire profile. The exact mechanisms shaping this discrepancy remain unknown, but may relate to the presence (under grasslands and clusters) or absence (under groves) of a subsurface argillic horizon that could interact with vegetation change to drive the vertical patterns of soil $^{15}$N. Our results also highlight the benefits of spatially-specific deep soil sampling for the study of soil $^{15}$N in arid and semiarid ecosystems. Further studies aimed at improving our understanding of the mechanisms and controls over spatial variations in soil $^{15}$N will enhance our ability to apply $^{15}$N as a tool for inferring soil N dynamics across multiple spatial scales. Since woody plant encroachment is a graphically widespread phenomenon in dryland regions which cover 41% of Earth’s land surface (Reynolds et al., 2007; Stevens et al., 2017), this land cover change has the potential to significantly alter patterns of soil $^{15}$N at regional to global scales. Ultimately, a better characterization of global patterns of soil $^{15}$N values may facilitate our ability to predict changes in N cycling processes that can influence global biogeochemistry and the climate system (Craine et al., 2015a,b).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2018.01.012.

References


