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Spatial patterns of soil δ^{13}C reveal grassland-to-woodland successional processes

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ABSTRACT

Many grasslands and savannas around the world have experienced woody plant encroachment in recent history. In the Rio Grande Plains of southern Texas, subtropical woodlands dominated by C_3 trees and shrubs have become significant components of landscapes once dominated almost exclusively by C_4 grasslands. In this study, spatial variation of soil δ^{13}C to was used to investigate patterns of transformation. Previous research has shown that grassland-to-shrubland transitions are initiated when discrete, multi-species shrub clusters organized around a honey mesquite (*Prosopis glandulosa*) tree nucleus established in grassland. It is inferred from space-for-time substitution and modeling studies that as new shrub clusters are initiated and existing clusters enlarge, coalescence will occur, leading to the formation of groves; and that groves will eventually merge to form woodlands. The hypothesis that present-day mesquite groves represent areas where individual discrete shrub clusters have proliferated and coalesced was evaluated by comparing patterns of soil δ^{13}C within isolated shrub clusters ($n = 6$) to those in nearby groves ($n = 3$). Mean soil δ^{13}C within discrete clusters was lowest in the center (−23.3‰), increased exponentially toward the dripline (−20.1‰), and stabilized at a relatively high value approximately 15 cm beyond the dripline (−18.9‰). The spatial structure of soil δ^{13}C in groves was consistent with that which would be expected to occur if present-day grove communities were a collection of what once were individual discrete clusters that had fused. As such, it provides direct evidence in support of conceptual and mathematical models derived from indirect assessments. However, spatial patterns of soil δ^{13}C suggest that groves are not simply a collection of clusters with respect to primary production and SOC turnover. This study illustrates how soil δ^{13}C values can be used to reconstruct successional processes accompanying vegetation compositional change, and its consequences for ecosystem function.

1. Introduction

In recent history, many grasslands and savannas in Africa, Asia, Australia, Europe, South America, and North America have experienced woody plant encroachment (Archer, 1995; Archer et al., 2001; Arnold, 1995; Bai et al., 2009; Boutton et al., 1998; Maestre et al., 2009; Mast et al., 1997; Menaut et al., 1990; van Auken, 2000). This dramatic change in tree-shrub-grass composition appears to be driven by human manipulation of fire frequency and livestock grazing (Archer, 1995; Asner et al., 2004; Boutton et al., 1998; Jeltsch et al., 2000; Midgley and Bond, 2001), but may also be influenced by changes in climate, atmospheric composition, and atmospheric deposition (Archer et al., 2001; Bond, 2008; Bond and Midgley, 2000; Tape et al., 2006; Wigley et al., 2009). Increased abundance of woody plants has the potential to profoundly influence grassland biodiversity, hydrology, biogeochemistry, and landscape evolution (Boutton et al., 1998; Breshears, 2006; Nobel, 1997; Rappole et al., 1986; Schlesinger et al., 1990; Shachak et al., 2008). Due to its impacts on commercial livestock grazing and other land uses, this phenomenon has long been a concern to natural resource managers (Fisher, 1950, 1977; Rappole et al., 1986). Although this vegetation change has been widely recognized, relatively little is known of the rates, dynamics, and patterns of woody plant proliferation and its impact on ecosystem processes.

Subtropical woodlands dominated by C_3 trees and shrubs in the Rio Grande Plains of southern Texas have become significant components of landscapes once dominated almost exclusively by C_4 grasslands (Boutton et al., 1998). Reports from early settlers indicated that much of southern Texas was open grassland or savanna in the mid-1800s (Inglis, 1964), but today the vegetation in this region is savanna parkland in which subtropical woodlands cover large portions of the landscape (Archer et al., 2001; Bai et al., 2009). Previous studies in this region have shown that woody encroachment is initiated by the establishment of *Prosopis glandulosa* (honey mesquite – an N_2-fixing tree legume) in the grassland. As these mesquite trees grow, they serve as recruitment foci, facilitating the establishment of other woody species beneath their

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canopies (Archer, 1995; Archer et al., 1988). This process has led to the formation of multi-species woody clusters that may range up to ~10 m in diameter. It has been hypothesized that as new clusters form and existing clusters expand, coalescence occurs, leading to the formation of mesquite groves that range from ~20–100 m in diameter in size (Archer, 1995; Stokes, 1999). Woodlands would eventually develop as shrub clusters and groves continue to form and expand. This hypothetical successional scenario is based on "space-for-time" and modeling studies of vegetation structure, wherein shrub clusters of different sizes and compositional attributes were inferred to represent the stages of successional development. Though widely used in studies of plant succession such inferences can be misleading (Austin, 1977; Johnson and Miyanishi, 2008; Shugart et al., 1981). Confirmation with direct measurements is thus desirable.

The stable carbon isotopic composition (δ^{13}C) of soil organic carbon (SOC) is a powerful tool for making direct assessments of vegetation changes where C_3 ($\delta^{13}C \approx -27‰$) and C_4 ($\delta^{13}C \approx -13$ ‰) plants coexist. Spatial variation in δ^{13}C of SOC provides direct evidence of patterns and processes of woodland development in bioclimatic regions where C_3 woody plants occur with C_4 grasses (Bai et al., 2009; Biedenbender et al., 2004; Boutton et al., 1999; Jessup et al., 2003; Krull et al., 2005; McPherson et al., 1993). While some studies have reported that there can be carbon isotopic fractionation during decomposition of soil organic matter (Wynn, 2007; Novak et al., 2009) and selective preservation of C_3-derived SOC over C_4-derived SOC (Wynn and Bird, 2007), prior studies in our study area showed no significant isotopic fractionation during soil organic carbon formation (Boutton, 1996; Boutton et al., 1998, 1999). In the Rio Grande Plains of southern Texas, all woody plants use the C_3 photosynthetic pathway ($\delta^{13}C \approx -27‰$) and all grasses use the C_4 pathway ($\delta^{13}C \approx -13‰$) (Boutton et al., 1999). Thus, soil δ^{13}C values can provide information regarding both the shift from C_4 to C_3 dominance, and the relative contribution of the invading C_3 woody plants to SOC. Since vegetation change from C_4 grassland to C_3 shrubland has been relatively recent in this region, the soil organic carbon pool beneath wooded areas is comprised of carbon derived from both the original C_4-dominated grassland and from the more recent C_3 woody plants, with δ^{13}C values varying as a function of time of woody plant occupation (e.g., Boutton et al., 2009; Liao et al., 2006b;).

The purpose of this study was to quantify within-patch variability of the δ^{13}C of soils associated with discrete shrub cluster and grove communities as a direct test of hypothesized grassland-to-woodland successional processes. We examine the following possible scenarios of woody cluster and grove formation within a grassland matrix (see legend of Fig. 1 for additional details): (1) If woody clusters form via recruitment of shrubs beneath and around mesquite canopies, then soil δ^{13}C should increase along transects from the bole of mesquite plants in cluster centers (the point of longest woody plant occupancy) out into the adjacent grasslands (Fig. 1A); and (2) if groves develop via the establishment, expansion and coalescence of discrete mesquite clusters, then spatial patterns of soil δ^{13}C within groves should mimic those observed along bole-to-dripline transects within discrete clusters; and should vary as a function of the size (age) of the mesquite plants within the groves (Fig. 1B).

2. Materials and methods

2.1. Study area

Research was conducted at the Texas AgriLife La Copita Research Area in Jim Wells County, 15 km SW of Alice, TX (27° 40′ N; 98° 12′ W) in the eastern Rio Grande Plains of the Tamaulipan Biotic Province. The climate is subtropical with a mean annual temperature of 22.4 °C and mean annual precipitation of 680 mm. Rainfall maxima occur in May–June and September.

The landscape grades (1–3% slopes) from sandy loam uplands to clay loam and clay lowlands, and elevations range from 75–90 m. Soils are primarily Typic Argiustolls with a subsurface argillic horizon; however, patches of Typic Haplustepts lacking an argillic horizon are also found in the uplands (Archer, 1995). The vegetation is subtropical savanna parkland comprised of discrete shrub clusters (consisting of a single honey mesquite tree with up to 15 understory shrub species) and larger groves (consisting of multiple mesquite tree with associated understory shrubs) embedded within a grassland matrix. The grasslands consist mainly of rhizomatous and weakly caespitose C_4 grasses, and C_3 forbs. Clusters and groves are dominated by honey mesquite and *Zanthoxylum fagara* (lime prickly ash). Honey mesquite and

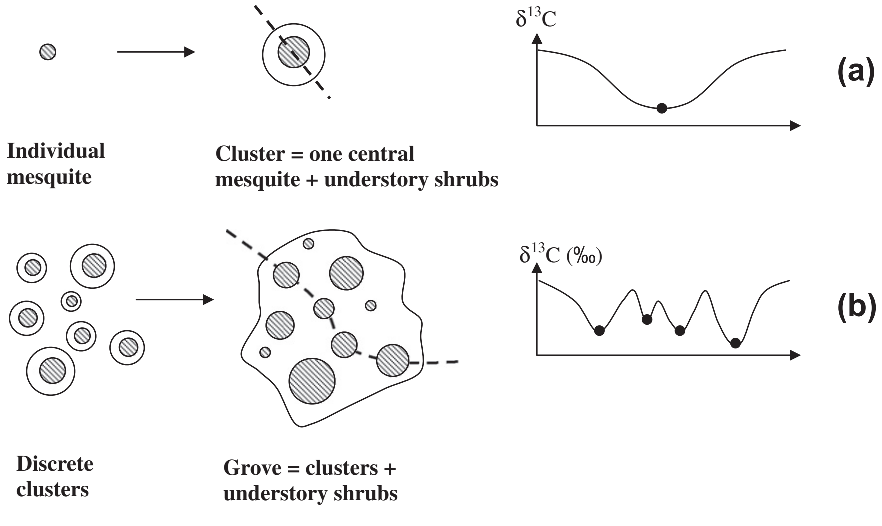

Fig. 1. Hypothesized developmental patterns of woody patches in uplands of the Rio Grande Plains of southern Texas (based on Archer (1995) and Stokes (1999)): (a) A shrub cluster is initiated by mesquite (cross-hatched circle) and the subsequent recruitment of other shrub species beneath and around its canopy; (b) A grove consisting of multiple mesquite trees is formed by the establishment, expansion and coalescence of discrete clusters. Graphs illustrate the spatial pattern of soil δ^{13}C that would be expected along transects of discrete clusters; and along mesquite tree-to-mesquite tree transects in groves (indicated by the dashed lines) if groves were formed from the coalescence of discrete clusters (low points depict location of mesquite boles).

several other leguminous trees and shrubs present in wooded areas are capable of symbiotic N_2-fixation (Zitzer et al., 1996). See Archer (1995) and Boutton et al. (1998) for additional details on soils, vegetation and climate.

2.2. Field sampling procedures

Six discrete shrub clusters and three groves were randomly selected in the sandy loam upland portion of a landscape. 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 130 spatially explicit soil samples (0–15 cm) were collected in the six cluster plots as shown in Fig. 2. Groves ($n = 3$) were sampled using tree-to-tree transects originating from the approximate center of the grove and zig-zaging from one mesquite tree to the next mesquite tree and towards the grove perimeter and into the grasslands. These transect samples were supplemented with additional random samples. The total numbers of sampling points were: (a) 45 transect points and 17 random points in Grove 1; (b) 19 transect points and four random points in Grove 2; and (c) 29 transect points and seven random points in Grove 3. Points along the grove transects were classified into one of four categories based on their positions relative to mesquite trees: (1) base: next to the bole of a mesquite tree, (2) mid: ca. half-way between the canopy edge and a bole, (3) edge: within 15 cm of a mesquite canopy edge and (4) grass: in grassland matrix (Fig. 3b).

To assess spatial variation within the grassland matrix, three 6 m × 10 m plots located at least 5 m away from any woody patch were established. Each plot was subdivided into 2 m × 2 m grid cells. Two soil samples (0–15 cm) were taken at random in each grid cell, resulting in a total of 180 samples.

Transect end points and the corners of the grassland plots were georeferenced with a global positioning system (Trimble GPS pathfinder Pro XRS, Trimble Navigation Limited, Sunnyvale, CA). The distances from sampling points to the nearest two georeferenced points were measured to determine their exact spatial coordinates. Locations of all mature mesquite trees (basal diameter >15 cm) within the three groves were similarly georeferenced and their basal diameters recorded. Distance from the sampling points to the bole of the nearest mesquite tree was calculated using ArcView GIS Spatial Analyst (ESRI, 1998).

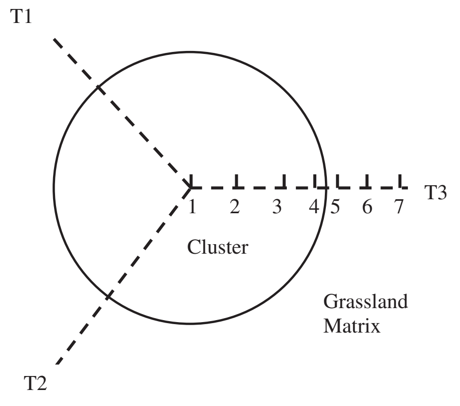

Fig. 2. The sampling regime in upland discrete shrub clusters. Soil samples were taken along transects extending from the center (location 1) and into the surrounding grassland (location 7). Locations 2 and 3 were 1/3 and 2/3 of distance from center to drip line. Locations 4 and 5 were ~15 cm inside and beyond the canopy dripline, respectively. Locations 6 and 7 were beyond the cluster drip line at distances corresponding to 1/3 and 2/3 the cluster center to drip line distance.

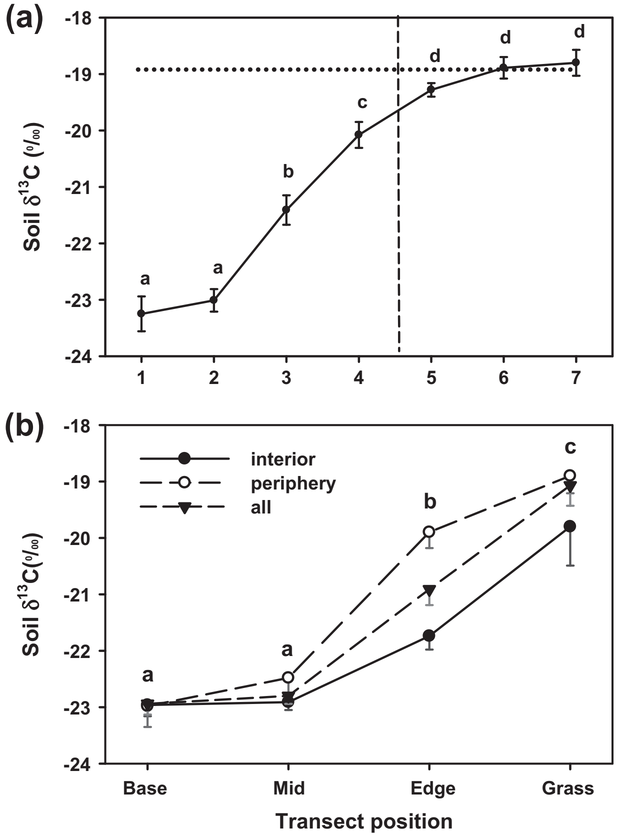

Fig. 3. Mean (±SE) soil $\delta^{13}C$ (‰) along transects within discrete shrub cluster (a) and grove (b) patches. Transects within the clusters extend from their centers into the surrounding grasslands (numbers on the x-axis correspond to sampling locations described in Fig. 2). The dashed vertical line and the dotted horizontal line in (a) represent the location of the shrub cluster margin and the mean soil $\delta^{13}C$ value in the grassland plots respectively. Within groves (b), "base" represents sampling points at the boles of the mesquite trees; "mid" represents points midway between the bole and the edge of the mesquite canopy; "edge" represents points at the mesquite canopy drip line; and "grass" represents samples in the adjoining grassland community. "Periphery" points (open circles) are from mesquite plants along the perimeter of the grove ($n = 36$); "Interior" points are those from mesquite plants within the groves ($n = 51$). "All" represents all points sampled on transects ($n = 87$). Different letters indicate differences between means (test results were the same for the three categories in groves) (Fisher's LSD; $\alpha = 0.05$).

2.3. Soil analyses

Soils were sieved through a 2 mm screen to remove coarse organic fragments and gravel, dried at 60 °C for 48 h and ground in a centrifugal mill (Angstrom, Inc., Belleville, MI). Subsamples were weighed into silver capsules using a microbalance, treated with HCl vapor in a desiccator (Harris et al., 2001) to volatilize inorganic C present as $CaCO_3$, then analyzed for $\delta^{13}C$ of SOC using a Carlo Erba EA-1108 (CE Elantech, Lakewood, NJ) elemental analyzer interfaced with a Delta Plus isotope ratio mass spectrometer operating in continuous flow mode (ThermoFinnigan, San Jose, CA).

Carbon isotope ratios are presented in standard δ notation:

$$\delta = [(R_{\mathrm{SAMPLE}} - R_{\mathrm{STD}})/R_{\mathrm{STD}}] \times 10^3‰ \qquad (1)$$

where R_{SAMPLE} is the $^{13}C/^{12}C$ ratio of the sample and R_{STD} is the $^{13}C/^{12}C$ ratio of the V-PDB standard (Coplen, 1996). Precision of duplicate $\delta^{13}C$ measurements was 0.1‰.

2.4. Statistical analyses

One-way ANOVA was used to test for the differences in soil $\delta^{13}C$ with respect to vegetation type (grassland, discrete shrub cluster, and grove) and transect locations in discrete shrub clusters and groves. Fisher's LSD was used to distinguish differences among means. Regression analyses were performed using Sigma Plot 8.0 (SPSS Inc., 2002) to determine the relationship between mesquite tree GPI (see definition below) values and soil $\delta^{13}C$.

In contrast to discrete clusters, which represent isolated, widely dispersed mesquite plants, groves represent associations consisting of multiple mesquite plants of various sizes/ages growing in relatively close proximity to each other. Gustafson and Parker (1992) developed a proximity index to distinguish isolated patches from those which are part of a complex of patches by considering the size and distance of all like patches whose edges are within a specified distance from the edge of the focal patch. We generalized the concept of their proximity index to measure the influence of objects (mesquite plants in our case) in the neighborhood of a focal point (soil sample location in our case) on the attributes measured at that focal point ($\delta^{13}C$ in our case). This generalized proximity index (GPI) was thus used to estimate the influence of mesquite trees on soil $\delta^{13}C$ as follows:

$$\mathrm{GPI} = \sum_{i=1}^{n} Di/Zi \tag{2}$$

where Z_i is the distance in meters from a sampling point to each surrounding mesquite within 9 m (when distance >9 m, mesquite has no influence on soil $\delta^{13}C$, as determined by iterative solution of the relationship between GPI and soil $\delta^{13}C$; data not shown), and D_i is mesquite basal diameter in meters. Therefore, this index considers the number, size, and distance of surrounding mesquite trees, weighted in favor of the nearer and larger trees. The GPI is large when the soil sampling point is surrounded by closer and/or more and/or larger mesquite trees and decreases when mesquite trees in the neighborhood are farther and/or sparser and/or smaller.

3. Results

The mean $\delta^{13}C$ of grassland soils (-19.0 ± 0.1‰) was significantly greater than that of soils within discrete shrub clusters (-21.0 ± 0.2‰) and groves (-21.6 ± 0.2‰); and values for groves were significantly lower than those for clusters ($F = 106.0$; $p < 0.001$; Table 1). The coefficient of variation of soil $\delta^{13}C$ was highest in clusters (9.5%) and lowest in grasslands (5.3%). There was no evidence of past shrub occupation in the grassland grids because soil $\delta^{13}C$ values were relatively homogeneous and higher than −20.9‰.

Soil $\delta^{13}C$ increased exponentially from the center of the cluster (−23.3‰) to the edge of the canopy (−20.1‰), and then reached a value typical of grassland (−19.3‰) at a distance approximately 1/3 of the cluster diameter beyond the canopy (Fig. 3a). Within groves, mean $\delta^{13}C$ values were lowest at the base of mesquite trees, increasing towards the perimeters of their canopies (Fig. 3b). This was the case for both interior and periphery mesquite trees. However, with the exception of soils near the base of the mesquite trees, interior soils had consistently lower soil $\delta^{13}C$ values than periphery soils (Fig. 3b). $\delta^{13}C$ values for soils at the base of mesquite trees in groves were an inverse function of mesquite basal diameter (Fig. 4a). The GPI, which accounts for the number, distance and size of mesquite trees in the neighborhood of non-mesquite bole soil sampling points, was also inversely related to soil $\delta^{13}C$ (Fig. 4b),

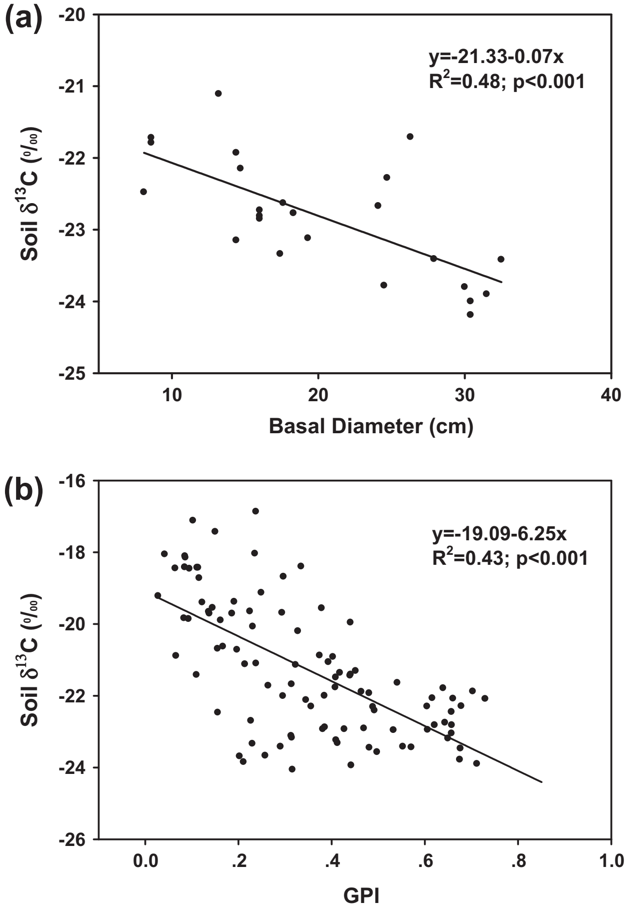

Fig. 4. Relationship between soil $\delta^{13}C$ (‰) and mesquite basal diameter for bole samples (a), and soil $\delta^{13}C$ as a function of the generalized proximity index values (GPI) for all points except the bole samples (b). GPI values increase as the abundance/size of mesquite plants in the neighborhood of grove sampling points increase (see text for details).

To what extent do the mesquite plants in groves influence the $\delta^{13}C$ of soils in the surrounding grassland matrix? The mean $\delta^{13}C$ of soil samples collected 10 m from the grove margins (-18.9 ± 0.32‰) was significantly higher than those of soils occurring at the canopy margins of mesquite plants near the perimeter of the grove (-19.9 ± 0.35‰), but was comparable to grassland average (−19.0‰, Table 1), indicating soil $\delta^{13}C$ of grassland was no longer affected by woody patches when it was more than 10 m away.

Soil $\delta^{13}C$ values were spatially variable within groves, with lowest values generally occurring at the base of mesquite trees (Fig. 5). Some bole locations had higher soil $\delta^{13}C$ than mid- and edge-points (e.g., last tree of Transect 3 of Grove 1, Fig. 5).

Table 1
Descriptive statistics for soil $\delta^{13}C$ (‰) in grassland, discrete cluster and grove landscape elements. Different letters beside means indicate significant differences (Fisher's LSD, $p = 0.05$).

Landscape element	N	Mean	Coefficient of variation	Standard error	Minimum	Maximum
Grassland	180	-19.0^{a}	5.3	0.1	−23.1	−15.8
Cluster	130	-21.0^{b}	9.5	0.2	−24.8	−17.3
Grove	121	-21.6^{c}	8.3	0.2	−24.9	−16.9

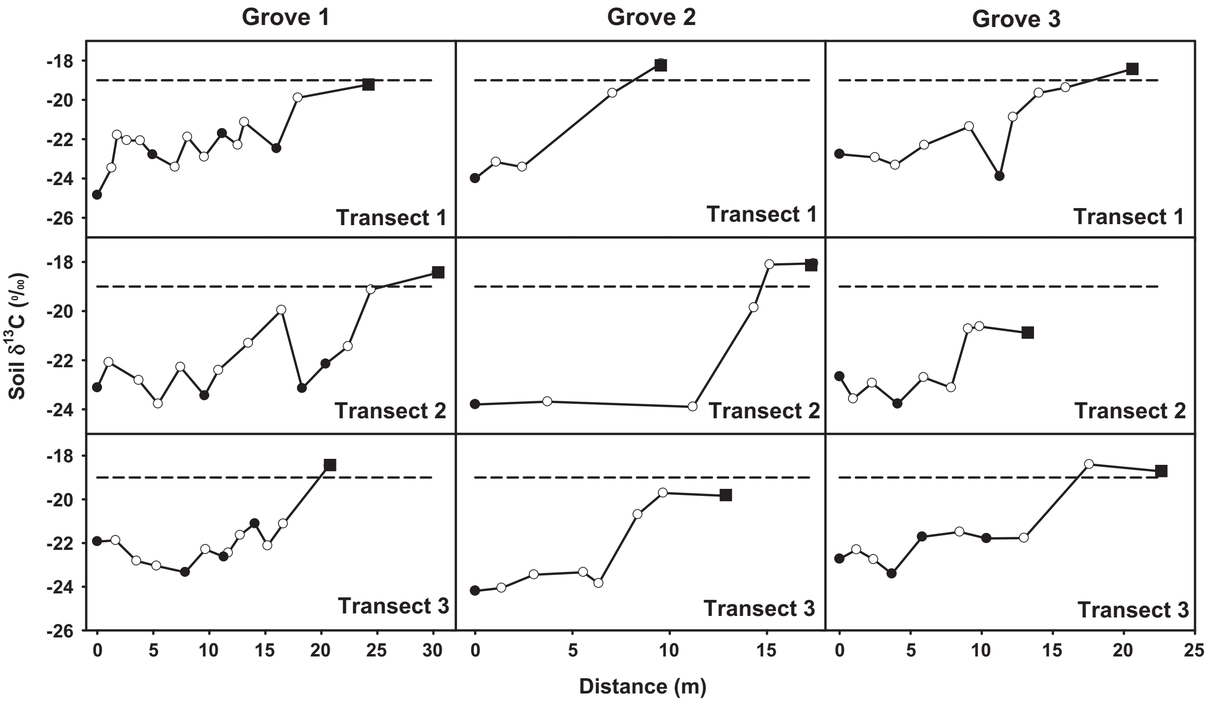

Fig. 5. Soil $\delta^{13}C$ (‰) as a function of distance (m) from the geographic center (distance = 0) of each grove. Solid squares represent points in grasslands. Solid black dots are points adjacent to the bole of mesquite trees. Dashed horizontal lines indicate the mean soil $\delta^{13}C$ value of grassland calculated from all grassland samples (Table 1).

4. Discussion

4.1. Soil $\delta^{13}C$ values of grasslands vs. woody patches

Present grasslands in the Rio Grande Plains of southern Texas are comprised of a mixture of C_3 forbs and C_4 grasses. $\delta^{13}C$ values of soils in the grassland matrix at our study site averaged −19.0 ± 1.0‰ (Table 1), similar to values reported previously for grassland litter (−19‰) and roots (−20.5‰) in this and many other C_4-dominated grasslands around the world (Boutton et al., 1998). Following woody plant invasion into the grassland, mean soil $\delta^{13}C$ values decreased to −21.0‰ in shrub clusters and −21.6‰ in groves (Table 1), reflecting the simultaneous loss of SOC derived from the original C4 grassland and the accumulation of SOC derived from the invading C3 woody vegetation. Soil $\delta^{13}C$ values indicated that a substantial proportion of the SOC presently in both clusters and groves was derived from C_4 plants (grasses). This is consistent with previous isotopic studies in this region (Boutton et al., 1998, 1999; Gill and Burke, 1999; Liao et al., 2006a) and supports the conclusion that C_4 grass communities have been replaced by C_3 woody plant communities. Analyses of repeat aerial photography at this site (Archer et al., 1988) and others (Browning et al., 2008) suggests that while there may be net increases in woody plant cover within grasslands at decadal time scales, shrubs and shrub patches do turnover and revert back to grass domination. However, we found no isotopic evidence for this in our detailed grid-sampling within grasslands. This may simply indicate insufficient sampling ($n = 3–6 \times 10$ m areas); or it may indicate that shrub to grass transitions most often occur where shrubs have not occupied the site long enough to have substantively influenced soil $\delta^{13}C$.

4.2. Spatial patterns of soil $\delta^{13}C$ in discrete shrub clusters

Soil $\delta^{13}C$ was lowest in shrub cluster centers and increased sigmoidally towards the cluster/grassland boundary (Fig. 3a), consistent with the hypothesis that honey mesquite initially colonizes the grassland, and then other shrub species subsequently establish beneath its canopy (Fig. 1A) (Archer, 1995; Archer et al., 1988). Patterns of soil $\delta^{13}C$ have also been shown to be strongly coupled to the distribution of mesquite canopies in semiarid grassland in southeastern Arizona (Biggs et al., 2002) and to vary with time since mesquite occupation (McClaran et al., 2008; Throop and Archer, 2008; Wheeler et al., 2007).

The isotopic impact of clusters on adjacent grasslands extended only about 0.15 m beyond the cluster/grassland edge. This is consistent with data from Watts (1993) which showed that lateral extension of woody plant roots beyond cluster canopies was minimal at this site. In contrast, the influence of mesquite on soil $\delta^{13}C$ values in New Mexico's Jornada Basin extended approximately 3 m beyond the edge of the tree canopy, paralleling the distribution pattern of fine roots (Connin et al., 1997).

4.3. Spatial patterns of soil $\delta^{13}C$ in groves

Soil $\delta^{13}C$ was heterogeneous in groves and strongly affected by the presence of mesquite. The inverse relationship between soil $\delta^{13}C$ and mesquite basal diameter (Fig. 4a) indicates points with lower soil $\delta^{13}C$ have a longer history of C_3 organic matter inputs from mesquite. This is consistent with other studies showing a negative relationship between the size/age of mesquite trees and soil $\delta^{13}C$ (Liao et al., 2006a; Tieszen and Archer, 1990; Wheeler et al., 2007). The negative relationship between soil $\delta^{13}C$ and the mesquite GPI (Fig. 4b) further reflects the fact that areas within groves with larger/denser mesquite plants have a longer history of shrub occupation, a higher C_3 shrub net primary productivity, lower C_4 grass production or a combination of these. Therefore, we conclude that the present-day pattern of soil $\delta^{13}C$ values reflects successional processes leading to the formation of shrub clusters and groves on this landscape.

If present-day groves were formed by coalescence of discrete clusters as inferred from space-for-time and modeling

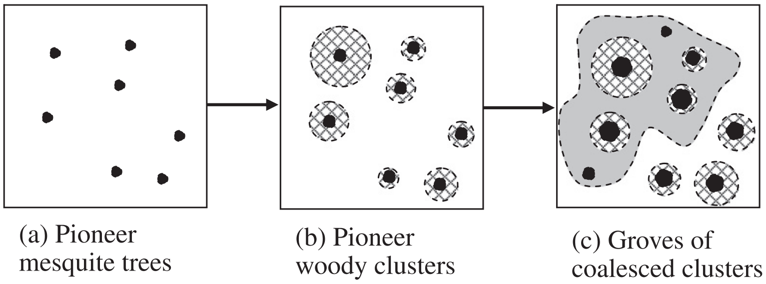

Fig. 6. Conceptual model of successional processes in the conversion of grassland-to-woodland (based on Archer (1995) and Stokes (1999) and data from this study). Black dots represent pioneer mesquite trees within a C_4 grassland matrix (white background, highest soil $\delta^{13}C$ values). Cross-hatched areas of various sizes represent shrub cluster age-states organized around mesquite plants (lowest soil $\delta^{13}C$ values). Grey areas represent groves of coalesced clusters (intermediate soil $\delta^{13}C$ values). Some newly established mesquite tree may get into the system during the grove development.

reconstructions (Archer, 1995; Stokes, 1999), patterns of soil $\delta^{13}C$ would mimic those in Fig. 3a, be repeated for each mesquite tree in the grove, and thus vary as depicted in Fig. 1B, with bole-to-mesquite dripline gradients being attenuated with decreasing mesquite plant size. Results support the proposed mechanism of woody plant community development in that soil $\delta^{13}C$ in groves was lowest next to the boles of mesquite plants, and increased steadily toward the grassland (Fig. 3b), similar to the isotopic pattern in discrete clusters (Fig. 3a). As with discrete clusters, soils at the base of mesquite plants in groves typically had the lowest $\delta^{13}C$ values relative to sample points around them, indicating the specific locations mesquite plants initially colonized within the grassland matrix; with spatial variation in mesquite bole $\delta^{13}C$ (Fig. 5) reflecting variation in time of occupation (as inferred from plant size; Fig. 4a). Direct evidence from isotopic patterns therefore supports the hypothesis that mesquite groves are comprised of shrub cluster age-states, and that they have developed via the coalescence of discrete woody clusters.

Doecosystem processes change as discrete clusters develop into groves? Or put another way, are groves merely a collection of clusters from an ecosystem function standpoint? Fig. 3b suggests 'yes' and 'no', respectively, to these two questions. The bole points generally had lower soil $\delta^{13}C$ compared to other points along the transects, showing the locations of the pioneer clusters. However, the amplification of the C_3 signal amongst samples associated with interior shrub clusters relative to those at the grove perimeter suggests that processes and environmental factors influencing SOC pools and fluxes (e.g., primary production, decomposition, microclimate) are likely to differ between discrete clusters and groves. The occurrence of lower-than-expected soil $\delta^{13}C$ values in locations without mesquite trees may also be indicative of divergence in ecological function. For example, the 4th point on Transect 2 of Grove 1 (an "edge" point), the 11th point on Transect 3 of Grove 1 (a "mid" point), points 2 and 3 on Transect 2 in Grove 3, and points 2–5 on Transect 2 in Grove 3 had comparable or lower soil $\delta^{13}C$ values than their nearest bole point (Fig. 5). It is also possible that there were mesquite trees located at these points sometime in the past, but they have since died. Previous studies indicated that shallow-rooted understory shrubs may accelerate the mortality of mesquite trees (Archer, 1995; Archer et al., 1988; Barnes and Archer, 1999). We speculate these "anomalous" soil $\delta^{13}C$ values within groves may represent locations where founding mesquite plants have died and are no longer evident aboveground. This is analogous to "ghost trees" in burned grasslands of Arizona where aboveground evidence for trees once present in grasslands had been obliterated by fire (Biggs et al., 2002). Thus, while it appears that groves are an amalgamation of discrete cluster age-states that have fused, they appear to have emergent properties that distinguish them from a simple collection of isolated discrete clusters.

4.4. Synthesis and conceptual model of woody patch development in grasslands

Spatial patterns of soil $\delta^{13}C$ values provided direct evidence for successional processes resulting in the formation of woody patches in grasslands of the Rio Grande Plains of Texas, and supported prior inferences based on plant community analyses and space-for-time studies of vegetation dynamics (Archer et al., 1988; Bai et al., 2009). Based on the results of this study and previous findings (Stokes, 1999), we propose a conceptual model (Fig. 6) that appears to account for the successional processes of woody encroachment into upland grasslands. Woody clusters are initiated when mesquite trees establish in the grassland (Fig. 6a); as these mesquite grow, other woody species establish beneath their canopies, and multi-species woody clusters develop (Fig. 6b). Discrete clusters then grow in size as the canopies of the mesquite plant and its associated shrubs develop and as new individuals are recruited around the canopy perimeter. As new clusters are initiated and existing clusters grow, coalesce occurs and leads to the formation of groves (Fig. 6c). Based on ages of mesquite trees, historical aerial photographs, turnover rates of SOC, and natural ^{14}C measurements on SOC, it appears that mature woody clusters and groves can form within 150 years or less (Boutton et al., 1998; Archer et al., 2001).

Spatial patterns of soil $\delta^{13}C$ provide not only chemical evidence of vegetation successional patterns, but also a strong spatial context for future studies aimed at understanding the factors controlling vegetation dynamics and predicting the future dynamics of the landscape. These methods and approaches should be applicable in any ecosystem or landscape where successional processes have led to shifts in the distribution and abundance of C_3–C_4 functional types.

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