SPATIAL VARIATIONS IN SOIL AND PLANT $\delta^{13}$C AND $\delta^{15}$N VALUES IN A SUBTROPICAL SAVANNA: IMPLICATIONS FOR VEGETATION CHANGE AND NUTRIENT DYNAMICS

A Dissertation

by

E BAI

Submitted to the Office of Graduate Studies of Texas A&M University in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY

August 2007

Major Subject: Rangeland Ecology and Management
SPATIAL VARIATIONS IN SOIL AND PLANT $\delta^{13}$C AND $\delta^{15}$N VALUES IN A SUBTROPICAL SAVANNA: IMPLICATIONS FOR VEGETATION CHANGE AND NUTRIENT DYNAMICS

A Thesis

by

E BAI

Submitted to the Office of Graduate Studies of Texas A&M University in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY

Approved by:

Co-Chairs of Committee, Thomas W. Boutton
               X. Ben Wu
Committee Members,  Fred E. Smeins
               C. Tom Hallmark
Head of Department, Steven G. Whisenant

August 2007

Major Subject: Rangeland Ecology and Management
ABSTRACT

Spatial Variations in Soil and Plant $\delta^{13}C$ and $\delta^{15}N$ Values in a Subtropical Savanna: Implications for Vegetation Change and Nutrient Dynamics. (August 2007)

E Bai, B. Sc. Nankai University

Co-Chairs of Advisory Committee: Dr. Thomas W. Boutton
Dr. X. Ben Wu

Grass-dominated ecosystems in many regions around the world have experienced increased abundance of woody plants during the past 100 yrs. In the Rio Grande Plains of southern Texas, subtropical woodlands, dominated by $C_3$ trees/shrubs capable of symbiotic N$_2$-fixation, have become significant components of landscapes that were once dominated by $C_4$ grasslands. Upland areas in this region now consist of small discrete clusters and large groves of woody vegetation embedded in a grassy matrix, while lower-lying portions of the landscape are dominated by closed-canopy woodlands. I used soil $\delta^{13}C$ in conjunction with aerial photography and geostatistics to quantify landscape-scale vegetation dynamics in uplands of this savanna parkland.

Spatial patterns of soil $\delta^{13}C$ in grids and transects traversing woody patches indicated larger woody groves were formed from small discrete clusters of woody plants that spread laterally and eventually coalesced. Soil $\delta^{13}C$ contour maps revealed some clusters are currently growing rapidly towards each other and might coalesce into groves in the near future, while some clusters remained relatively stable. Kriged maps of soil $\delta^{13}C$ provided a strong spatial context for future studies aimed at understanding the functional consequences of this change in landscape structure. The dominant invading
woody plant, honey mesquite (*Prosopis glandulosa*), was important in determining the spatial pattern of soil $\delta^{13}$C, supporting the hypothesis that they serve as recruitment foci and facilitate the establishment of subordinate woody species. Leaf $\delta^{15}$N values suggested that the N$_2$-fixing mesquite influenced the N nutrition of nearby non-N$_2$-fixing shrubs, thus, suggesting a mechanism by which mesquite could facilitate establishment of other woody species. In closed-canopy drainage woodlands, however, spatial patterns of soil $\delta^{13}$C were no longer controlled by the presence of mesquite, but by the amount of soil organic carbon and soil texture. The positive correlation between silt+clay and soil $\delta^{13}$C indicates that the formation of organomineral complexes and microaggregates may slow SOC turnover rates and favor the persistence of C$_4$-derived SOC from the original grassland. This study enhances our understanding of potential patterns, causes and consequences of grassland to woodland conversions which are underway today in many grass-dominated ecosystems around the world.
ACKNOWLEDGMENTS

I am most grateful to Dr. Boutton for his assistance during my stay at Texas A&M. He has spent countless hours helping me through this Ph.D. program and offered me lots of patience, guidance, and encouragement. I also would like to thank Dr. Wu for his guidance and support throughout the course of this research. I appreciate Dr. Smeins and Dr. Hallmark for serving on my graduate committee. They were always willing to provide advice when needed. Thanks are also extended to Dr. Archer for his help in discussion of ideas and revision of manuscripts.

I want to acknowledge my colleague Feng Liu for his generous help with sample processing and data analyses. Many other people helped in the collection of field data and processing of laboratory samples, including Kirk Jessup, Lisa Alexander, Steve Reagan, Bing Zhao, Berglind Orradóttir, Johann Thorsson. In addition, Terri Rosol, Andrew Boutton, and Heather Jahnsen worked as NSF/REU undergraduate research fellows to determine soil texture under the guidance of Donna Prochaska in the Soil Characterization Laboratory directed by Dr. Tom Hallmark. The McKown family was very hospitable during my stays at La Copita Research Area. Emily Hollister, Lori Biederman, Darrin Moore, Ilsa Kantola and Emily Martin have provided comments and recommendations on drafts of my dissertation chapters. I appreciated the help from all of these people.

This research was supported by the National Science Foundation, a Regents Fellowship from the Texas A&M University, a Tom Slick Fellowship from the Texas
College of Agriculture, and a Wasko Fellowship from the Ecosystem Science and Management Department.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II</td>
<td>LANDSCAPE-SCALE VEGETATION DYNAMICS INFERRED FROM SPATIAL PATTERNS OF SOIL $\delta^{13}$C IN A SUBTROPICAL SAVANNA PARKLAND</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><strong>Synopsis</strong></td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><strong>Introduction</strong></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td><strong>Materials and methods</strong></td>
<td>12</td>
</tr>
<tr>
<td></td>
<td><strong>Results</strong></td>
<td>20</td>
</tr>
<tr>
<td></td>
<td><strong>Discussion</strong></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td><strong>Conclusions</strong></td>
<td>34</td>
</tr>
<tr>
<td>III</td>
<td>SPATIAL VARIATION OF SOIL $\delta^{13}$C VALUES IN A SUBTROPICAL SAVANNA PARKLAND REVEALS PATTERN OF WOODLAND DEVELOPMENT</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td><strong>Synopsis</strong></td>
<td>36</td>
</tr>
<tr>
<td></td>
<td><strong>Introduction</strong></td>
<td>37</td>
</tr>
<tr>
<td></td>
<td><strong>Materials and methods</strong></td>
<td>40</td>
</tr>
<tr>
<td></td>
<td><strong>Results</strong></td>
<td>48</td>
</tr>
<tr>
<td></td>
<td><strong>Discussion</strong></td>
<td>55</td>
</tr>
<tr>
<td></td>
<td><strong>Conclusions</strong></td>
<td>63</td>
</tr>
<tr>
<td>IV</td>
<td>SPATIAL PATTERNS OF SOIL $\delta^{13}$C IN A SUBTROPICAL WOODLAND...</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td><strong>Synopsis</strong></td>
<td>65</td>
</tr>
<tr>
<td></td>
<td><strong>Introduction</strong></td>
<td>66</td>
</tr>
<tr>
<td></td>
<td><strong>Materials and methods</strong></td>
<td>69</td>
</tr>
</tbody>
</table>
CHAPTER                                                                                                                      Page

Results ................................................................................................................ 76
Discussion .......................................................................................................... 86
Conclusions ........................................................................................................ 94

V SPATIAL VARIATION OF δ^{15}N OF WOODY PLANTS ALONG A
TOPOEDAPHIC GRADIENT IN A SUBTROPICAL SAVANNA
PARKLAND LANDSCAPE .................................................................................. 96

Synopsis ............................................................................................................. 96
Introduction ........................................................................................................ 97
Materials and methods ..................................................................................... 101
Results .............................................................................................................. 107
Discussion ........................................................................................................ 120
Conclusions ...................................................................................................... 126

VI VARIATION IN δ^{13}C VALUES OF WOODY PLANTS ALONG A
TOPOEDAPHIC GRADIENT IN A SUBTROPICAL SAVANNA
PARKLAND LANDSCAPE .................................................................................. 128

Synopsis ............................................................................................................. 128
Introduction ........................................................................................................ 129
Materials and methods ..................................................................................... 132
Results .............................................................................................................. 138
Discussion ........................................................................................................ 146
Conclusions ...................................................................................................... 152

VII SUMMARY AND CONCLUSIONS .................................................................. 154

REFERENCES ..................................................................................................... 158

VITA ..................................................................................................................... 177
LIST OF FIGURES

FIGURE                                                                                                                          Page
1.  Color infrared aerial view of study area. Red areas represent woody vegetation, while light gray areas represent grasslands. Blue dots indicate soil sampling locations within the 100 X 160 m grid. Numbered woody patches were evaluated in detail for evidence of expansion in size over time. Yellow lines indicate the edge of the woody patches. ........................................................................................................... 14
2.  Components of a semivariogram........................................................................................................... 19
3.  Elevation map computed from field survey for the 120×100m upland grid at La Copita Research Area in southern Texas. .......................................................................................... 23
4.  Kriged map of soil δ¹³C (‰) in uplands. .......................................................................................... 24
5.  Aerial photos taken in 1930, 1941, 1982 and 2003 illustrate vegetation cover change. Lighter areas are herbaceous vegetation; darker areas are woody vegetation. .......................................................................................... 26
6.  Soil δ¹³C contours superimposed on the vegetation change map calculated from aerial photos. Red color represents vegetation increase from 1930 to 2003. Yellow color represents vegetation cover in 1930. Arrows indicate the estimated fastest direction of expansion of each patch. ........................................................................... 28
7.  Hypothesized developmental patterns of woody patches in uplands of the Rio Grande Plains of southern Texas: (A) A cluster is initiated by honey mesquite and is formed by following recruitment of other understory shrubs; (B) A grove is coalescence of discrete clusters; (C) A grove is the migration around the boundary of the pioneer patch recruiting newly established invading mesquite trees and shrubs. Cross hatched areas represent the canopies of mesquite trees. Diagrams on the right suggest the spatial pattern of soil δ¹³C along a transect (indicated by the dashed line) traversing the present-day woody patch. ........................................................................... 41
8.  Map of sampling points in upland superimposed on aerial photo. Clusters and groves of woody vegetation (red in aerial view) are embedded within a grassland matrix (gray). The three groves sampled in this study are circled with blue lines, and the blue dots are the sampling points in groves and adjacent grasslands. Similarly, the six clusters sampled in this study are circled by yellow lines, and the sampling points within those clusters and their adjacent grasslands are indicated by yellow dots........................................................................................................... 43
9. The sampling regime in upland clusters. Soil samples were taken from the center (location 1) to the adjacent grassland (location 7). Locations 2 and 3 were 1/3 and 2/3 of distance from center to dripline. Location 4 was just inside dripline (~15cm inside) and location 5 was just outside dripline (~15cm outside). Location 6 and 7 were beyond the cluster dripline at distances corresponding to 1/3 and 2/3 the distance from cluster center to dripline.

10. Mean soil $\delta^{13}C$ (‰) along the transects in woody clusters. The transects in the clusters went from the center of the clusters to the adjacent grassland (Fig. 9). Dashed line indicates the cluster/grassland boundary. Different letters indicate differences between means (Fisher’s LSD analysis; $\alpha = 0.05$). Error bars are standard errors of the mean.

11. Relationship between soil $\delta^{13}C$ (‰) and PI of mesquite trees in woody groves.

12. Soil $\delta^{13}C$ (‰) from the last tree on the transects in groves to the adjacent grassland area. Base represents sampling points adjacent to the boles of the mesquite trees on the transects; mid represents points midway between the bole and the edge of the canopy; edge represents points at the canopy dripline; and grass represents grassland points adjacent to the groves. Dashed line represents the mean value of soil $\delta^{13}C$ of grassland calculated from the samples in the grassland plots (Table 4). Different letters indicate differences between means (Fisher’s LSD analysis; $\alpha = 0.05$). Error bars are standard errors of the mean.

13. Soil $\delta^{13}C$ (‰) along transects from the center of each grove out into the adjacent grassland. Values along the X-axes are distances (m) from the center of each grove. Black dots represent sampling points in the grove and red dots represent points in the grassland. Solid black dots indicate points adjacent to the bole of mesquite trees. Dashed lines indicates the mean soil $\delta^{13}C$ value of grassland calculated from all grassland samples (Table 4).

14. Kriged map of soil $\delta^{13}C$ (‰) for the three groves. Blue dots represent the location of mesquite trees, and the size of the dots is proportional to the basal diameters of those trees.

15. Reconstruction of grove expansion simulated from the spatial pattern of current soil $\delta^{13}C$ values. Yellow color represents grass cover and other colors represent woody cover. Darker colors represent longer time of occupation by woody plants.

16. Aerial view of the woodland study area in southern Texas. Darker area represents denser woody vegetation cover. Yellow lines (T1, T2) connecting dots represent the two transects. Yellow dots represent soil sampling points within the grid and along the two transects.
17. Kriged map of soil $\delta^{13}C$ (‰) (a), soil silt+clay content (%) (b), and SOC (g C kg$^{-1}$). Red dots represent mesquite trees. .......................................................................................... 81

18. Soil $\delta^{13}C$ (‰) along the two transects in the drainage woodlands. Values of X-axes are the actual distances (m). Solid black dots indicate points adjacent to mesquite tree trunks. .......................................................................................... 84

19. Soil $\delta^{13}C$ (‰) values along the transects in woodlands. Base represents sampling points adjacent to mesquite tree trunks; mid represents points in the middle between the bole and the edge of the canopy; edge represents points on the edge of the canopy. Different letters above points indicate differences among means of different transect positions from Fisher’s LSD (level of significance is 0.01). Error bars are standard errors of the mean. .......................................................................................... 85

20. Effects of SOC (g C kg$^{-1}$) and silt+clay (%) on soil $\delta^{13}C$ (‰). Fitted surface is based on linear regression equation: $z = a + bx + cy$. X-axis is SOC; Y-axis is Silt+clay; z-axis is $\delta^{13}C$. $a = -21.5, b = -0.20, c = 0.136. R^2 = 0.73; p<0.001............ 87$

21. Locations of individual plants and along the transect. Three woody species Prosopis glandulosa, Condalia hookeri, and Zanthoxylum fagara were sampled along a 308m long, 12m wide (6m each side of the transect) belt. The enlarged section shows the details of a small portion of the belt............................................. 104

22. Monthly mean temperature (°C) and rainfall (mm) of NOAA weather station at Alice, TX (www.ncdc.noaa.gov). Solid line and darker bars represent the monthly mean temperature and rainfall of the year of sampling (2005), while dashed line and lighter bars represent 110-year-mean. ............................................. 109

23. Changes in elevation (m) (A), soil particle size distribution (g kg$^{-1}$) (B) and soil volumetric water content and soil available water (m$^3$ m$^{-3}$) (C) along the transect. Dashed lines in A indicate locations of different landscape elements along the transect. ..................................................................................................................... 110

24. Soil total N (g N kg$^{-1}$) and $\delta^{15}N$ (‰) (A), and leaf $\delta^{15}N$ (‰) of three woody species (Prosopis glandulosa, Condalia hookeri, and Zanthoxylum fagara) along the transect in April (B) and August (C), 2005......................................................... 112

25. Relationships between leaf $\delta^{15}N$ (‰) and soil GWC (mg mg$^{-1}$) (A), soil N concentration (g N kg$^{-1}$) (B), and soil $\delta^{15}N$ (‰) (C) for P. glandulosa, C. hookeri, and Z. fagara in April and relationships between leaf $\delta^{15}N$ and leaf [N] of all the three species in April (solid dots and lines) and August (hollow dots and dashed lines) (D). Regression statistics are in Table 11....................................................... 117
FIGURE

26. Relationships between leaf δ\(^{15}\)N of *P. glandulosa*, *C. hookeri*, and *Z. fagara* and soil NH\(_4\)\(^+\)-N (mg N kg\(^{-1}\)), soil NO\(_3\)\(^-\)-N (mg N kg\(^{-1}\)), and nitrification rates (mg N kg\(^{-1}\) d\(^{-1}\)). Data were measured in both April (solid symbols) and August (hollow symbols) of 2005. Soil nitrification rates, soil NH\(_4\)\(^+\)-N, and NO\(_3\)\(^-\)-N data were obtained from (McCulley et al., 2004). ................................................................................................................................. 119

27. Leaf δ\(^{15}\)N (‰) of *C. hookeri* (upper panel) and *Z. fagara* (lower panel) along the proximity index gradient. The proximity index (PI) is a measure of isolation of the sampled plant to neighboring *P. glandulosa*. Thus, high PI means *P. glandulosa* neighbors are closer to the sampled plant and/or denser near the sampled plant........................................................................................................................................ 121

28. Color IR aerial view (2006) of the 308 m transect at the savanna parkland study site showing clusters (C) and groves (G) embedded within an herbaceous grassland matrix (H) in upland areas, and drainage woodland (W) and playa (P) located in lower portions of the landscape. Red tones indicate woody plant cover, while gray and white tones indicate herbaceous grassland vegetation (largely senescent) and bare ground, respectively. Green dots (10 m apart) indicate the location of the transect. ........................................................................................................................................ 134

29. Changes in elevation (m) (A), soil particle size distribution (g kg\(^{-1}\)) (B), soil volumetric water content (total and available) (m\(^3\) m\(^{-3}\)) (C), soil total nitrogen (g N kg\(^{-1}\)) (D) and *P. glandulosa*, *C. hookeri* and *Z. fagara* leaf δ\(^{13}\)C (‰ vs. V-PDB) (E-G) along the transect........................................................................................................................................ 140

30. Relationships between leaf δ\(^{13}\)C (‰) and plant available volumetric water content (VWC; m\(^3\) m\(^{-3}\)) (a), specific leaf area (SLA; m\(^2\) kg\(^{-1}\)) (b), and leaf [N] (g kg\(^{-1}\)) (c) for *Prosopis glandulosa*, *Condalia hookeri*, and *Zanthoxylum fagara* in April (—●—) and August (---○---) of 2005. ........................................................................................................................................ 145
## LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Area of the woody patches (groves) in 1903 and 2003 (m²)</td>
<td>17</td>
</tr>
<tr>
<td>2.</td>
<td>Descriptive statistics of soil $\delta^{13}$C values (0-15cm) (% vs. V-PDB) under different types of vegetation cover</td>
<td>22</td>
</tr>
<tr>
<td>3.</td>
<td>Parameters for best-fitted semivariogram of soil $\delta^{13}$C</td>
<td>22</td>
</tr>
<tr>
<td>4.</td>
<td>Descriptive statistics of soil $\delta^{13}$C (%) in different landscape elements. Different letters beside means indicate differences between means from Fisher’s LSD (p=0.05)</td>
<td>49</td>
</tr>
<tr>
<td>5.</td>
<td>Parameters for best-fit omni-directional semivariogram of soil $\delta^{13}$C of upland groves</td>
<td>56</td>
</tr>
<tr>
<td>6.</td>
<td>Descriptive statistics for soil chemical and physical properties in transect and grid samples (n=207)</td>
<td>77</td>
</tr>
<tr>
<td>7.</td>
<td>Omni-directional variogram of soil $\delta^{13}$C (%) and soil particle size (%) and omni-directional cross-variogram of the two variables</td>
<td>78</td>
</tr>
<tr>
<td>8.</td>
<td>Correlation coefficients (r) for soil $\delta^{13}$C, PI, SOC, root biomass and soil particle size distribution from grid data</td>
<td>80</td>
</tr>
<tr>
<td>9.</td>
<td>Leaf $\delta^{15}$N (%) in different species (S), landscape locations (L) and sample periods (P). Different letters indicate differences between means (Fisher’s LSD analysis; $\alpha = 0.05$). Since the interactions for L × P, and S × P × L were not significant, detailed comparisons are omitted</td>
<td>114</td>
</tr>
<tr>
<td>10.</td>
<td>Pearson’s correlation coefficients between elevation vs. transect properties, and elevation vs. plant properties</td>
<td>115</td>
</tr>
<tr>
<td>11.</td>
<td>Regression parameters for changes in leaf $\delta^{15}$N with soil N concentration (g N kg⁻¹), soil $\delta^{15}$N (%), leaf [N] (g N kg⁻¹), and soil GWC (mg mg⁻¹). (symbols: $b_0$ intercept, $b_1$ slope, F calculated F-ratio. Regression equations are of the form $y = b_0 + b_1(x)$).</td>
<td>118</td>
</tr>
<tr>
<td>12.</td>
<td>Leaf $\delta^{13}$C (%) in different species (S), landscape locations (L) and sample periods (P). Different letters indicate differences between means (Fisher’s LSD analysis; $\alpha = 0.05$). Since the interactions effects of S × L, L × P, and S × P × L was not significant, detailed comparison was not listed</td>
<td>142</td>
</tr>
<tr>
<td>TABLE</td>
<td>Page</td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>------</td>
<td></td>
</tr>
<tr>
<td>13. Pearson’s correlation coefficients for elevation vs. soil properties, and elevation vs. plant properties along a hillslope gradient</td>
<td>143</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION

Woody plant encroachment into grassland and savanna ecosystem has been reported in many areas including North and South America, Australia, Africa, and southeast Asia over the past century (Archer et al., 2001; Arnold, 1995; Boutton et al., 1998; Mast et al., 1997; Menaut et al., 1990; Nobel, 1997). Heavy grazing and fire suppression have often been implicated as causes of this geographically widespread vegetation change (Archer, 1995; Boutton et al., 1998; Jeltsch et al., 2000; Midgley and Bond, 2001). This woody plant encroachment has long been a concern of land managers and ecologists (Fisher, 1950; Fisher, 1977; Rappole et al., 1986) because it has the potential to profoundly influence grassland biodiversity, commercial livestock grazing, hydrology, biogeochemistry and landscape evolution (Boutton et al., 1998; Nobel, 1997; Rappole et al., 1986), and could impact ecosystem services, land use options, and livelihoods of almost 20% of the world’s population (Turner et al., 1990). Despite the global significance of this land cover change, we know relatively little regarding the pattern and extent of this vegetation change.

In the Rio Grande Plains of southern Texas, subtropical woodlands dominated by C₃ plants have become significant components of landscapes that were once almost

This dissertation follows the style and format of the Soil Science Society of America Journal.
exclusively dominated by C₄ grasslands (Archer et al., 1988; Boutton et al., 1998). Historical accounts suggest that this conversion began in the mid- to late 1800s (Johnston, 1963) and coincided with the intensification of livestock grazing and fire suppression (Archer, 1995; Archer et al., 2001). In this region, small discrete woody clusters organized around a central honey mesquite (*Prosopis glandulosa*) tree, and larger groves of woody vegetation (apparently comprised of clusters that have grown together and fused) are embedded in an herbaceous matrix in uplands (Whittaker et al., 1979). The size, cover and density of woody plants are influenced by interactions between rainfall and disturbance (herbivory and fire) as constrained by soils (Archer, 1995; House et al., 2003; Rodriguez-Iturbe et al., 1999; Scholes and Archer, 1997). Previous studies suggested that the increases of woody cover were initiated by the establishment of honey mesquite, an unpalatable, stress-tolerant N₂-fixing tree. As mesquite trees establish and grow, they serve as recruitment foci, facilitating the recruitment and establishment of other woody species beneath their canopies (Archer, 1995; Archer et al., 1988). Studies conducted at the La Copita Research Area (situated in the eastern portion of the Rio Grande Plains of southern Texas) have shown this initiation of new shrub clusters and the expansion of established clusters increased woody cover by 150-338% since the 1940s (Archer, 1995; Archer et al., 2001). However, we still know little about the development of the successional process. Understanding these vegetation dynamics at the landscape-scale will aid in reconstructing patterns of woody plant encroachment in the past, and in anticipating future changes which may occur on landscapes in this region.
Stable isotopes provide useful tools for examining the pattern and impacts of vegetation change on ecosystem processes (Boutton et al., 1999). Stable isotopic distributions in nature reflect physical, chemical, and biological processes that fractionate stable isotopes, and often record information about the environmental conditions prevailing at the time those processes occurred. They also record information about the origins of the samples (Peterson and Fry, 1987). The natural abundance of stable carbon isotopes in soils can be utilized to document changes in ecosystem structure where C₃ woody plant encroachment into C₄ grassland has occurred (Boutton et al., 1999; Krull et al., 2005). C₃ and C₄ plants have unique δ¹³C values which are incorporated into the soil without significant fractionation during soil organic carbon formation (Boutton, 1996; Boutton et al., 1998; Fernandez et al., 2003; Wedin et al., 1995). In the Rio Grande Plains of southern Texas, all woody plants have the C₃ photosynthetic pathway (δ¹³C ≈ -27‰) and all grasses have the C₄ pathway (δ¹³C ≈ -13‰) (Boutton et al., 1999). Therefore, soil δ¹³C values reflect the relative contributions of plant species with C₃ and C₄ photosynthetic pathways to community net primary productivity, and should be useful in reconstructing this vegetation change from C₄ grassland to C₃ woodland. The difference between the isotopic composition of the current plant community and that of the soil organic matter created by the vegetation change will persist for a length of time determined by the soil organic matter turnover rate (Boutton et al., 1998). Previous studies have demonstrated the usefulness of δ¹³C natural abundance for documenting vegetation change and estimating the turnover rate.
of soil organic carbon (Balesdent et al., 1987; Bernardes et al., 2004; Krull and Bray, 2005; Liao et al., 2006b).

The natural abundance of $^{15}$N has been studied in an attempt to understand patterns of N cycling (Dijkstra et al., 2003; Handley and Scimgeour, 1997; Hobbie and Hobbie, 2006; Hogberg, 1997; Hopkins et al., 1998; Robinson, 2001). Nitrogen isotope fractionation occurs during nitrogen transformations mediated by microbes, such as mineralization, nitrification, denitrification, $N_2$ fixation, and immobilization (Högberg et al., 1996; Kawanashi et al., 1993; Shearer and Kohl, 1986), and the $\delta^{15}N$ values of soils are known to be influenced by the relative rates of N inputs vs. N losses from the system. Nearly all ecosystem gaseous N-losses that occur during ammonification (NH$_3$), nitrification (NO, N$_2$O), and denitrification (N$_2$O, N$_2$) are depleted in $^{15}$N, causing $^{15}$N enrichment of residual ecosystem N. In addition, most N-inputs to ecosystems (e.g., atmospheric deposition, N-fixation) are depleted in $^{15}$N. Hence, relative rates of N-losses vs. N-inputs play an important role in determining the natural abundance of $^{15}$N in plants and soils (Amundson et al., 2003; Garten and van Miegroet, 1994; Hopkins et al., 1998). Previous studies in the Rio Grande Plains have shown that woody plant encroachment into grassland has altered N-storage in soil (Liao et al., 2006a; McCulley et al., 2004), the abundance of nitrogen fixing trees and shrubs (Zitzer et al., 1996), as well as soil microbial biomass and rates of N-cycling processes (Archer et al., 2001; Hibbard et al., 2001; McCulley et al., 2004). These dramatic changes in nitrogen storage and nitrogen cycling processes have likely left an imprint on the pattern of $\delta^{15}N$ in plants.
and soils that encodes information related to the dynamics of the nitrogen cycle in this
landscape.

Geostatistical approaches have received increased attention in ecology in the last
decade. The basic idea of geostatistics is that of spatial continuity. That is, on average,
samples close together are more similar than those further apart (Rossi et al., 1992).
Geostatistics can provide powerful tools for: (a) quantifying spatial structures and
autocorrelation for unbiased measurements; (b) estimation of the minimum distance
required for the spacing of independent sampling; (c) interpolating values at unsampled
locations; and (d) assessing uncertainty of spatial predictions (Goovaerts, 1997;
Goovaerts, 1998; Rossi et al., 1992; Sutherland et al., 1991). The use of geostatistics in
conjunction with soil and plant isotopic composition should be a powerful approach for
extending isotopic analyses of vegetation dynamics to the landscape scale; however, the
union of these methodologies still remains largely unexplored.

The purpose of this study is to use $\delta^{13}$C and $\delta^{15}$N values of soil organic matter
and plant tissue in conjunction with geostatistics to quantify the landscape-scale
vegetation dynamics in the Rio Grande Plains of southern Texas. To further optimize
this useful methodology, I also examined the factors controlling spatial variations of
plant and soil isotopic composition in an effort to understand the biotic and edaphic
mechanisms that influence soil and plant $\delta^{13}$C and $\delta^{15}$N values at ecosystem and
landscape scales. Specific objectives are to: (1) Use spatial patterns of soil $\delta^{13}$C to
reconstruct historical vegetation cover and estimate the direction of woody plant
expansion; (2) Investigate the within-patch variability of soil $\delta^{13}C$ and the factors that may regulate this variability under woody plant canopies in upland clusters and groves and in lower-lying drainage woodlands; (3) Document spatial and temporal variation in leaf $\delta^{13}C$ and $\delta^{15}N$ of three dominant woody species along a topoedaphic gradient; and (4) Identify the factors that may be controlling spatial and seasonal variation in leaf $\delta^{13}C$ and $\delta^{15}N$ of these woody species.
CHAPTER II

LANDSCAPE-SCALE VEGETATION DYNAMICS INFERRED FROM SPATIAL PATTERNS OF SOIL $\delta^{13}$C IN A SUBTROPICAL SAVANNA PARKLAND

SYNOPSIS

Grass-dominated ecosystems in many regions around the world have experienced increased woody plant abundance during the past 100 yrs. In the Rio Grande Plains of southern Texas, subtropical woodlands dominated by $C_3$ trees/shrubs have become significant components of landscapes that were once almost exclusively dominated by $C_4$ grasslands. However, we know relatively little regarding the pattern and extent of this vegetation change at the landscape-scale. $C_3$ and $C_4$ plants have unique $\delta^{13}$C values which are incorporated into the soil without significant fractionation during soil organic carbon (SOC) formation. In this study, I used soil $\delta^{13}$C in conjunction with sequential aerial photography and spatial statistics to quantify landscape-scale vegetation dynamics in southern Texas. A 160 x 100 m plot was georeferenced and subdivided into 10 x 10 m cells on a sandy loam upland site (1-3% slope) comprised of woody patches in a grassland matrix. Two soil samples (0-15cm) were collected at points selected randomly within each grid cell, and analyzed for $\delta^{13}$C. Semivariogram analysis indicated that soil $\delta^{13}$C had an anisotropic spatial pattern, with stronger spatial structure [(sill-nugget)/sill= 70.3%] and shorter range (11.90 m) parallel to the slope and weaker spatial structure [(sill-nugget)/sill= 57.5%] and longer range (19.09m) perpendicular to the slope. Kriged
maps of soil δ^{13}C revealed the pattern of woody plant encroachment across this landscape. On soil δ^{13}C contour maps, centers of closed contour lines represented centers of woody patches, and revealed that larger woody patches were formed from small discrete clusters of woody plants that spread laterally and eventually coalesced. Areas where woody patches were expanding into grassland were characterized by low densities of soil δ^{13}C contour lines, and indicated the direction and extent of woody plant encroachment. Conversely, areas with high contour densities represented grassland-woodland boundaries that were relatively stable over time. Indeed, aerial photos from 1930, 1941, 1982 and 2003 confirmed that woody patches with low spatial variability in δ^{13}C corresponded to areas where C_3 woody plants had encroached during the past 30-75 yrs. While aerial photos can only reflect the vegetation cover at the photo acquisition time, kriged maps of soil δ^{13}C allowed us to accurately reconstruct long-term temporal dynamics of woody plant encroachment into grassland. This approach can also be reliably applied to reconstruct large-scale changes in woody cover in areas where historical aerial photography or satellite imagery are not available. These data provide a strong spatial context for future studies aimed at understanding the functional consequences of this change in landscape structure.

INTRODUCTION

Grass-dominated ecosystems in many regions around the world have experienced increased woody plant abundance during the past 100 yrs (Archer et al., 2001; Asner et al., 2004; van Auken, 2000). This woody plant encroachment has long been a concern
of land managers and ecologists (Fisher, 1950; Fisher, 1977; Rappole et al., 1986) because it has the potential to profoundly influence grassland biodiversity, commercial livestock grazing, hydrology, biogeochemistry and landscape evolution (Boutton et al., 1998; Nobel, 1997; Rappole et al., 1986), and could impact ecosystem services and the livelihoods of almost 20% of the world’s population (Turner et al., 1990). Despite the global significance of this land cover change, we know relatively little regarding the pattern and extent of this vegetation change.

In the Rio Grande Plains of southern Texas, subtropical woodlands dominated by C₃ plants have become significant components of landscapes that were once almost exclusively dominated by C₄ grasslands (Archer et al., 1988; Boutton et al., 1998). Historical accounts suggest that this conversion began in the mid- to late 1800s (Johnston, 1963) and coincided with the intensification of livestock grazing and fire suppression (Archer, 1995; Archer et al., 2001). In this region, small discrete woody clusters organized around a central honey mesquite (*Prosopis glandulosa*) tree, and larger groves of woody vegetation (apparently comprised of clusters that have grown together and fused) are embedded in an herbaceous matrix in uplands. The size, cover and density of woody plants are influenced by interactions between rainfall and disturbance (herbivory and fire) as constrained by soils (Archer, 1995; House et al., 2003; Rodriguez-Iturbe et al., 1999; Scholes and Archer, 1997).

Previous studies suggested that the increases of woody cover were initiated by the establishment of honey mesquite, an unpalatable, stress-tolerant N₂-fixing tree. As
mesquite trees establish and grow, they serve as recruitment foci, facilitating the recruitment and establishment of other woody species beneath their canopies (Archer, 1995; Archer et al., 1988). Studies conducted at the La Copita Research Area (situated in the eastern portion of the Rio Grande Plains of southern Texas) have shown this initiation of new shrub clusters and the expansion of established clusters increased woody cover by 150-338% since the 1940s (Archer, 1995; Archer et al., 2001). However, we still know little about the development of the successional process.

Understanding the vegetation dynamics at the landscape-scale will aid in reconstructing the pattern of woody plant encroachment in the past, and in anticipating future changes which may occur on landscapes in this region.

The natural abundance of stable carbon isotopes in soil can be utilized to document changes in ecosystem structure where C₃ woody plant encroachment into C₄ grassland has occurred (Boutton et al., 1999; Krull et al., 2005). C₃ and C₄ plants have unique δ¹³C values which are incorporated into the soil without significant fractionation during soil organic carbon formation (Boutton, 1996; Boutton et al., 1998; Fernandez et al., 2003; Wedin et al., 1995). In the Rio Grande Plains of southern Texas, all woody plants have the C₃ photosynthetic pathway (δ¹³C ≈ -27‰) and all grasses have the C₄ pathway (δ¹³C ≈ -13‰) (Boutton et al., 1999). Therefore, soil δ¹³C values reflect the relative contributions of plant species with C₃ and C₄ photosynthetic pathways to community net primary productivity, and should be useful in reconstructing this vegetation change from C₄ grassland to C₃ woodland. The difference between the isotopic composition of the current plant community and that of the soil organic matter
created by the vegetation change will persist for a length of time determined by the soil organic matter turnover rate (Boutton et al., 1998). Previous studies have demonstrated the usefulness of $\delta^{13}$C natural abundance for documenting vegetation change and estimating soil turnover rate (Balesdent et al., 1987; Bernardes et al., 2004; Krull and Bray, 2005; Liao et al., 2006b). However most of these previous studies were conducted at the ecosystem level and were not spatially explicit. The use of quantitative spatial methods (e.g. geostatistics) in conjunction with soil $\delta^{13}$C analyses should be a powerful approach for extending isotopic analyses of vegetation dynamics to the landscape scale; however, these methodologies have been merged in only two previous studies (Biggs et al., 2002; van Kessel et al., 1994).

Use of satellite images or aerial photographs to study woody plant encroachment and the spatial pattern of vegetation have received increased attention (Archer et al., 2004; Fensham and Fairfax, 2003; Laliberte et al., 2004; Witt et al., 2006). However, aerial photos represent the vegetation at the time the image is acquired, and do not reveal past dynamics. In areas with no historical aerial photos or with only low-resolution satellite images, our knowledge of the rates, patterns, and biogeochemical consequences of tree and shrub proliferation is limited. If spatial pattern of soil $\delta^{13}$C can accurately reconstruct the dynamics of woody plant encroachment, it can provide not only direct evidence for the successional development of woody patches, but also a strong spatial context for future studies aimed at understanding the functional consequences of this change in landscape structure. Therefore, in this study, I used $\delta^{13}$C values of soil organic matter in conjunction with sequential aerial photography and geostatistics to quantify the
landscape-scale vegetation dynamics in the Rio Grande Plains of southern Texas. My objectives were to: (1) Quantify spatial variation of soil $\delta^{13}C$ across the landscape; (2) Reconstruct historical vegetation cover and estimate the direction of woody plant expansion using soil $\delta^{13}C$ kriging map; and (3) Predict the future evolution of the landscape based on results from objectives 1 and 2.

MATERIALS AND METHODS

Study area

Research was conducted at the Texas Agricultural Experiment Station La Copita Research Area in Jim Wells County, 15 km SW of Alice, TX (27° 40’ N; 98° 12’ W; elevation 80m) 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. This study was confined to upland portions of the landscape. Upland 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 a grassland matrix, with discrete woody clusters (comprised of a single mesquite tree with up to 15 understory tree/shrub species, 3–10 m diameter), and larger groves (comprised of several woody clusters that have fused
together, 10 to >20 m diameter) embedded within that matrix. The grasslands consist mainly of rhizomatous and weakly caespitose C₄ grasses, and C₃ forbs. Clusters and groves are dominated by *P. glandulosa* and *Zanthoxylum fagara* (lime pricklyash). Mesquite and several other leguminous trees and shrubs present in wooded areas are capable of symbiotic N₂-fixation (Zitzer et al., 1996). Archer (1995) and Boutton et al. (1998) have additional details on soils, vegetation, and climate.

**Field sampling design**

A 100m × 160m plot consisting of 10m × 10m grid cells was established on an upland landscape (Fig. 1) which included all of the upland landscape elements: clusters, groves, and grasslands. Elevations within the study area were determined by terrain surveying and kriging interpolation. Ground control points (n = 48) were established throughout the study area, and their exact locations determined by GPS. The relative elevation of one point (Point A) was set at 0 m. The elevation change from one control point to another was measured by field surveying. The absolute elevation of Point A was determined on USGS 1:24,000-scale 10-meter resolution DEM in UTM projection. Then the absolute elevations of the other ground control points were calculated based on their elevations relative to point A. A topographic map of the whole area was developed using kriging interpolation based on the elevations of the ground control points in ArcView GIS Spatial Analyst (ESRI, 1998). Elevation was highest at the northeast corner (90.67 m) and lowest at the southwest corner (87.93 m), resulting in a gentle northeast to southwest slope. Soil samples (0-15 cm x 2.24 cm in diameter) were
Fig. 1. Color infrared aerial view of study area. Red areas represent woody vegetation, while light gray areas represent grasslands. Blue dots indicate soil sampling locations within the 100 X 160 m grid. Numbered woody patches were evaluated in detail for evidence of expansion in size over time. Yellow lines indicate the edge of woody patches.
collected at two randomly selected points within each cell over a four-day period in January 2003. A total of 320 points were sampled. All the grid sampling points were generated in a GIS and located in the field using GPS.

**Soil δ¹³C analyses**

Soils were first passed through a 2 mm sieve to remove coarse organic fragments and gravel, then dried at 60°C for 48 hours and ground in a centrifugal mill (Angstrom, Inc., Belleville, MI). Ground soils were treated with HCl vapor in a desiccator to remove carbonates (Harris et al., 2001) and then dried. The acid-treated samples were used to measure δ¹³C of soil organic matter, which were determined using a Carlo Erba EA-1108 interfaced with a Delta Plus isotope ratio mass spectrometer operating in continuous flow mode (ThermoFinnigan, San Jose, CA).

Carbon isotope ratios are presented in δ notation:

\[
\delta = \left[ \frac{(R_{\text{SAMPLE}} - R_{\text{STD}})}{R_{\text{STD}}} \right] \times 10^3
\]  

(1)

where \( R_{\text{SAMPLE}} \) is the \(^{13}\text{C}/^{12}\text{C} \) ratio of the sample and \( R_{\text{STD}} \) is the \(^{13}\text{C}/^{12}\text{C} \) ratio of the V-PDB standard (Coplen, 1996). Precision of duplicate measurements was 0.1 ‰ for δ¹³C.

The proportion of carbon derived from grassland was estimated by the mass balance equation:

\[
\delta^{d\text{C}} = (\delta^{\text{C}_4})(x) + (\delta^{\text{C}_3})(1-x)
\]

(2)

where \( \delta^{\text{C}_3} \) is the \( \delta^{\text{C}} \) value of the whole soil organic matter, \( \delta^{\text{C}_4} \) is the average \( \delta^{\text{C}} \)
value of the C₄ components of the sample, x is the proportion of carbon from C₄ plant sources, δ¹³Cᵥ is the average δ¹³C value of the C₃ components, and (1-x) is the proportion of carbon derived from C₃ plant sources. In this study, we used -14‰ as δ¹³Cᵥ and -27‰ as δ¹³Cᵥ based on a previous study in the same area (Boutton et al., 1998).

### Aerial photography and image analysis

Black and white (B&W) aerial photos taken in 1930, 1941 and 1982 and a color-infrared (IR) aerial photo taken in 2003 were used to examine changes in vegetation cover. Aerial photos were scanned and georeferenced in UTM projection with 1m resolution using ERDAS Imagine (ERDAS, 1998). The aerial photo images were subjected to an unsupervised classification using ERDAS Imagine (ERDAS, 1998). Forty classes were first grouped based on reflectance value similarities of pixels. These classes were further classified into two categories (woody vs. non-woody) and area of the woody patches were calculated in ArcView GIS Spatial Analyst (ESRI, 1998). Ten woody patches whose present area is more than 100 m² (Fig. 1) were selected as representative of invading woody patches to study the relationship between soil δ¹³C values and vegetation dynamics revealed by sequential aerial photography. All ten plots currently are large groves comprised of multiple mesquite trees and other woody plants in the understory. The area of the ten patches is provided in Table 1.
Table 1. Area of the woody patches (groves) in 1930 and 2003 (m²).

<table>
<thead>
<tr>
<th>ID</th>
<th>Area in 1930 (m²)</th>
<th>Area in 2003 (m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>181.7</td>
<td>227.0</td>
</tr>
<tr>
<td>2</td>
<td>105.7</td>
<td>148.9</td>
</tr>
<tr>
<td>3</td>
<td>227.9</td>
<td>475.7</td>
</tr>
<tr>
<td>4</td>
<td>1257.6</td>
<td>2330.3</td>
</tr>
<tr>
<td>5</td>
<td>116.8</td>
<td>246.6</td>
</tr>
<tr>
<td>6</td>
<td>240.6</td>
<td>348.1</td>
</tr>
<tr>
<td>7</td>
<td>143.4</td>
<td>183.1</td>
</tr>
<tr>
<td>8</td>
<td>206.0</td>
<td>631.0</td>
</tr>
<tr>
<td>9</td>
<td>88.3</td>
<td>181.8</td>
</tr>
<tr>
<td>10</td>
<td>91.0</td>
<td>131.7</td>
</tr>
</tbody>
</table>
Statistical analysis and mapping

Descriptive statistical analyses were performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL). Variographic analyses were conducted using VARIOWIN (Pannatier, 1996). All GIS analyses were conducted using ArcView GIS Spatial Analyst (ESRI, 1998).

Variogram was used to determine the spatial autocorrelation pattern for soil δ13C. Experimental semivariogram for the lag distance h was calculated according to:

\[ \gamma(h) = \frac{1}{2N(h)} \sum_{i=N(h)}^{i=N(h)} \left( Z(X_i) - Z(X_i + h) \right)^2 \]  

(3)

where \( Z(x_i) \) and \( Z(x_{i+h}) \) are the values of measured properties at spatial location \( x_i \) and \( x_{i+h} \), \( N(h) \) is the number of pairs with lag distance \( h \). The model we chose was the spherical model. Nugget variance (\( C_0 \)), range (A), structure variance (C) and sill (\( C_0 + C \)) were the parameters used to interpret spatial autocorrelation (Fig. 2). The variance at lag distance zero, called “nugget” variance, is caused by measurement error or variation at scales smaller than the sampling unit. Usually semivariance increases with lag distance and then levels off to a constant value called the sill. The lag distance at which the sill is approached is called the range of the spatial continuity. Beyond the range, the properties can be considered spatially unrelated. The difference between the sill and the nugget is called the structural variance. The ratio of the structural variance and sill, representing the proportion of the total variance explained by the spatial structure, reveals the
Fig. 2. Components of a semivariogram.
structure strength (Dent and Grimm, 1999). Kriging was used for spatial interpolation of values at unsampled locations based on sample data and their spatial structure determined using variogram analysis.

RESULTS

Descriptive statistics

In the upland grid sampling area, soil δ¹³C values beneath both clusters and groves had a mean of -21.10‰. Soil δ¹³C values beneath grassland patches averaged -18.98‰ (Table 2). The mean values of soil δ¹³C beneath woody clusters and grove patches were higher than those of the current organic matter inputs (-26 to -28 ‰, (Boutton et al., 1998)), indicating a proportion of soil carbon beneath present C₃ woodlands was derived from C₄ grasses.

Soil δ¹³C values in grasslands, clusters, and groves were negatively skewed, indicating the median was less than the mean with a long tail of small values to the left (Table 2). However, in general, skewness values in Table 1 are near zero, indicating an approximately symmetric distribution. Coefficients of variation for soil δ¹³C values were 4.90% in grasslands, 11.56% in cluster patches, and 7.82% in groves (Table 2). The wider range of δ¹³C in cluster and grove patches (9.56‰ and 8.68‰) also indicated that heterogeneity of soil δ¹³C is greater beneath herbaceous vegetation cover.
Variographic analysis

Variographic analysis was performed to assess spatial autocorrelation of soil $\delta^{13}C$ (Table 3). The elevation within the grid was higher at the northeast corner and lower at the southwest corner (Fig. 3). Therefore, we used omni-directional, 45° from north (i.e. parallel to slope), and 135° from north (i.e. perpendicular to slope) directional models to examine if topography caused differences in spatial patterns of soil $\delta^{13}C$. All the models were spherical with a clear limit of spatial correlation (range). The range of the omni-directional model was 12.6 m, which means that there was no spatial autocorrelation of soil $\delta^{13}C$ beyond this distance. The proportion of the sill explained by spatial dependence $[(\text{sill} – \text{nugget})/\text{sill}]$, which indicates the spatial structure at the sampling scale, was 70.3% for the omni-directional model. Soil $\delta^{13}C$ had an anisotropic spatial pattern, with stronger spatial structure $[(\text{sill-nugget})/\text{sill}= 95.7\%]$ and shorter range (11.90 m) in the 45° direction (parallel to slope) and weaker spatial structure $[(\text{sill-nugget})/\text{sill}= 57.5\%]$ and longer range (19.09 m) in the 135° direction (perpendicular to slope). Therefore, differences in spatial pattern of soil $\delta^{13}C$ appear to be at least partially due to topography.

Mapping

Ordinary kriging provided estimates of soil $\delta^{13}C$ values at locations which had not been sampled. Using kriging based on the variographic analysis, soil $\delta^{13}C$ values were mapped across this landscape. When kriged maps of soil $\delta^{13}C$ (Fig. 4) are
Table 2. Descriptive statistics of soil $\delta^{13}C$ values (0-15cm) (‰ vs. V-PDB) under different types of vegetation cover.

<table>
<thead>
<tr>
<th></th>
<th>Grassland</th>
<th>Cluster</th>
<th>Grove</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>-18.98</td>
<td>-21.10</td>
<td>-21.10</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>-18.90</td>
<td>-21.15</td>
<td>-21.52</td>
</tr>
<tr>
<td><strong>Minimum</strong></td>
<td>-23.31</td>
<td>-26.59</td>
<td>-25.81</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>-16.48</td>
<td>-17.03</td>
<td>-17.13</td>
</tr>
<tr>
<td><strong>Standard Error</strong></td>
<td>0.07</td>
<td>0.45</td>
<td>0.21</td>
</tr>
<tr>
<td><strong>Standard Deviation</strong></td>
<td>0.93</td>
<td>2.44</td>
<td>1.65</td>
</tr>
<tr>
<td><strong>Coefficient of Variation</strong></td>
<td>4.90%</td>
<td>11.56%</td>
<td>7.82%</td>
</tr>
<tr>
<td><strong>Sample Variance</strong></td>
<td>0.87</td>
<td>5.96</td>
<td>2.72</td>
</tr>
<tr>
<td><strong>Skewness</strong></td>
<td>-1.07</td>
<td>-0.00</td>
<td>-0.22</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>6.83</td>
<td>9.56</td>
<td>8.68</td>
</tr>
</tbody>
</table>

Table 3. Parameters for best-fitted semivariogram of soil $\delta^{13}C$.

<table>
<thead>
<tr>
<th>Model</th>
<th>Range (m)</th>
<th>Nugget (‰)</th>
<th>Sill (‰)</th>
<th>(Sill – Nugget)/Sill (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omni-directional</td>
<td>Spherical</td>
<td>12.60</td>
<td>0.62</td>
<td>2.09</td>
</tr>
<tr>
<td>45°</td>
<td>Spherical</td>
<td>11.90</td>
<td>0.11</td>
<td>2.58</td>
</tr>
<tr>
<td>135°</td>
<td>Spherical</td>
<td>19.09</td>
<td>0.71</td>
<td>1.67</td>
</tr>
</tbody>
</table>
Fig. 3. Elevation map computed from field survey for the 120×100m upland grid at La Copita Research Area in southern Texas.
Fig. 4. Kriged map of soil $\delta^{13}C$ (‰) in uplands.
compared with aerial photos of the study area (Fig. 5), it is clear that soil δ\textsuperscript{13}C values in woody patches are lower than those in grassland patches.

An isotopic mass balance model was utilized to compute the relative proportions of SOC derived from C\textsubscript{4} grasses vs. C\textsubscript{3} woody plants (Eq. 2). In grasslands, approximately 60\% to 75\% SOC was derived from C\textsubscript{4} plants, while in the woody patches the value could be as low as 30\%. Patches with lower soil δ\textsuperscript{13}C values should represent older woody patches where much of the original grassland-derived SOC has decayed out of the soil system and been replaced by newer woody plant-derived SOC. Therefore, it appears that woody patches 5 and 7 were relatively older, and patch 3 was relatively younger.

A contour map of soil δ\textsuperscript{13}C values (Fig. 6) obtained by ordinary kriging revealed the development of woody patches. Centers of concentric contour lines may represent centers of woody patches. Woody patches 1, 2, 3, 5, 6, 7, 9 and 10 each contained one contour center. Patches 4 and 8 both appear to have developed from at least three discrete smaller patches based on the existence of three obvious isotopic centers in each of those wooded areas (Fig. 6). Indeed, aerial photos confirmed that patches 4 and 8 each evolved from three discrete patches between 1930-2003 (Fig. 5).

Interestingly, a dirt road which was not present in either 1930 (Fig. 5a) or 1941 (Fig. 5b), was observed in 1982 in patch 4 (Fig. 5c). However, this road was subsequently abandoned and overgrown by woody plants, and the road was not evident
Fig. 5. Aerial photos taken in 1930, 1941, 1982 and 2003 illustrate vegetation cover change. Lighter areas are herbaceous vegetation; darker areas are woody vegetation.
in the 2003 aerial photo (Fig. 5d). This change was not reflected in the kriged map of soil δ\textsubscript{13}C (Fig. 4) or the contour map of soil δ\textsubscript{13}C (Fig. 6).

Woody plant expansion into grassland causes a reduction in soil δ\textsubscript{13}C values as C\textsubscript{3} woody plant carbon is added to a pool of soil organic matter derived largely from C\textsubscript{4} grasses. The change in soil δ\textsubscript{13}C values should be greatest near the oldest central portion of a woody patch, and least near the grassland-woody patch boundary, resulting in concentric contour lines on the kriged map of δ\textsubscript{13}C values (Fig. 6). High densities of contour lines means there is an abrupt boundary with respect to soil δ\textsubscript{13}C values. Therefore, areas with high contour densities represent grassland-woodland boundaries that have been relatively stable over time. Conversely, areas where woody patches are encroaching into grassland should have low densities of soil δ\textsubscript{13}C contour lines. For example, patches 2 and 7 appear to have been relatively stable due to their higher densities of contour lines near their boundaries with grassland (Fig. 6). In contrast, woody cover appears to have increased significantly in patches 4 and 8 based on lower densities of contour lines near their boundaries with grassland (Fig. 6). Raster calculations based on aerial photos taken in 1993 and 2003 showed that woody cover of patch 7 increased only 28% while that of patches 4 and 8 increased 85% and 206%, respectively (Table 1).

The asymmetric shapes of the concentric contour lines of each patch indicate the anisotropic expansion of the cluster. In other words, each patch may have variable growth rates in different directions. Denser contour lines of soil δ\textsubscript{13}C near the edges of
Fig. 6. Soil δ¹³C contours (at 0.4‰ intervals) superimposed on the vegetation change map calculated from aerial photos. Red color represents vegetation increase from 1930 to 2003. Yellow color represents vegetation cover in 1930. Arrows indicate the estimated fastest direction of expansion of each patch.
woody patches represent slower woody invasion into grassland, while sparser contour lines represented faster woody plant expansion. We estimated the direction of fastest woody expansion for each patch by selecting the direction with sparsest contour lines and indicated them by arrows starting from the center of the contour lines (Fig. 6). Because patches 4 and 8 had more than one contour center, they had 3 and 2 arrows, respectively. Patch 1 appears to expanding rapidly to the southeast and patch 2 appears to expanding rapidly to the southwest, suggesting that they may coalesce in the future. Patch 4 was the result of coalescence of three expanding discrete clusters, which had different directions of conjectural expansion. Patch 7 was relatively stable with the least woody expansion. Patch 8 was formed by coalescence of three smaller patches. Densities of contour lines indicated that left and lower right one expanded rapidly to northwest; while the upper left one remained relatively stable (Fig. 6).

To assess the accuracy of the estimation based on contour map of soil δ¹³C, we compared the conjectural fastest woody expansion direction of each patch to the direction of vegetation changes computed from aerial photos taken in 1930 and 2003 (Fig. 6). The conjectural directions were similar to the expansion directions for patch 1, 2, 4, 6 and 7. The conjectural directions for patch 9 and 10 were not clearly substantiated by the aerial photos, probably because they were located on the edge of the study area and were therefore sampled incompletely. Aerial photos indicated patch 3 and 5 expanded largely around the edge in all directions. The sparser contour lines of soil δ¹³C may be caused by the smaller fast growing clusters near the groves. In patch 8,
the conjectural directions of the left and lower right pioneer isolated patches were confirmed by the computation of the aerial photos (Fig. 6).

**DISCUSSION**

**Overall landscape pattern of soil $\delta^{13}C$**

Descriptive statistics revealed that grassland soils had a $\delta^{13}C$ value (mean ± SE) of $-18.98 \pm 0.07 \%$, while that of cluster and grove soils was $-21.10 \pm 0.45 \%$ and $-21.10 \pm 0.21 \%$, respectively (Table 1). These values were consistent with previous studies in the same area (Boutton et al., 1999; Boutton et al., 1998; Liao et al., 2006b). These data indicated that at least some of the spatial variability of soil $\delta^{13}C$ was related to vegetation cover, and that variability was slightly higher in the woody patches than in the grassland. However, spatial statistics are needed to further explore the nature of this variability.

The omni-directional semivariogram analysis showed that the range of soil $\delta^{13}C$ was 12.6 m, indicating that values were no longer autocorrelated at distances $> 12.6$ m. Similarly, in one of the few geostatistical studies of spatial variability of soil $\delta^{13}C$, Marriott et al. (1997) found that the patch size of soil $\delta^{13}C$ in a temperate grassland was 13.5 m based on variogram models. In the present study, soil $\delta^{13}C$ had an anisotropic spatial pattern, with stronger spatial structure [(sill-nugget)/sill= 95.7\%] and shorter range (11.90 m) parallel to the slope and weaker spatial structure [(sill-nugget)/sill= 57.5\%] and longer range (19.09 m) perpendicular to the slope (Table 1). Therefore,
topography appears to have some control over the spatial pattern of soil $\delta^{13}C$ values at this site. Topography often influences soil texture as well as water and nutrient availability, all of which can influence $\delta^{13}C$ values of plants and soil organic matter inputs (Bird et al., 2003; Panek and Waring, 1995; Schulze et al., 1998; Van de Water et al., 2002). This is consistent with Cerri et al. (2004), who suggested that spatial variability of soil $\delta^{13}C$ in a tropical pasture was highly influenced by topography and management. van Kessel et al. (1994) also suggested that topographic and hydrologic factors had large effects on spatial patterns of $\delta^{13}C$ of crop residues and soil organic matter in an agricultural landscape in the northern Great Plains.

However, in this savanna landscape characterized by discontinuous C$_3$ woody patches within a C$_4$ herbaceous matrix, spatial variability of soil $\delta^{13}C$ is mainly determined by the isotopic composition of soil organic matter inputs (litter and roots). Visual assessment of the kriged map of soil $\delta^{13}C$ (Fig. 4) and aerial photographs (Fig. 5d) revealed the strong resemblance between the spatial pattern of soil $\delta^{13}C$ and vegetation cover. Areas with lower soil $\delta^{13}C$ values corresponded to areas with C$_3$ woody plant cover. This finding is consistent with previous studies documenting the strong controlling effects of plant cover on soil $\delta^{13}C$ variability (Biggs et al., 2002; van Kessel et al., 1994).

**Formation of wooded landscape elements revealed by soil $\delta^{13}C$**

Previous studies have hypothesized that the groves, larger woody patches in the upland savanna, were formed through coalescence of expanding discrete clusters, and
there had been some limited supporting evidence from historical aerial photos (Archer, 1995; Scanlan and Archer, 1991; Wu and Archer, 2005). However, we still know relatively little regarding the successional dynamics that lead to the formation of groves. In this study, the kriged map of soil δ¹³C based on the intensively sampled grid provided strong evidence in support of the proposed mechanism of grove formation by coalescence of clusters (Fig. 6). Concentric contour lines within a grove appear to delineate once discrete clusters that coalesced to form the grove. Historical aerial photos confirmed that the centers of concentric contour lines of soil δ¹³C were once discrete clusters that have coalesced to form larger groves (Fig. 6).

Previous studies at La Copita Research Area proposed that rates of cluster development and patterns of distribution were regulated by subsurface variations in clay content and by variations in annual rainfall (Archer, 1995; Scanlan and Archer, 1991). Therefore, each woody patch may have variable growth rates in different directions due to the influences of edaphic and hydrological factors. In our study, we found the asymmetric shapes of the concentric contour lines indicated the anisotropic expansion of clusters. Sparser contour lines of soil δ¹³C indicated that the cluster expanded further for the same change of soil δ¹³C, therefore representing faster invasion. On the contrary, denser contour lines meant that the cluster boundaries remained relatively stable, thereby establishing a strong isotopic contrast between the interior of the cluster and the adjacent grassland. McAuliffe (1994) found edaphic features such as soil texture controlled the vertical movement and distribution of soil water, in turn affecting the distribution of woody plants in arid and semiarid environments. Archer (1995) suggested variations in
annual rainfall and soil clay content might regulate the rate of woody expansion. Wu and Archer (2005) suggested that rainfall, topography and soil texture were factors potentially influencing woody cover changes. However, the mechanism of the anisotropic expansion of woody clusters is still unclear. Therefore, contour maps of soil $\delta^{13}C$ may provide a strong spatial context for future studies aimed at understanding the factors regulating the rates of cluster development and patterns of woody plant distribution.

While aerial photographs can only reflect the vegetation at the time the photos were taken, the maps of soil $\delta^{13}C$ provide longer-term, integrated records of the cumulative impact of woody encroachment on soils, and these records should be somewhat insensitive to short-term dynamics or recent changes in vegetation. For example, a road, which was not present in 1930 (Fig. 5a) or 1941 (Fig. 5b) was observed in 1982 in patch 4 (Fig. 5c). If we had only the 1982 photo, we would not know if the road was covered by woody plants in the past. However, in the kriged map of soil $\delta^{13}C$ (Fig. 4), the spatial pattern of soil $\delta^{13}C$ for patches 4 and 8 indicated the road was covered by C$_3$ woody vegetation. Therefore, it may be fruitful to use maps of soil $\delta^{13}C$ to investigate vegetation change even if the area has recently encountered disturbance such as fire, mechanical cutting or grazing. For instance, Biggs et al. (2002) used maps of soil $\delta^{13}C$ to document evidence for C$_3$ “ghost trees” that were once present in burned grassland where aboveground evidence for these trees had been obliterated.
**Future landscape evolution**

Landscape-scale analyses of soil $\delta^{13}C$ may also afford glimpses of future landscape evolution. Some of the present discrete clusters appear destined to coalesce and form larger groves. For example, patches 1 and 2 seem likely to coalesce because they are actively expanding towards each other, as revealed by the contour map of soil $\delta^{13}C$ (Fig. 6). Some woodland margins have been relatively stable over the past 50-70 years, indicating that the grasslands at those margins may continue to remain open in the future. Simulations using transition probabilities suggested the present landscape may develop into closed-canopy woodland within the next 180 years (Archer, 1995). Wu and Archer (2005) suggested in order to accurately predict the future rate and pattern of changes in woody abundance, rainfall-topography-soil texture relationships and scale-dependent mechanisms needed to be considered. Our study confirmed that different woody patches may have different expansion rates in the future.

These results could also have implications for ecosystem management practices aimed at controlling woody plant abundance in grasslands. Because the fate of faster growing pioneer clusters may have a greater impact on woody cover increases than the establishment of new clusters (Scanlan and Archer, 1991), removal of faster growing clusters may slow or reverse the trend toward a closed-canopy woodland in the future.

**CONCLUSIONS**

Woody plant cover has increased since the 1930s in the Rio Grande Plains of
southern Texas. Geostatistical analyses showed that soil $\delta^{13}C$ values were autocorrelated within a range of 12.6m. Spatial patterns of soil $\delta^{13}C$ were related to both vegetation (primarily woody vs. grassland cover) and topography. A contour map of soil $\delta^{13}C$ confirmed that the large upland groves were formed in the past by coalescence of expanding discrete clusters, and revealed that some clusters are currently growing rapidly towards each other and might coalesce into groves in the near future. This pattern of woody patch development was substantiated by sequential aerial photographs taken during the past 80 years. Kriged maps of soil $\delta^{13}C$ allowed us to accurately reconstruct landscape-scale spatial pattern and temporal dynamics of woody plant encroachment into grassland, suggesting this approach can be reliably applied to reconstruct large-scale changes in woody cover in areas where historical aerial photography or satellite imagery are not available. Kriged maps of soil $\delta^{13}C$ can provide a strong spatial context for future studies aimed at understanding the functional consequences of this change in landscape structure.
CHAPTER III

SPATIAL VARIATION OF SOIL $\delta^{13}C$ VALUES IN A SUBTROPICAL SAVANNA PARKLAND REVEALS PATTERN OF WOODLAND DEVELOPMENT

SYNOPSIS

In recent history, many grasslands and savannas around the world have experienced woody plant encroachment. In the Rio Grande Plains of southern Texas, subtropical woodlands dominated by $C_3$ trees and shrubs have become significant components of landscapes that were once almost exclusively dominated by $C_4$ grasslands. In this study, we used spatial variation of soil $\delta^{13}C$ to investigate patterns of vegetation change. In an upland portion of the landscape, we set up 6 plots in small woody clusters organized around a central mesquite tree, and 3 plots in larger woody groves which appear to be comprised of clusters that have fused together. Three transects were established from the center of each woody patch type out into the adjacent grassland area. There were significant spatial patterns in soil $\delta^{13}C$ associated with these woody patches. In clusters, soil $\delta^{13}C$ was lowest in the center (mean $\delta^{13}C = -23.25\%$), increased exponentially to the edge of the canopy (mean $\delta^{13}C = -20.08\%$), and stabilized at a relatively high value approximately 15 cm outside of the dripline (mean $\delta^{13}C = -18.89\%$). In groves, soil $\delta^{13}C$ generally increased along the transect from the grove center to the adjacent grassland, but the lowest soil $\delta^{13}C$ values were not always in the grove center. Spatial structure of soil $\delta^{13}C$ in groves appears to reflect the age and
spatial patterns of the individual discrete clusters that fused to form the larger grove. Based on these patterns and a kriged map of soil δ^{13}C within woody groves, I found evidence for successional processes of woody encroachment consistent with prior interpretations based on plant community structure. This study shows that the union of soil stable isotope analyses with geostatistical techniques can generate sophisticated insights regarding patterns of vegetation change at the landscape scale.

INTRODUCTION

In recent history, many grasslands and savannas in Africa, Asia, Australia, South America and North America have experienced woody plant encroachment (Archer, 1995; Archer et al., 2001; Arnold, 1995; Boutton et al., 1998; Mast et al., 1997; Menaut et al., 1990; van Auken, 2000). This dramatic change in tree-shrub-grass composition is strongly influenced by human manipulation of fire frequency and livestock grazing (Archer, 1995; Boutton et al., 1998; Jeltsch et al., 2000; Midgley and Bond, 2001). Increased abundance of woody plants has the potential to profoundly influence grassland biodiversity, hydrology, biogeochemistry and landscape evolution (Boutton et al., 1998; Nobel, 1997; Rappole et al., 1986; Schlesinger et al., 1990). 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; Fisher, 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.
In the Rio Grande Plains of southern Texas, subtropical woodlands dominated by C\textsubscript{3} plants have become significant components of landscapes that were once almost exclusively dominated by C\textsubscript{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; Archer et al., 1988). Previous studies in this region have shown that woody encroachment is initiated by the establishment of *Prosopis glandulosa* (honey mesquite – an N\textsubscript{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 canopies (Archer, 1995; Archer et al., 1988). This process has led to the formation of multispecies 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 of clusters might occur to form larger woody groves that may range from ~20-100 m in diameter (Archer, 1995; Stokes, 1999). Rates of cluster and grove development and their patterns of distribution across the landscape may be regulated by subsurface variations in clay content and by variations in annual rainfall (Archer, 1995; Scanlan and Archer, 1991). However, this hypothesized successional process was based on “space-for-time” studies of vegetation structure in which ages of mesquite trees were used to represent the ages of the woody clusters.

Because the stable carbon isotope composition ($\delta^{13}$C) of soil organic carbon (SOC) reflects the contributions of C\textsubscript{3} shrubs vs. C\textsubscript{4} grasses to the SOC pool following
woody plant encroachment into grassland, spatial variation in $\delta^{13}C$ of SOC may provide more direct evidence of patterns and processes of woodland development in this region (Biedenbender et al., 2004; Boutton et al., 1999; Jessup et al., 2003; Krull et al., 2005; McPherson et al., 1993). $C_3$ and $C_4$ plants have unique $\delta^{13}C$ values which are incorporated into the soil without significant isotopic fractionation during soil organic carbon formation (Boutton, 1996; Boutton et al., 1999; Boutton et al., 1998; Fernandez et al., 2003; Wedin et al., 1995). In the Rio Grande Plains of southern Texas, all woody plants have the $C_3$ photosynthetic pathway ($\delta^{13}C \approx -27\%$) and all grasses have the $C_4$ pathway ($\delta^{13}C \approx -13\%$) (Boutton et al., 1999). Thus, soil $\delta^{13}C$ 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 still comprised of carbon derived from both $C_3$ and $C_4$ plants. Lower soil $\delta^{13}C$ values indicate that more of the carbon is derived from $C_3$ trees/shrubs, therefore implying that site has been occupied by woody plants for a longer duration.

Spatial patterns of soil $\delta^{13}C$ should provide direct evidence for patterns of woodland development in areas that were once grassland. Accordingly, the purpose of this study was to quantify within-patch variability of soil $\delta^{13}C$ in both upland clusters and groves to reveal grassland-to-woodland successional processes on upland portions of the landscape. More specifically we examined the following possible scenarios of woody patch development: (1) If clusters originate first by the establishment of a
mesquite tree and then by the recruitment of other woody species beneath and around its canopy, then soil $\delta^{13}C$ values should increase along a transect from the cluster center into the surrounding grassland (Fig. 7A); (2) If groves developed via the coalescence of discrete clusters, distinct spatial patterns of soil $\delta^{13}C$ should be evident around the individual clusters of different age that merged to form the larger grove (Fig. 7B); and (3) On the other hand, if a grove developed from a single discrete cluster by continued recruitment of new woody plants around the periphery of that cluster, then soil $\delta^{13}C$ values should be lowest in the center and increase gradually from the center to the adjacent grasslands reflecting the direction of woody invasion (Fig. 7C).

MATERIALS AND METHODS

Study area

Research was conducted at the Texas Agricultural Experiment Station 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
Fig. 7. Hypothesized developmental patterns of woody patches in uplands of the Rio Grande Plains of southern Texas: (A) A cluster is initiated by honey mesquite and is formed by following recruitment of other understory shrubs; (B) A grove is coalescence of discrete clusters; (C) A grove is the migration around the boundary of the pioneer patch recruiting newly established invading mesquite trees and shrubs. Cross hatched areas represent the canopies of mesquite trees. Diagrams on the right suggest the spatial pattern of soil $\delta^{13}C$ along a transect (indicated by the dashed line) traversing the present-day woody patch.
subtropical savanna parkland comprised of a grassland matrix, with discrete woody clusters (comprised of a single \textit{Prosopis glandulosa} [honey mesquite] tree with up to 15 understory shrub species) and larger groves (comprised of more than one mesquite tree with associated understory shrubs) embedded within that matrix. The grasslands consist mainly of rhizomatous and weakly caespitose C\textsubscript{4} grasses, and C\textsubscript{3} forbs. Clusters and groves are dominated by honey mesquite and \textit{Zanthoxylum fagara} (lime pricklyash). Honey mesquite and several other leguminous trees and shrubs present in wooded areas are capable of symbiotic N\textsubscript{2}-fixation (Zitzer et al., 1996). Archer (1995) and Boutton et al. (1998) have additional details on soils, vegetation, and climate.

**Field sampling procedures**

Three grove plots, each including an entire grove and adjacent grassland areas within 10m of the grove/grassland edge were established (Fig. 8). Three transects from the center to the edge of each grove were established in three random directions at least 90° apart. Soil samples (0-15cm x 2.24 cm in diameter) were collected at 0.5m intervals along each transect. Additional soil samples away from transects were taken randomly within each grove plot. The total numbers of sampling points were: (a) 45 transect points and 17 random points in Grove #1; (b) 19 transect points and 4 random points in Grove #2; and (c) 29 transect points and 7 random points in Grove #3. All sampling points were classified into one of four categories based on their positions relative to mesquite trees: (a) base or bole of the tree, (b) mid point between the canopy edge and bole, (c) canopy edge, and (d) grassland.
Fig. 8. Map of sampling points in upland superimposed on aerial photo. Clusters and groves of woody vegetation (red in aerial view) are embedded within a grassland matrix (gray). The three groves sampled in this study are circled with blue lines, and the blue dots are the sampling points in groves and adjacent grasslands. Similarly, the six clusters sampled in this study are circled by yellow lines, and the sampling points within those clusters and their adjacent grasslands are indicated by yellow dots.
Six cluster plots, each consisting of an entire cluster and a buffer area with a width 1/3 of the cluster’s diameter, were established in the uplands. Three transects from the center to the edge of each cluster were established in three random directions at least 90° apart. The sampling regime was similar to that for grove plots and shown in detail in Fig. 9. A total of 130 soil samples (0-15cm) were collected in the six cluster plots (Fig. 8).

Three 6m × 10m grassland plots located at least 5m from any woody patch were established in the uplands. Each plot was subdivided into 0.5m × 0.5m grid cells. Soil samples (0-15cm x 2.24 cm in diameter) were taken in 60 randomly selected cells in each of the three grassland grids, resulting in a total of 180 soil samples.

Transect end points and the corners of the grassland plots were georeferenced using a Trimble GPS pathfinder Pro XRS system (Trimble Navigation Limited, Sunnyvale, CA). A measuring tape was used to measure the distance between other points and the ends or corner, therefore determining the exact spatial coordinates of all sample points on transects and grassland plots. All random sample points away from transects were also georeferenced using GPS. Locations of all the mature trees (basal diameter ≥ 15cm) of mesquite within the three grove plots were mapped and their basal diameters were recorded. Distance from the sampling points to the nearest mesquite tree was calculated using ArcView GIS Spatial Analyst (ESRI, 1998).

Soil analyses

Soils were first sieved through a 2 mm sieve to remove coarse organic fragments
Fig. 9. The sampling regime in upland clusters. Soil samples were taken from the center (location 1) to the adjacent grassland (location 7). Locations 2 and 3 were 1/3 and 2/3 of distance from center to dripline. Location 4 was just inside dripline (~15cm inside) and location 5 was just outside dripline (~15cm outside). Locations 6 and 7 were beyond the cluster dripline at distances corresponding to 1/3 and 2/3 the distance from cluster center to dripline.
and gravel. Then soils were dried at 60°C for 48 hours and ground in a centrifugal mill (Angstrom, Inc., Belleville, MI). Soils were weighed into silver capsules using a microbalance, then treated with HCl vapor in a desiccator (Harris et al., 2001) to volatilize inorganic C present as CaCO₃. Samples were dried thoroughly, then analyzed for δ¹³C of SOC using a Carlo Erba EA-1108 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 δ notation:

\[ \delta = \left( \frac{R_{\text{SAMPLE}} - R_{\text{STD}}}{R_{\text{STD}}} \right) \times 10^3 \quad (4) \]

where \( R_{\text{SAMPLE}} \) is the \(^{13}\text{C}/^{12}\text{C} \) ratio of the sample and \( R_{\text{STD}} \) is the \(^{13}\text{C}/^{12}\text{C} \) ratio of the V-PDB standard (Coplen, 1996). Precision of duplicate measurements was 0.1 ‰ for δ¹³C.

**Statistical analyses**

A proximity index (PI) was originally developed by Gustafson and Parker (1992) to distinguish isolated patches from those which are part of a complex of patches. We used a transformation of PI to estimate the influence of mesquite trees in the neighborhood on the soil properties at a sampling point:

\[ PI = \sum_{i=1}^{n} \frac{D_i}{Z_i} \quad (5) \]

where \( Z_i \) is the distance in meters from the sampling point to each surrounding mesquite,
and $D_i$ is the basal diameter of mesquite in meters. Therefore, this index considers the number, size, and distance of surrounding mesquite trees, weighted in favor of the nearer and larger trees. $PI$ is large when the soil sampling point is surrounded by closer and/or more and/or bigger mesquite and decreases as mesquite become further and/or sparser and/or smaller.

One-way ANOVA was used to test for the differences in soil $\delta^{13}C$ with respect to vegetation cover (grassland, cluster and grove) and transect location in woody clusters and groves. Fisher’s LSD method was used to distinguish differences following ANOVA. Regression analyses were performed using Sigma Plot 8.0 (SPSS Inc., 2002) to determine the significance of the relationship between $PI$ of mesquite trees and soil $\delta^{13}C$.

Variogram was used to determine the spatial autocorrelation pattern for soil $\delta^{13}C$ of upland groves. Experimental semivariogram for the lag distance $h$ was calculated according to:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z(X_i) - Z(X_i + h)]^2$$  \hspace{1cm} (6)

where $Z(x_i)$ and $Z(x_{i+h})$ are the values of measured properties at spatial location $x_i$ and $x_{i+h}$, $N(h)$ is the number of pairs with lag distance $h$. Nugget variance ($C_0$), range ($A$), structure variance ($C$) and sill ($C_0+C$) were the parameters used to interpret spatial autocorrelation. The variance at lag distance zero, called “nugget” variance, is caused
by measurement error or variation at scales smaller than the sampling unit. Usually semivariance increases with lag distance and then levels off to a constant value called the sill. The lag distance at which the sill is approached is called the range of the spatial continuity. Beyond the range, the properties can be considered spatially unrelated. The difference between the sill and the nugget is called the structural variance. The ratio of the structural variance and sill, representing the proportion of the total variance explained by the spatial structure, reveals the structure strength (Dent and Grimm, 1999). The semivariogram analysis was conducted using VARIOWIN (Pannatier, 1996).

RESULTS

Descriptive statistics of soil $\delta^{13}$C values

The mean soil $\delta^{13}$C of grasslands (-19.00 ‰) was significantly greater than mean values of soil $\delta^{13}$C under woody clusters (-20.96 ‰) and groves (-21.60 ‰) (Table 4). Soil $\delta^{13}$C was significantly lower in groves than in clusters. The standard deviation of soil $\delta^{13}$C was highest in clusters (1.95) and lowest in grasslands (0.96).

Spatial pattern of soil $\delta^{13}$C in upland clusters

Soil $\delta^{13}$C increased exponentially from centers of clusters (-23.25‰) to the edge of the canopy (-20.08‰), and then reached a value typical of grassland (-19.28‰) at a distance approximately 1/3 of the cluster diameter outside of canopy (Fig. 10).
Table 4. Descriptive statistics for soil $\delta^{13}$C (‰) in different landscape elements. Different letters beside means indicate differences between means from Fisher’s LSD (p=0.05).

<table>
<thead>
<tr>
<th>Landscape element</th>
<th>N</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Standard Error</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grassland</td>
<td>180</td>
<td>-19.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.96</td>
<td>0.07</td>
<td>-23.12</td>
<td>-15.75</td>
</tr>
<tr>
<td>Cluster</td>
<td>130</td>
<td>-20.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.95</td>
<td>0.16</td>
<td>-24.78</td>
<td>-17.30</td>
</tr>
<tr>
<td>Grove</td>
<td>121</td>
<td>-21.60&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.84</td>
<td>0.17</td>
<td>-24.85</td>
<td>-16.87</td>
</tr>
</tbody>
</table>
Spatial pattern of soil $\delta^{13}$C in upland groves

The proximity index (PI), which accounts for the number, distances, and basal diameters of mesquite trees near each soil sampling point, was used to evaluate the potential influence of the mesquite trees on soil $\delta^{13}$C values in groves. We found a positive correlation between soil $\delta^{13}$C and the PI of mesquite trees ($y = -19.09 - 6.25x; r^2 = 0.43; p<0.001$) (Fig. 11), indicating the strong influence of mesquite on soil $\delta^{13}$C in groves.

Changes in soil $\delta^{13}$C between the last mesquite tree along the grove transects (i.e. the tree closest to the grove/grassland boundary) and the adjacent grassland area provided an assessment of the impact of the grove on the $\delta^{13}$C of adjacent grasslands (Fig. 12). Soil $\delta^{13}$C increased from -23.20 ‰ at the base of the tree to -19.90 ‰ at the edge of the canopy. Grassland sampling points approximately 10m away from the grove canopy had mean soil $\delta^{13}$C values of -18.90 ‰, indicating that groves do not influence the isotopic composition of grasslands located >10m beyond grove/grassland margins. However, no samples were taken between the grove/grassland edge and 10 m away from the grove, so this distance over which groves influence grassland $\delta^{13}$C values may be less than 10 m.

Soil $\delta^{13}$C generally increased along the tree-to-tree transects from the centers of the groves to the adjacent grasslands (Fig. 13). However, the lowest soil $\delta^{13}$C values along the transects were not always in the grove centers. For example, in transect 2 and
Fig. 10. Mean soil $\delta^{13}$C (‰) along the transects in woody clusters. The transects in the clusters went from the center of the clusters to the adjacent grassland (Fig. 9). Dashed line indicates the cluster/grassland boundary. Different letters indicate differences between means (Fisher’s LSD analysis; $\alpha = 0.05$). Error bars are standard errors of the mean.
Fig. 11. Relationship between soil $\delta^{13}C$ (‰) and PI of mesquite trees in woody groves.
Fig. 12. Soil $\delta^{13}C$ (‰) from the last tree on the transects in groves to the adjacent grassland area. Base represents sampling points adjacent to the boles of the mesquite trees on the transects; mid represents points midway between the bole and the edge of the canopy; edge represents points at the canopy dripline; and grass represents grassland points adjacent to the groves. Dashed line represents the mean value of soil $\delta^{13}C$ of grassland calculated from the samples in the grassland plots (Table 4). Different letters indicate differences between means (Fisher’s LSD analysis; $\alpha = 0.05$). Error bars are standard errors of the mean.
Fig. 13. Soil $\delta^{13}$C (‰) along transects from the center of each grove out into the adjacent grassland. Values along the X-axes are distances (m) from the center of each grove. Black dots represent sampling points in the grove and red dots represent points in the grassland. Solid black dots indicate points adjacent to the bole of mesquite trees. Dashed lines indicate the mean soil $\delta^{13}$C value of grassland calculated from all grassland samples (Table 4).
3 of grove 1 and all three transects of grove 3, the lowest values were found 4-12 m away from the center. In grove 1, the presence of a tree on the transect did not cause a significant decrease in soil δ¹³C; however, in grove 3, we observed a decrease in soil δ¹³C every time the transect encountered a tree. Soil δ¹³C values of points next to tree boles were all lower than other points in grove 2 (Fig. 13).

Variographic analysis of soil δ¹³C indicated that the ranges of autocorrelation of upland groves were comparable (Grove 1 = 8.6m, Grove 2 = 8.6m, Grove 3 = 6.5m) (Table 5). The proportion of the sill explained by spatial dependence [(sill – nugget)/sill], which indicates the spatial structure at the sampling scale, was 85%, 96%, 96% respectively for the three groves, which indicated soil δ¹³C was heterogeneous in groves.

Interpolation of soil δ¹³C in the groves was conducted by kriging based on the variographic analysis. From the kriged map of soil δ¹³C, we observed at least 3 discrete isotopic centers in groves 1 and 3 (Fig. 14). Generally, soil δ¹³C was lower near the center of each grove, and increased from the center to the edge. Areas with more mesquite trees corresponded to areas with lower soil δ¹³C values based on visual observations (Fig. 14).

**DISCUSSION**

**Soil δ¹³C values of grasslands vs. woody patches**

Present day grasslands in the Rio Grande Plains of southern Texas are comprised
Table 5. Parameters for best-fit omni-directional semivariogram of soil $\delta^{13}C$ of upland groves.

<table>
<thead>
<tr>
<th></th>
<th>Model</th>
<th>Range (m)</th>
<th>Nugget (%)</th>
<th>Sill (%)</th>
<th>$(\text{Sill} – \text{Nugget}) / \text{Sill}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grove 1</td>
<td>Spherical</td>
<td>8.6</td>
<td>0.23</td>
<td>1.56</td>
<td>85.3</td>
</tr>
<tr>
<td>Grove 2</td>
<td>Gaussian</td>
<td>8.6</td>
<td>0.30</td>
<td>7.69</td>
<td>96.1</td>
</tr>
<tr>
<td>Grove 3</td>
<td>Spherical</td>
<td>6.5</td>
<td>0.13</td>
<td>3.10</td>
<td>95.8</td>
</tr>
</tbody>
</table>
Fig. 14. Kriged map of soil $\delta^{13}$C (‰) for the three groves. Blue dots represent the location of mesquite trees, and the size of the dots is proportional to the basal diameters of those trees.
of a mixture of C₃ forbs and C₄ grasses. δ¹³C values of soils in the grassland averaged -19.00 ± 0.96 ‰ (Table 4), which are similar to values reported previously for grassland litter (-19 ‰) and roots (-20.5 ‰) in this ecosystem and many other C₄-dominated grasslands around the world (Boutton et al., 1998). Following woody plant invasion of grassland, mean value of soil δ¹³C decreased to -20.96 ‰ in clusters and -21.60 ‰ in groves (Table 4), reflecting the simultaneous loss of SOC derived from the original C₄ grassland and the accumulation of SOC derived from the invading C₃ woody vegetation.

δ¹³C values indicate that a substantial proportion of the SOC found in both clusters and groves is derived from C₄ grassland, and support the conclusion that C₄ grasslands have been replaced by C₃ woody plants. This is consistent with previous isotopic studies conducted in this region (Boutton et al., 1999; Boutton et al., 1998; Gill and Burke, 1999; Liao et al., 2006b).

**Spatial patterns of soil δ¹³C in upland clusters**

Soil δ¹³C was lowest in the cluster centers and increased exponentially towards the cluster/grassland edge (Fig. 10), which is consistent with the hypothesis that honey mesquite first enter the system and subordinate shrubs follow to form clusters (Fig. 7A). Biggs et al. (2002) also found the distribution pattern of soil δ¹³C was strongly related to the invading mesquite canopies in semiarid grassland in southeastern Arizona.

The isotopic impact of clusters on adjacent grasslands extended approximately 0.15m beyond the cluster/grassland edge. Watts (1993) showed that lateral root
extension beyond cluster canopies was minimal at La Copita Research Area, confirming results from this study showing that the influence of woody plants on soil $\delta^{13}C$ outside of the canopies should be limited. In contrast, Connin et al. (1997) found that following mesquite establishment in New Mexico's Jornada Basin, soil $\delta^{13}C$ was lowest beneath shrub canopies and extended approximately 3 m beyond canopy margins, similar to the distribution of fine roots.

**Spatial patterns of soil $\delta^{13}C$ in upland groves**

Soil $\delta^{13}C$ was heterogeneous in groves and was strongly affected by the presence of mesquite. Regression analysis of soil $\delta^{13}C$ and PI of mesquite trees in groves indicated the size, distance, and number of neighboring mesquite trees was strongly related to soil $\delta^{13}C$ values ($r^2=0.43; p<0.001$; Fig. 11). Kriged maps of soil $\delta^{13}C$ further confirmed that areas near older/larger mesquite trees tended to have lower soil $\delta^{13}C$ values (Fig. 14). This is consistent with Liao et al. (2006b) and Tieszen and Archer (1990), who also found negative relationships between the size/age of mesquite trees and soil $\delta^{13}C$.

However, in some portions of groves with lower soil $\delta^{13}C$ values, older mesquite trees were not found. For example, at the northeast corner of grove 1, only young trees were found in the area with low soil $\delta^{13}C$ values; in the middle of grove 3, no mesquite trees were found although the area had low $\delta^{13}C$ values (Fig. 14). Previous studies indicated that understory shrubs may accelerate the mortality of mesquite trees (Archer, 1995; Archer et al., 1988). We speculate these areas with lower $\delta^{13}C$ values may
actually have a longer history of woody cover, but the original mesquite tree(s) in that portion of the grove may have already died and decomposed and are no longer evident. This is similar to results obtained by Biggs et al. (2002) who found isotopic evidence for “ghost trees” in burned grasslands of Arizona where aboveground evidence for these trees had been obliterated.

We hypothesized that if present-day groves were formed by coalescence of discrete clusters, we would expect to see fluctuations in soil $\delta^{13}C$ along a transect traversing through a grove, as shown in Fig. 7B. According to this conceptual model, the lowest value of soil $\delta^{13}C$ occurs in the oldest cluster contained within the grove, which may or may not be at the center of the grove. On the other hand, if present-day groves were formed by recruitment around the edge of a single founding cluster, we would expect to see the lowest value near the center of the grove (Fig. 7C). In order to test these hypotheses, we first examined soil $\delta^{13}C$ from the last tree on each transect to the adjacent grassland. Results showed soil $\delta^{13}C$ was lowest next to the bole of the mesquite, and increased gradually from the bole to the grassland, which was similar to the isotopic pattern in discrete clusters (Fig. 12). However, the spatial pattern of soil $\delta^{13}C$ across entire groves was neither homogeneous nor regular. We found that although soil $\delta^{13}C$ generally increased along the transect from the grove center to the adjacent grassland, it was not always lowest at the center (Fig. 13). Generally, soil $\delta^{13}C$ decreased when the sampling point was next to a mesquite tree. Variographic analyses also indicated soil $\delta^{13}C$ is heterogeneous within groves. The range of autocorrelation was 6.5 to 8.6m (Table 5).
We suggest that points along the transect with lower soil $\delta^{13}C$ values were located close to centers of the clusters that fused sometime in the past to form the groves. Therefore, the hypothesis that groves developed via the coalescence of individual discrete woody clusters appears to be correct. The kriged maps of soil $\delta^{13}C$ values in each of the three groves (Fig. 14) also support this hypothesis. Groves 1 and 3 clearly have multiple soil $\delta^{13}C$ minima which represent the centers of the older patches. In addition, areas closer to the edge of the grove generally has higher soil $\delta^{13}C$ values, which means the grove has also expanded around the boundary after the coalescence of individual clusters.

Previous studies indicated that upland woody patches were initiated when mesquite trees establish in the grassland. As these mesquite grew, other woody species established beneath their canopies, and small, multispecies woody clusters developed (Archer et al., 1988). Based on this conceptual model and the spatial pattern of soil $\delta^{13}C$ within the woody groves, we simulated the successional processes of this woody encroachment (Fig. 15). This simulation is currently qualitative to illustrate the location of the initially established mesquite trees and the subsequent grove development. This simplistic model could be improved by parameterizing the rate of change of soil $\delta^{13}C$ to quantitatively predict the time point associated with each model step. Grove formation may be constrained by limitations to mesquite seed dispersal and establishment. Archer (1995) suggested groves developed most rapidly on non-argillic inclusions in uplands. Other studies indicated woody plant proliferation should be influenced by interactions...
Fig. 15. Reconstruction of grove expansion simulated from the spatial pattern of current soil $\delta^{13}$C values. Yellow color represents grass cover and other colors represent woody cover. Darker colors represent longer time of occupation by woody plants.
between climate and disturbance such as grazing and fire (Higgins et al., 2000; Rodriguez-Iturbe et al., 1999; Sankaran et al., 2004; Scholes and Archer, 1997). Thus, the future of the landscape may depend on edaphic constraints at local scales and climate and disturbance at larger scales. 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 the future dynamics of the landscape.

CONCLUSIONS

Prior studies based on current vegetation patterns, space for time substitutions, and simulation models of vegetation dynamics in the Rio Grande Plains of southern Texas suggested that woody patches were initiated when mesquite established in grassland. As mesquite grew, other woody species established beneath and around its canopy, resulting in the formation of discrete clusters. As clusters grew and expanded laterally, they often coalesced to form larger groves. In this study, spatial variation of soil $\delta^{13}C$ values within woody patches provided direct evidence for this successional process. Presence of mesquite had strong influence on soil $\delta^{13}C$ values in both upland clusters and groves. Soil $\delta^{13}C$ was lowest in the center of clusters, as would be expected if the central mesquite was the original (and therefore oldest) colonist of the woody cluster. In groves, soil $\delta^{13}C$ reflected the age and spatial patterns of the individual discrete clusters that fused to form the larger grove; therefore soil $\delta^{13}C$ was not always lowest in the center of each grove. Our study shows that the union of soil stable isotope
analyses with geostatistical techniques can generate sophisticated insights regarding patterns of vegetation change at the landscape scale.
CHAPTER IV

SPATIAL PATTERNS OF SOIL $\delta^{13}C$ IN A SUBTROPICAL WOODLAND

SYNOPSIS

Soil carbon isotopic signatures ($\delta^{13}C$) are commonly used to reconstruct plant community history, determine sources of soil organic carbon (SOC), and quantify SOC turnover rates. To optimize this useful methodology, it is critical that we understand the biotic and edaphic mechanisms that influence spatial patterns of soil $\delta^{13}C$ at ecosystem and landscape scales. In this study, we examined spatial patterns of soil $\delta^{13}C$ in a $C_3$ subtropical woodland that developed during the past 100 yrs on a site that was once $C_4$ grassland in the Rio Grande Plains of southern Texas. A 50m×30m plot and two transects were established in a lowland woodland, and soil cores (0-15cm x 2.24 cm in diameter; n = 208) were collected, spatially referenced, and analyzed for $\delta^{13}C$, SOC and soil texture. Variogram analysis indicated that soil $\delta^{13}C$ was heterogeneous (nugget = 0.82 ‰, sill = 1.54 ‰, range = 7.50m). The distribution of mesquite trees (previously shown to initiate grassland to woodland conversion in uplands in this region) did not influence this spatial pattern. However, amount of SOC and soil silt+clay content accounted for 73% of the variance of soil $\delta^{13}C$ values. Cross-variogram analysis and kriging interpolation also indicated that soil $\delta^{13}C$ was spatially correlated with SOC and silt+clay. The negative correlation between SOC and soil $\delta^{13}C$ indicates that higher amount of SOC are derived primarily from the invading $C_3$ woody plants. The positive correlation between silt+clay and soil $\delta^{13}C$ indicate the importance of this soil physical
fraction in the formation of relatively stable organomineral complexes and microaggregates which have slow SOC turnover rates and favor the persistence of C4-derived SOC from the grassland that once dominated this site approximately 100 yrs ago. This study reveals the importance of soil texture in controlling spatial patterns of soil δ¹³C in areas where C₃ to C₄ vegetation changes have occurred, and highlights the need to consider this edaphic property in isotopic studies of vegetation history or SOC dynamics.

INTRODUCTION

Natural stable isotope ratios are widely used in ecological research as an intrinsic tracer to investigate the structural and functional characteristics of ecosystems and their responses to human activities (Amundson et al., 1998; Boutton et al., 1999; Ehleringer et al., 2000; Pataki et al., 2003). Soil carbon isotopic signatures (δ¹³C) are commonly used to reconstruct plant community history, determine sources of soil organic carbon (SOC), and quantify SOC turnover rates (Balesdent et al., 1987; Bernoux et al., 1998; Boutton et al., 1998; Choi et al., 2001; Sanaiotti et al., 2002; Tieszen and Archer, 1990). Soil carbon isotope composition corresponds closely to the carbon isotope composition of plant residues entering the system (Ludlow et al., 1976; Nissenbaum and Schallinger, 1974; van Kessel et al., 1994). After plant residues enter the soil, δ¹³C values are modified slightly from this initial value as decomposition proceeds (Agren et al., 1996; Blair et al., 1985; Santruckova et al., 2000). Therefore, variation in soil δ¹³C values and
their evolution over time are controlled primarily by carbon inputs from vegetation and secondarily by biological decay processes (Nadelhoffer and Fry, 1988).

C3/C4 distributions produce the highest variations of soil $\delta^{13}C$ at landscape to global scale. Due to the natural isotopic differences between plant species with C3 and C4 photosynthetic pathways (Farquhar et al., 1989), soil $\delta^{13}C$ values reflect the relative contributions of C3 and C4 plants to community net primary productivity (Balesdent and Mariotti, 1996; Boutton, 1996; Troughton et al., 1974). $\delta^{13}C$ values of C3 plants range from -32 to -22 ‰, while those with C4 photosynthesis range from -17 to -9 ‰ (Farquhar et al., 1989). Climatic factors, primarily MAP and MAT, control soil $\delta^{13}C$ through control of the ratio of C3 to C4 productivity (Anderson et al., 1998; Ometto et al., 2002; Stewart et al., 1995) and decomposition rate (Garten et al., 2000; Lloyd and Farquhar, 1994; Reichstein et al., 2003). In areas where vegetation has changed from one photosynthetic pathway type to another (e.g. C3 $\rightarrow$ C4, or vice versa), SOC is derived from both the vegetation in the past and the current vegetation, and the isotopic signal from the original vegetation will persist in the SOC pool for a duration dependent on the turnover rate of SOC in that ecosystem. Soil $\delta^{13}C$ values have been utilized successfully to document vegetation changes in a variety of ecosystem types around the world where plant cover has changed from C4 to C3 or vice versa (Boutton, 1996; Boutton et al., 1998; Krull et al., 2005; Martin et al., 1990).

In areas with C3 woody plant encroachment into C4 grasslands, both carbon input from vegetation and decomposition of SOC are expected to affect soil $\delta^{13}C$ (Boutton et
Firstly, because the new litter input is more $^{13}$C depleted, areas with $C_3$ carbon input derived from woody plant cover should have lower soil $\delta^{13}$C values. Therefore, the distribution of woody plants and the amount of SOC should affect soil $\delta^{13}$C values. Secondly, under similar climatic conditions, the dominant factor controlling decomposition processes within the soil is soil texture (Jobbagy and Jackson, 2000; Schimel et al., 1994), with slower rates of SOC turnover in fine-textured soils because clay protects organic matter from mineralization (Anderson and Paul, 1984; Feller and Beare, 1997; Hassink, 1997). The slower SOC turnover rate of clay-associated organic carbon would favor the persistence of carbon derived from original vegetation and affect soil $\delta^{13}$C values (Balesdent and Mariotti, 1996; Balesdent et al., 1987; Bird et al., 2003; Desjardins et al., 1994; Liao et al., 2006b).

In the Rio Grande Plains of southern Texas, subtropical thorn woodlands dominated by $C_3$ plants have become significant components of landscapes that were once almost exclusively dominated by $C_4$ grasslands (Archer et al., 1988; Boutton et al., 1998). Previous studies showed that this vegetation change was initiated by the establishment of the $C_3$ woody plant honey mesquite ($Prosopis glandulosa$), an $N_2$-fixing tree legume. Mesquite appears to facilitated the establishment of other $C_3$ woody species beneath its canopy, resulting in the formation of discrete woody clusters within the grassland matrix (Archer, 1995; Archer et al., 1988). Rates of cluster development and patterns of distribution were regulated by subsurface variations in clay content and by variations in annual rainfall (Archer, 1995; Scanlan and Archer, 1991). This successional pattern is still in progress in uplands, where small discrete clusters
organized around one central mesquite tree and larger groves of woody vegetation
(apparently comprised of fused clusters) are embedded in the C₄ grassland. On lower-
lying portions of the landscape, grassland-to-woodland conversion has gone to
completion and formed closed-canopy drainage woodlands. However, it is not clear
whether or not the mesquite facilitation model and the edaphic constraints theory evident
in the uplands also applies to these lower-lying drainage woodlands. Spatial variation of
soil $\delta^{13}$C may provide information to better understand the biotic and edaphic processes
that have resulted in the formation of drainage woodlands. Specifically, the objective of
this study is to: (1) determine spatial variation of soil $\delta^{13}$C in drainage woodlands; and (2)
investigate spatial correlations between soil $\delta^{13}$C values and the distribution of mesquite
trees, amount of SOC, and soil silt + clay content.

MATERIALS AND METHODS

Study area

Research was conducted at the Texas Agricultural Experiment Station La Copita
Research Area in Jim Wells County, 15 km SW of Alice, TX (27° 40’ N; 98° 12’ W;
elevation 80m) 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
lowlands which receive runoff from the uplands and have intermittent water flow in high
rainfall periods. The elevation ranges from 75-90 m. Upland soils are primarily Typic Argiustolls with a subsurface argillic horizon (Archer, 1995). Soils in lower-lying portions of the landscape are finer-textured clay loams (Pachic Argiustolls). Upland vegetation is subtropical savanna parkland comprised of a grassland matrix, with discrete woody clusters (comprised of a single mesquite tree with up to 15 understory tree/shrub species, 3–10 m diameter) and larger groves (comprised of several woody clusters that have fused together, 10 to >20 m diameter) embedded within that matrix. Lowland drainage areas are covered by closed-canopy woodlands similar in composition to upland wooded areas. Mesquite and *Zanthoxylum fagara* (lime pricklyash) dominate all woody landscape elements. Mesquite and several other leguminous trees and shrubs present in wooded areas are capable of symbiotic N₂-fixation (Zitzer et al., 1996). Woody plant encroachment in the study area over the past 75-100 years due to the interaction of heavy livestock grazing and reduced fire frequency has been well documented (Archer, 1995; Archer et al., 2001). Archer (1995) and Boutton et al. (1998) have additional details on soils, vegetation, and climate.

**Field sampling design**

A 50m × 30m plot consisting of 5m × 5m grid cells was established in a lowland drainage woodland (Fig. 16). The corners of the grid and ten points scattered within the grid were georeferenced using a Trimble GPS pathfinder Pro XRS system (Trimble Navigation Limited, Sunnyvale, CA). The locations of the remaining points in the grids and the transect points were calculated based on their distance to the georeferenced
Fig. 16. Aerial view of the woodland study area in southern Texas. Darker area represents denser woody vegetation cover. Yellow lines (T1, T2) connecting dots represent the two transects. Yellow dots represent soil sampling points within the grid and along the two transects.
Locations of all mature mesquite trees (basal diameter > 15 cm) within the plot were mapped and their basal diameters were recorded.

Two points were randomly selected and their locations were mapped within each grid cell. In addition, two tree-to-tree transects were set up in the drainage woodland (Fig. 16). Transect one started in the southeast corner of the grid and went towards the north. Forty-one sampling points were selected along transect one. Transect two started in the southwest corner and extended towards the north. Forty-seven sampling points were selected along transect two. All sampling points on both transects were classified into one of three categories based on their positions: (i) base of the mesquite tree, (ii) mid-point between the mesquite canopy edge and the tree bole, and (iii) mesquite canopy edge. At each sampling point in the grid and along the transects, three soil cores (0-15 cm x 2.24 cm in diameter) were collected. Distances from the soil sampling points to the nearest mesquite tree were calculated using ArcView GIS Spatial Analyst (ESRI, 1998).

Soil analysis

One of the three soil cores collected at each sample point was used for root biomass analysis by washing soil cores through a hydropneumatic elutriation system (Gillison’s Variety Fabrications Inc., Benzonia, MI, USA) (Smucker et al., 1982) using 410 µm mesh filters. The collected roots were dried for at least 72 hours at 60ºC to determine dry weight, and then ashed at 400ºC in a muffle furnace to obtain ash-free root biomass. The second soil core was used for bulk density and texture analysis. Soil bulk
density was determined by the core method (Soil Survey Staff, 1996). Then the organic
fragments and gravel > 2 mm were removed. Aggregates were crushed by hand, and the
fraction >2 mm was removed by sieving. The percentages of sand, silt, and clay were
determined by pipette method (Gee and Bauder, 1986). All results of particle-size
analysis are expressed as the percentage, by weight, of the oven-dried soil.

The third soil core was used for pH, elemental and isotopic analysis. Soils were
first passed through a 2 mm sieve to remove coarse organic fragments and gravel. Soils
were thoroughly mixed. 12g soil was added to 0.01 M CaCl₂ solution for pH analysis.
The pH of the liquid was determined with an Accumet Basic pH meter (Fisher Scientific).
The remainder was dried at 60°C for 48 hours and ground in a centrifugal mill
(Angstrom, Inc., Belleville, MI) for elemental and isotopic analysis. Soil organic carbon
and total nitrogen concentrations, and δ¹³C values, were determined on a Delta Plus
/Carlo Erba EA-1108 continuous flow isotope ratio mass spectrometer (ThermoFinnigan,
San Jose, CA). Samples were weighed into silver capsules (5 x 7 mm) using a
microbalance, treated with HCl vapor in a desiccator (Harris et al., 2001) to volatilize
inorganic carbon, and then dried and sealed in the capsules. These acid treated samples
were used to measure organic carbon concentration and δ¹³C of soil organic matter.

Carbon isotope ratios are presented in δ notation:

\[ \delta = \frac{\left( \frac{R_{SAMPLE}}{R_{STD}} - 1 \right) \times 10^3}{R_{STD}} \]  \hspace{1cm} (7)
where $R_{\text{SAMPLE}}$ is the $^{13}\text{C}/^{12}\text{C}$ ratio of the sample and $R_{\text{STD}}$ is the $^{13}\text{C}/^{12}\text{C}$ ratio of the V-PDB standard (Coplen, 1996). Precision of duplicate measurements was 0.1 ‰ for $\delta^{13}\text{C}$.

**Statistical analysis and mapping**

A proximity index (PI) was originally developed by Gustafson and Parker (1992) to distinguish isolated patches from those which are part of a complex of patches. We used a transformation of PI to estimate the influence of mesquite trees in the neighborhood on the soil properties at a sampling point:

$$PI = \sum_{i=1}^{n} \frac{D_i}{Z_i}$$

(8)

where $Z_i$ is the distance in meters from the sampling point to each surrounding mesquite, and $D_i$ is the basal diameter of mesquite in meters. Therefore, this index considers both the number of surrounding mesquite trees and their sizes and distances, weighted in favor of the nearer and larger trees. PI is larger when the soil sampling point is surrounded by closer and/or more and/or bigger mesquite and decreases as mesquite become further and/or sparser and/or smaller.

Descriptive statistical analyses were performed on each variable using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL). Modified t-test for correlation (Clifford et al., 1989; Dutilleul et al., 1993) and Pearson’s correlation coefficients were used to calculate correlation coefficients between soil $\delta^{13}\text{C}$, SOC, PI and soil particle size variables using PASSAGE (Rosenberg, 2001). Stepwise multiple linear regression
was performed using SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL) to determine if certain combinations of plant and/or soil variables could be used to predict soil $\delta^{13}C$. The 3D surface plots of soil $\delta^{13}C$ versus the selected variables were generated using TableCurve 3D, version 3 (SPSS Inc., Chicago, IL) using the linear equation $z = a + bx + cy$. Variogram analyses were conducted using VARIOWIN (Pannatier, 1996). GIS analyses were conducted using ArcView GIS Spatial Analyst (ESRI, 1998).

Semivariogram was used to determine the spatial autocorrelation pattern for soil $\delta^{13}C$. Experimental semivariogram for the lag distance $h$ was calculated according to:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{i=N(h)} \left[ Z(X_i) - Z(X_i + h) \right]^2$$  \hspace{1cm} (9)

where $Z(x_i)$ and $Z(x_{i+h})$ are the values of measured properties at spatial location $x_i$ and $x_{i+h}$, $N(h)$ is the number of pairs with lag distance $h$. The model we chose was the spherical model. Nugget variance ($C_0$), range (A), structure variance (C) and sill ($C_0+C$) were the parameters used to interpret spatial autocorrelation. The variance at lag distance zero, called “nugget” variance, is caused by measurement error or variation at scales smaller than the sampling unit. Usually semivariance increases with lag distance and then levels off to a constant value called the sill. The lag distance at which the sill is approached is called the range of the spatial continuity. Beyond the range, the properties can be considered spatially unrelated. The difference between the sill and the nugget is called the structural variance. The ratio of the structural variance and sill, representing the proportion of the total variance explained by the spatial structure, reveals the structure strength (Dent and Grimm, 1999).
Ordinary kriging was used as a spatial interpolation tool for predicting values at unsampled locations based on sample data and their spatial structure determined using variogram analysis.

RESULTS

Soil chemical and physical properties

Coefficients of variation for most soil chemical and physical properties were relatively high, ranging from 8% for soil $\delta^{13}$C values to 58% for root biomass, indicating considerable spatial heterogeneity in edaphic properties in this drainage woodland (Table 6). Soil $\delta^{13}$C values varied by approximately 8 ‰ and revealed that SOC was almost entirely derived from C$_4$ plants in some portions of this woodland ($\delta^{13}$C of SOC = -16.24 ‰), but almost entirely C$_3$-derived in other portions of the woodland ($\delta^{13}$C of SOC = -23.83 ‰).

Spatial patterns of soil properties

Variogram analysis of soil $\delta^{13}$C, SOC and soil silt + clay content of the grid data indicated that the ranges of autocorrelation were 7.50m, 25.65m and 12.97m respectively. The proportion of the sill explained by spatial dependence [(sill – nugget) /sill], which indicates the spatial structure at the sampling scale, was 47% for soil $\delta^{13}$C 29% for SOC and 93% for silt+clay (Table 7).
Table 6. Descriptive statistics for soil chemical and physical properties in transect and grid samples (n=207).

<table>
<thead>
<tr>
<th></th>
<th>SOC (g C kg⁻¹)</th>
<th>Total Nitrogen (g N kg⁻¹)</th>
<th>pH</th>
<th>Bulk Density (g cm⁻³)</th>
<th>δ¹³C (%)</th>
<th>Root Biomass (g m⁻²)</th>
<th>Clay (%)</th>
<th>Silt (%)</th>
<th>Sand (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>19.3</td>
<td>1.67</td>
<td>6.15</td>
<td>1.11</td>
<td>-19.46</td>
<td>785.4</td>
<td>22.7</td>
<td>20.9</td>
<td>56.4</td>
</tr>
<tr>
<td>Median</td>
<td>19.1</td>
<td>1.60</td>
<td>6.18</td>
<td>1.11</td>
<td>-19.42</td>
<td>638.2</td>
<td>22.7</td>
<td>20.4</td>
<td>57.2</td>
</tr>
<tr>
<td>Minimum</td>
<td>9.3</td>
<td>0.70</td>
<td>4.37</td>
<td>0.75</td>
<td>-23.83</td>
<td>144.4</td>
<td>13.5</td>
<td>14.7</td>
<td>40.2</td>
</tr>
<tr>
<td>Maximum</td>
<td>46.1</td>
<td>4.50</td>
<td>7.67</td>
<td>1.39</td>
<td>-16.24</td>
<td>2645.7</td>
<td>39.8</td>
<td>32.4</td>
<td>71.8</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>6.2</td>
<td>0.62</td>
<td>0.79</td>
<td>0.14</td>
<td>1.51</td>
<td>456.9</td>
<td>4.8</td>
<td>3.2</td>
<td>6.6</td>
</tr>
<tr>
<td>Sample Variance</td>
<td>39.5</td>
<td>0.38</td>
<td>0.63</td>
<td>0.02</td>
<td>2.29</td>
<td>208762.5</td>
<td>22.8</td>
<td>10.4</td>
<td>43.1</td>
</tr>
<tr>
<td>Skewness</td>
<td>0.9</td>
<td>1.24</td>
<td>-0.08</td>
<td>-0.28</td>
<td>-0.26</td>
<td>1.6</td>
<td>0.6</td>
<td>0.8</td>
<td>-0.1</td>
</tr>
<tr>
<td>Coefficients of Variation</td>
<td>33%</td>
<td>37%</td>
<td>13%</td>
<td>13%</td>
<td>8%</td>
<td>58%</td>
<td>21%</td>
<td>15%</td>
<td>12%</td>
</tr>
</tbody>
</table>
Table 7. Omni-directional variogram of soil $\delta^{13}C$ (‰) and soil particle size (%) and omni-directional cross-variogram of the two variables.

<table>
<thead>
<tr>
<th></th>
<th>Nugget</th>
<th>Sill</th>
<th>Range (m)</th>
<th>(Sill-Nugget)/Sill</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil $\delta^{13}C$</td>
<td>0.82‰</td>
<td>1.54‰</td>
<td>7.50</td>
<td>47%</td>
</tr>
<tr>
<td>SOC</td>
<td>18.10 g C kg$^{-1}$</td>
<td>25.56 g C kg$^{-1}$</td>
<td>25.65</td>
<td>29%</td>
</tr>
<tr>
<td>Silt+clay</td>
<td>3.32%</td>
<td>44.40%</td>
<td>12.97</td>
<td>93%</td>
</tr>
<tr>
<td>Soil $\delta^{13}C$ – SOC</td>
<td>-0.36</td>
<td>-5.17</td>
<td>4.40</td>
<td>93%</td>
</tr>
<tr>
<td>Soil $\delta^{13}C$ – Silt+clay</td>
<td>0.08</td>
<td>4.20</td>
<td>12.75</td>
<td>98%</td>
</tr>
</tbody>
</table>
From the kriged maps, we found soil $\delta^{13}$C was heterogeneous (Fig. 17a). It was relatively higher in the center of the plot. The northwest and northeast corners had patches with lower values. Silt+clay was also higher in the center of the plot, while two patches in the east were found with lowest values (Fig. 17b). SOC was relatively uniform over the plot (Fig. 17c), but higher values were found in the west and lower values were on the east of the plot.

Relationships between soil $\delta^{13}$C and mesquite distribution

The PI of mesquite trees was not significantly correlated with soil $\delta^{13}$C in the grid in the drainage woodland (Table 8). Soil $\delta^{13}$C ranged from -23 to -17‰ along the two transects, and did not appear to be related to the presence or absence of mesquite trees at the sampling location (Fig. 18). Similarly, there were no significant differences between soil $\delta^{13}$C values obtained at the base, mid-canopy, or canopy edge of mesquite trees (Fig. 19).

Relationships between soil $\delta^{13}$C and soil properties

Modified T-tests corrected for spatial auto-correlation and Pearson’s correlation coefficients indicated that soil $\delta^{13}$C was significantly correlated with root biomass ($r = -0.447$, $p < 0.01$), SOC ($r = -0.568$, $p < 0.01$), and silt+clay ($r = 0.398$, $p < 0.01$). SOC was positively correlated with root biomass ($r = 0.439$, $p < 0.01$), but was not correlated with either PI or silt+clay.
Table 8. Correlation coefficients (r) for soil $\delta^{13}$C, PI, SOC, root biomass and soil particle size distribution from grid data.

<table>
<thead>
<tr>
<th></th>
<th>$\delta^{13}$C (%)</th>
<th>PI</th>
<th>Root (g m$^{-2}$)</th>
<th>SOC (g C kg$^{-1}$)</th>
<th>Slit + Clay (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\delta^{13}$C</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PI</td>
<td>-0.079</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>-0.447**</td>
<td>0.120</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOC</td>
<td>-0.568**</td>
<td>0.156</td>
<td>0.439**</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Silt + Clay</td>
<td>0.398**</td>
<td>0.074</td>
<td>-0.036</td>
<td>0.249</td>
<td>1.000</td>
</tr>
</tbody>
</table>

** p < 0.01  
* p < 0.05
Fig. 17. Kriged map of soil $\delta^{13}C$ (‰) (a), soil silt+clay content (%) (b), and SOC (g C kg$^{-1}$). Red dots represent mesquite trees.
Fig. 17. Continued
Fig. 17. Continued.
Fig. 18. Soil $\delta^{13}C$ (‰) along the two transects in the drainage woodlands. Values of X-axes are the actual distances (m). Solid black dots indicate points adjacent to mesquite tree trunks.
Fig. 19. Soil $\delta^{13}C$ (‰) values along the transects in woodlands. Base represents sampling points adjacent to mesquite tree trunks; mid represents points in the middle between the bole and the edge of the canopy; edge represents points on the edge of the canopy. Different letters above points indicate differences among means of different transect positions from Fisher’s LSD (level of significance is 0.01). Error bars are standard errors of the mean.
Using SOC, silt+clay and PI as independent variables, stepwise multiple regression revealed that SOC and silt+clay together accounted for a significant proportion of the variation ($R^2 = 0.73$, $p < 0.01$) in soil $\delta^{13}C$ values (Fig. 20). SOC accounted for 32% of the variation, while silt+clay accounted for 41% of the variation. PI was not selected in the model.

The modeled cross-variogram of soil $\delta^{13}C$ and SOC showed that the two variables were negatively spatially correlated (nugget = -0.36, sill = -5.17) over a distance of 4.40m. When the separation distance was greater than 4.40m, soil $\delta^{13}C$ and SOC were no longer spatially correlated. The spatial dependence was 93% for soil $\delta^{13}C$ and SOC. Soil $\delta^{13}C$ and silt+clay were positively spatially correlated (nugget = 0.08, sill = 4.20) over a range of 12.75m and the spatial dependence was 98% (Table 7).

From the visual observation of kriged maps (Fig. 17), we found areas with higher soil $\delta^{13}C$ values were associated with the occurrence of higher soil silt+clay content. Areas with both higher SOC and lower silt+clay content (e.g. in northwest corner) tended to have lower soil $\delta^{13}C$ values.

**DISCUSSION**

**Spatial pattern of soil $\delta^{13}C$**

The areas chosen for the study had experienced vegetation change from C4 grassland to C3 subtropical woodlands dominated by *P. glandulosa*. This grassland-to-woodland shift occurred over the past 100 years and has finished in this low-lying
Fig. 20. Effects of SOC (g C kg\(^{-1}\)) and silt+clay (%) on soil $\delta^{13}\text{C}$ (‰). Fitted surface is based on linear regression equation: $z = a + bx + cy$. X-axis is SOC; Y-axis is silt+clay; z-axis is $\delta^{13}\text{C}$. $a = -21.5$, $b = -0.20$, $c = 0.136$. $R^2 = 0.73$; $p<0.001$. 
drainage area (Archer, 1995; Boutton et al., 1998). Soil δ^{13}C values ranged from -23.83‰ to -16.24‰ and averaged -19.46‰ (Table 6), which were higher than the δ^{13}C values of the ground surface litter (-28 to -26‰) and the roots (-25 to -24‰) in this same area (Boutton et al., 1998). Results indicated that although the aboveground vegetation is currently covered by continuous-canopy C_{3} woodlands, the soil had a strong memory of C_{4} grasslands that once occupied this area. This is consistent with many previous studies in the area (Archer, 1995; Archer et al., 2001; Boutton et al., 1999; Boutton et al., 1998; Liao et al., 2006b).

The spatial pattern of soil δ^{13}C indicated soil δ^{13}C was heterogeneous although there was a visually uniform distribution of aboveground vegetation cover (Table 7, Fig. 17a). Powers (2006) and Marriott et al. (1997) found vegetation contributed to the spatial patterns of soil δ^{13}C. Biggs et al. (2002) suggested that depleted soil δ^{13}C values were associated with C_{3} mesquite in semiarid grassland with mesquite encroachment. In addition, the heterogeneity of soil δ^{13}C could be related to topography and hydrology (van Kessel et al., 1994). We believe that both plant species distribution and edaphic properties were the most important factors creating heterogeneity in soil δ^{13}C in this study. On the one hand, newer carbon inputs from the C_{3} woody plants are more ^{13}C depleted, so that areas with larger inputs from woody plants should have lower soil δ^{13}C values. One the other hand, areas where edaphic properties stabilize and protect older soil carbon derived from the original C_{4} grassland should be more ^{13}C enriched. Therefore, the magnitude of carbon inputs derived from C_{3} vs. C_{4} plants and/or edaphic
properties that control the stabilization of $C_3$ vs. $C_4$ derived likely control spatial variations of soil $\delta^{13}C$ in this woodland.

The range of soil $\delta^{13}C$ based on semivariogram analysis was 7.5m, indicating soil $\delta^{13}C$ are no longer auto-correlated if separated by a distance of more than 7.5m (Table 7). In one of the few geostatistical studies of spatial variability of soil $\delta^{13}C$, Marriott et al. (1997) found the range of soil $\delta^{13}C$ was 13.5m based on variogram models in an upland Scottish grassland. The shorter range of autocorrelation in this study indicated that woody invasion may contribute to finer grain of patchiness and more heterogeneous spatial pattern of soil $\delta^{13}C$. This increase in spatial heterogeneity of soil properties has been reported as a consequence of woody encroachment into grassland in previous studies (Asfaw and Wayne, 2006; Asner et al., 2004).

**Plant community characteristics and spatial variations of soil $\delta^{13}C$**

In a previous study in the uplands at LaCopita Research Area, I found areas with lower soil $\delta^{13}C$ values were closely associated with size, number, and proximity of nearby mesquite trees due to the important role of this species in the genesis of woody patches in the uplands. Therefore, I originally hypothesized that spatial pattern of soil $\delta^{13}C$ in lower-lying drainage woodlands would also be related to the distribution of mesquite trees. I anticipated that sample points closer to a mesquite tree or surrounded by more and/or bigger mesquite trees would have lower soil $\delta^{13}C$ values. However, in the grid, PI was not correlated with soil $\delta^{13}C$ although points with higher PI tended to have lower $\delta^{13}C$ (Table 8). Similarly, the presence of mesquite trees along the two
transects was unrelated to soil $\delta^{13}$C values (Fig. 18). There was no significant difference between soil $\delta^{13}$C values of points adjacent to mesquite tree trunks vs. those located near the edge of the canopy (Fig. 19). Hence, the distribution of mesquite trees did not seem to have a significant impact on the spatial pattern of soil $\delta^{13}$C.

One possible explanation for this phenomenon is that these lower-lying drainage woodlands may be considerably older than upland woody patches. Previous studies indicated that later successional stages of this woody encroachment may be characterized by strong asymmetric competition resulting in the demise of mesquite trees (Archer, 1995; Archer et al., 1988). Therefore, some mesquite trees within the drainage woodlands may have already died, but left a legacy on soil $\delta^{13}$C. Hence, the lack of correlation between distributions of live mesquite trees and soil $\delta^{13}$C may be related to complex patterns of mortality and recruitment that are not evident in the present distribution of individual plants on the landscape.

**Soil texture and spatial variations of soil $\delta^{13}$C**

Soil $\delta^{13}$C values of this drainage woodland averaged -19.46 ‰, which was higher than values from upland clusters (-20.96 ‰) and groves (-21.60 ‰) (see earlier chapters). I anticipated that drainage woodlands would have lower soil $\delta^{13}$C values because C$_3$ woody plant cover is more extensive and more dense there, which should result in higher C$_3$ carbon input and lower soil $\delta^{13}$C values. Liao et al. (2006b) also found soil $\delta^{13}$C values were higher in drainage woodlands than in upland clusters and groves. Higher soil silt and clay content in the drainage woodlands may be an important cause of higher
soil $\delta^{13}$C in drainage woodlands. Correlation analysis indicated that there was a positive relationship between soil silt+clay content and soil $\delta^{13}$C values ($r = 0.398$, $p<0.01$, Table 8). This is consistent with many previous studies, which have found that SOC associated with finer soil particles has higher $\delta^{13}$C (Balesdent et al., 1987; Bird and Pousai, 1997; Bird et al., 2003; Desjardins et al., 1994). In addition, soil clay content may influence water-holding capacity, nutrient status, bioturbation rates, illuviation rates, oxygen diffusivity, litter quality, root penetration resistance, and net primary productivity (Bird et al., 2003). All of these affect physical, chemical and biological processes within soils, and therefore could impact rates of organic matter input/decay and soil $\delta^{13}$C values.

Variogram analysis indicated soil silt+clay content was heterogeneous (nugget = 3.32%, sill = 44.40%, range = 12.97m, Table 7), consistent with many other studies (Davidson, 1995; Hirobe et al., 2001; Shahandeh et al., 2005; Su et al., 2006). For example, Shahandeh et al. (2005) found a strong spatial pattern for soil clay content, with a range of 48.1m in a corn cropping system in south-central Texas; Su et al. (2006) found the range of autocorrelation of soil silt+clay content was 15.2m in a sandy grassland in inner Mongolia, China.

A cross-variogram model indicated soil $\delta^{13}$C and soil clay content were positively correlated at distances < 10.1m (nugget=0.08, sill=4.20, range=12.75m, Table 7), indicating spatial variability of soil $\delta^{13}$C is dependent on spatial variability of soil clay content. The kriged map of soil $\delta^{13}$C and soil silt+clay content further illustrated
the spatial correlation of these two variables (Fig. 17a, 17b). A comparison of the two kriged maps showed a similarity, especially the two patched in the mid-north and the two patches in the mid-south.

Before woody invasion, SOC was derived from the C₄ grassland that once covered this site (Boutton et al. 1998, 1999). Following woody invasion, the original grassland carbon has been decaying out of the system, and carbon derived from C₃ woody plants has been accumulating. Thus, soil $\delta^{13}C$ values reflect a mixture of older carbon derived from grassland and newer carbon derived from the current woodland. It is well-known that fine mineral particles (silts and clays) are critical for the formation of organomineral complexes and micro- and macroaggregates, all of which stabilize soil organic matter and protect it from mineralization (Anderson and Paul, 1984; Hassink, 1997; Jastrow and Miller, 1998; Ladd et al., 1985; Liao et al., 2006b). In the same drainage woodland as this study, Liao et al. (2006b) found the mean residence time (MRT) of SOC associated with free silt+clay was 589 years, while MRT of SOC associated with silt+clay within micro- and macroaggregates was 700-1000 years. Therefore, high silt+clay content would clearly tend to favor the stabilization and persistence of older SOC derived from the original grassland, causing higher soil $\delta^{13}C$ values in those areas. The geostatistical methods employed in this study confirmed clearly the significant control of soil texture on soil $\delta^{13}C$ values in this area which undergone grassland to woodland conversion.
Abundance of SOC and spatial variations of soil $\delta^{13}C$

The abundance of SOC is controlled by the balance between C inputs from plant biomass and output through decomposition (Amundson, 2001; Amundson et al., 2003; Jenny, 1941; Schlesinger, 1977). Higher amount of SOC can be a result of more C input and/or lower decomposition rate. We found a positive correlation between SOC and root biomass ($r = 0.439$, Table 8), indicating that carbon inputs via roots may be an important determinant of SOC storage in this landscape. However, the positive relationship between SOC and silt+clay was not significant ($r = 0.249$, Table 8), suggesting the impact of soil texture on decomposition rate was not as important as carbon inputs from roots in determining SOC storage.

In areas with woody invasion, changes in amount of litter input, tissue chemical composition and root biomass accompanying the development of the woody patches may have significant influences on SOC. SOC was heterogeneous although it appeared to vary more smoothly than soil $\delta^{13}C$ and soil particle size (Nugget = 18.10 g C kg$^{-1}$, Sill = 25.56 g C kg$^{-1}$, Table 7, Fig. 17c). The range of SOC was 25.65m, indicating beyond a distance of 25.65m, SOC is no longer autocorrelated. The larger range value and weaker spatial structure of SOC compared to soil $\delta^{13}C$ may indicate SOC was influenced more by the distribution of current vegetation, while soil $\delta^{13}C$ was influenced more by soil particle size.

The negative correlation between SOC and soil $\delta^{13}C$ values (Table 8, Fig. 20) in this study indicated higher amounts of SOC were caused by accrual of carbon derived
from C₃ woody plants, consistent with numerous previous studies of woody
encroachment (Connin et al., 1997; Hibbard et al., 2001; McPherson, 1997; Scholes and
Archer, 1997). Several plausible reasons can explain the fact that spatial pattern of soil
δ¹³C values was affected by amount of SOC, but not the distribution of mesquite trees.
SOC is an integrated value representative of the organic material incorporated into the
soil and its decay with time. Dead mesquite trees as well as other C₃ shrubs and forbs
may also play a role in determining SOC and soil δ¹³C. Other factors such as
topographic and hydraulic redistribution of soil may also affect the spatial variability of
SOC and soil δ¹³C (Burke et al., 1999).

CONCLUSIONS

This study indicated that soil particle size distribution and SOC storage played
important roles in determining spatial patterns of soil δ¹³C in this subtropical woodland
in the Rio Grande Plains of southern Texas. Multiple regression revealed that silt+clay
content and SOC together accounted for 73% of the variance in soil δ¹³C values.
Silt+clay explained a larger proportion of variance (41%) compared with SOC (32%).
The positive correlation between silt+clay and soil δ¹³C reflects the important role of
those fine particle size fractions in the formation of organomineral complexes and
micro- and macroaggregates which stabilize and protect SOC derived from the C₄
grasslands that dominated this site approximately 100 yrs ago. The negative correlation
between SOC and soil δ¹³C indicates that on those portions of the landscape with high
SOC, a significant proportion of that SOC is derived from the invading C₃ woody plants.
The spatial pattern of soil $\delta^{13}C$ values was not influenced by the distribution of mesquite trees, which are the dominant invading woody plants. The results have implications for the interpretation of soil $\delta^{13}C$ values that are being used to evaluate vegetation change at the landscape-scale.
CHAPTER V

SPATIAL VARIATION OF $\delta^{15}$N OF WOODY PLANTS ALONG A TOPOEDAPHIC GRADIENT IN A SUBTROPICAL SAVANNA PARKLAND LANDSCAPE

SYNOPSIS

Variation in the nitrogen (N) isotopic composition of plants and soils affords an important approach for understanding N cycling and the influence of environmental factors on the N cycle. In this study, $\delta^{15}$N values of leaves of three common woody species with different growth forms were determined along a topoedaphic gradient in a subtropical savanna ecosystem in southern Texas, USA. Leaf $\delta^{15}$N and N concentration ([N]) were measured in April and August 2005 on all individuals of *Prosopis glandulosa* (deeply-rooted summerrgreen legume), *Condalia hookeri* (facultative wintergreen non-legume), and *Zanthoxylum fagara* (shallow-rooted wintergreen non-legume) present within a belt transect 308 m long x 12 m wide. Soil gravimetric water content (GWC), total N and $\delta^{15}$N were also measured along the transect. At the landscape-scale, changes in GWC and soil [N] along the topoedaphic gradient appeared to contribute to spatial patterns of leaf $\delta^{15}$N. Leaf $\delta^{15}$N was negatively related to elevation for all the three plant species. In lower portions of the landscape, greater soil [N] and GWC are associated with higher rates of both N-mineralization and nitrification, resulting in a more open N cycle and plants with relatively high $\delta^{15}$N values. Both soil [N] and soil $\delta^{15}$N were
positively correlated with leaf $\delta^{15}N$ of *C. hookeri* and *Z. fagara* in April and August. These relationships did not occur for *P. glandulosa*, probably because it is capable of symbiotic N$_2$-fixation and less dependent on soil N sources. Consequently, leaf $\delta^{15}N$ values of *P. glandulosa* were closer to atmospheric N$_2$ and significantly lower than those of *C. hookeri* and *Z. fagara*. At smaller spatial scales, a proximity index (which reflected the density and distance of surrounding *P. glandulosa* trees) was negatively correlated with leaf $\delta^{15}N$ values of *C. hookeri* and *Z. fagara*, indicating that the N$_2$-fixing *P. glandulosa* may be important to the N nutrition of nearby non-N$_2$-fixing species. Our results indicate plant $^{15}N$ natural abundance can reflect the extent of N retention and help us better understand the N dynamics and plant-soil interaction in the ecosystem.

**INTRODUCTION**

The natural abundances of the light stable isotopes have been used as nonradioactive and nondestructive integrators of ecological processes (Boutton and Yamasaki, 1996; Handley and Raven, 1992; Hogberg, 1997; Robinson, 2001). They reflect the outcome of physical, chemical, and biological reactions that fractionate stable isotopes, and often record information about pools and fluxes in biogeochemical cycles (Peterson and Fry, 1987). $^{15}N$ natural abundance has been studied in an attempt to understand patterns of N cycling (Amundson et al., 2003; Dijkstra et al., 2003; Hobbie and Hobbie, 2006; Hogberg, 1997; Hopkins et al., 1998; Robinson, 2001). Nitrogen isotope fractionation occurs during nitrogen transformations mediated by microbes, such as mineralization, nitrification, denitrification, N$_2$ fixation, and immobilization (Högberg
et al., 1996; Kawanashi et al., 1993; Shearer and Kohl, 1986). During these processes, abiotic factors or interactions between abiotic and biotic factors could cause variation in nitrogen isotope fractionation. Due to this complexity, our understanding of the factors that control the 15N natural abundance in natural systems is incomplete.

$\delta^{15}N$ values are often related to the “openness” (rates of N input/output vs. internal N cycling) of the N cycle, which reflects the extent of N retention within an ecosystem over time (Austin and Vitousek, 1998). When the N cycle is more open, the accumulated losses of N are relatively large. Because nearly all N transformations lead to products that are depleted in 15N relative to the substrates (Peterson and Fry, 1987; Yoneyama, 1996), these N-losses leave the residual N more enriched in 15N (Nadelhoffer and Fry, 1994). Soil $\delta^{15}N$ values in most ecosystems are generally positive due to the cumulative loss of 15N-depleted N. Leaf $\delta^{15}N$ is often lower than soil values due to the uptake of 15N-depleted inorganic N. Therefore, while there is often uncertainty about the interpretation of $\delta^{15}N$ variations in nature, robust spatial patterns of plant and soil $\delta^{15}N$ exist at local to global scales (Robinson, 2001).

At biome to global scales, because the subtleties of N metabolism and N cycling can be ignored, plant and soil $\delta^{15}N$ mainly reflect mean annual precipitation (MAP) (Amundson et al., 2003; Robinson, 2001). With the increase of MAP, site-averaged plant $\delta^{15}N$ values were found to decrease significantly (Austin and Vitousek, 1998; Handley et al., 1999; Sah et al., 2006; Schuur and Matson, 2001). The fact that $\delta^{15}N$ is higher in arid and semiarid zones indicates the N cycle is more open and N losses
relative to pool sizes are greater in those regions. However, Martinelli et al. (1999) found that tropical forests were commonly enriched in $^{15}$N relative to temperate forests. They attributed the differences to a more open N cycle in tropical forests where N functions as an excess nutrient.

At ecosystem to landscape scales, topography appears to be a major influence on soil and plant $\delta^{15}$N due to its impacts on soil moisture availability (Garten and van Miegroet, 1994) and soil nutrient availability (Schmidt and Stewart, 2003). In topographic depressions or lower-lying landscape positions where soil moisture tends to be higher, soil $\delta^{15}$N values are generally higher, than in more upland portions of the landscape (Sutherland et al., 1993). This is probably because rates of denitrification and other N-losing transformations are higher and the N cycle is more open on those portions of the landscape (Farrell et al., 1996; Garten and van Miegroet, 1994). Similarly, soil nutrients (notably N and P) have been reported to be positively related to soil and leaf $\delta^{15}$N (Garten and van Miegroet, 1994; McKee et al., 2002; Schmidt and Stewart, 2003) because sites with higher soil nutrient concentrations usually have higher rates of N-transforming processes (Booth et al., 2005) that result in N-losses and $^{15}$N-enrichment of the residual ecosystem N. Disturbances and land use activities, such as fire (Cook, 2001; Handley et al., 1999), grazing (Schulze et al., 1999), forest production (Chang and Handley, 2000), and fertilization (Choi et al., 2003; McKee et al., 2002) are also known to influence spatial variation of plant $\delta^{15}$N at ecosystem and landscape scales.
At the scale of the individual plant, mycorrhizal symbioses (Michelsen et al., 1998; Schmidt and Stewart, 2003) and N$_2$-fixation (Schulze et al., 1991; Virginia and Delwiche, 1982) have been found to be related to plant $\delta^{15}$N. Plants associated with mycorrhizal fungi usually have lower $\delta^{15}$N than those non-mycorrhizal species because discrimination against $^{15}$N occurs during fungal N assimilation (Hobbie et al., 2000; Högberg et al., 1996). N$_2$-fixers have been reported to have both lower leaf $\delta^{15}$N and greater leaf [N] compared to non-fixing species because the $\delta^{15}$N of atmospheric N$_2$ (0 ‰) is usually lower than soil-derived N (Schmidt and Stewart, 2003; Schulze et al., 1991; Schulze et al., 1998). In addition, the depth(s) in soil from which N is acquired and the form(s) of soil-N used (organic N, NH$_4^+$, NO$_3^-$) may also affect plant $\delta^{15}$N (Hogberg, 1997).

In the Rio Grande Plains of southern Texas, subtropical woodlands dominated by N$_2$-fixing tree legumes have become significant components of landscapes that were once almost exclusively dominated by C$_4$ grasslands (Archer et al., 2001; Boutton et al., 1998). Previous studies have suggested that this vegetation change was driven largely by livestock grazing and reduced fire frequency, which enabled the establishment of honey mesquite (Prosopis glandulosa Torr. var. glandulosa), an unpalatable, stress-tolerant, evergreen N$_2$-fixing tree legume. As the P. glandulosa plants grow, they serve as recruitment foci, facilitating the establishment of subordinate woody species from other habitats (Archer, 1995; Archer et al., 1988), resulting in the development of subtropical thorn woodland. This dramatic vegetation change likely has a significant impact on nitrogen cycling processes because it alters: (a) rates of N-input to the
ecosystem through the fixation process (Zitzer et al., 1996), (b) the quantity and quality of organic matter inputs to the soil (Liao et al., 2006b), and (c) rates of nitrogen transformations such as ammonification, nitrification, denitrification, and NO flux (Archer et al., 2001; Hibbard et al., 2001; McCulley et al., 2004). These dramatic changes in nitrogen cycling processes have likely left an imprint on the pattern of δ15N in plants and soils in this landscape.

The objectives of this study were to: (1) Document spatial variation in leaf δ15N of three dominant woody species along a topographic gradient in a subtropical savanna: a deeply rooted, summergreen arborescent legume (*P. glandulosa*), a facultative wintergreen non-legume [*Condalia hookeri* M.C. Johnst. (bluewood)], and a shallow-rooted, evergreen non-legume [*Zanthoxylum fagara* (L.) Sarg. (lime prickly ash)]; (2) Describe seasonal variation in leaf δ15N of these species; and (3) Identify the factors that may be controlling spatial and seasonal variation in leaf δ15N at the landscape-scale.

**MATERIALS AND METHODS**

**Study area**

Research was conducted at the Texas Agricultural Experiment Station La Copita Research Area (27° 40’ N; 98° 12’ W; elevation 80m) in Jim Wells County, 15 km SW of Alice, TX in the eastern Rio Grande Plains of the Tamaulipan Biotic Province. The climate of the region 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;
July and August are typically the warmest, driest months of the year.

Landscapes at the La Copita typically grade (1-3% slopes) from sandy loam uplands to clay loam and clay lowlands which receive runoff from the uplands, and may have standing water after large rainfall events. Upland 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). Upland vegetation is savanna parkland consisting of discrete woody clusters (comprised of a single *P. glandulosa* trees with up to 15 understory tree/shrub species, 3–10 m diameter) and larger groves (comprised of several woody clusters that have fused together, 10 to >20 m diameter) embedded within a matrix of grassland vegetation. Upland grasslands consist mainly of rhizomatous and weakly caespitose C_{4} grasses, and C_{3} forbs. Clusters and groves are dominated by *P. glandulosa* and *Z. fagara*. *P. glandulosa* and several other leguminous trees and shrubs present in wooded areas are capable of symbiotic N_{2}-fixation (Zitzer et al., 1996).

Soils in lower-lying drainage woodlands are generally Pachic Argiustolls and are covered by closed-canopy woodlands similar in composition to upland clusters and groves. Playas are closed-basin depressions that occur within the drainage woodlands, and occupy the lowest portions of the landscape. Playa soils are Ustic Epiaquerts and Vertic Argiaquolls, and vegetation there consists of a continuous grass layer dominated by *Paspalum pubiflorum* and *Bothriochloa ischaemum*, with *P. glandulosa* and *Acacia farnesiana* trees of variable density (Farley, 2000).
Woody plant encroachment has occurred throughout the study area over the past 75-100 years due to the interaction of heavy livestock grazing and reduced fire frequency (Archer, 1995). Archer (1995), and Boutton et al. (1998) have additional details on soils, vegetation, and climate.

**Transect description**

A 308m transect spanning a hillslope gradient from an upland through a drainage woodland and into a playa was established (Fig. 21). All five of the major landscape elements at the study site (grasslands, clusters, groves, drainage woodlands, and playa) occurred along the transect. The transect was marked with steel posts at 1 m intervals, and georeferenced by Trimble GPS pathfinder Pro XRS system (Trimble Navigation Limited, Sunnyvale, CA) at 5 m intervals. Elevations were determined by surveying, and ranged from 86.5 m in the upland to 84.5 m in the playa.

**Plant and soil sampling and analysis**

Soils (0-15 cm) along the transect were sampled in April, 2005. Two soil cores were collected at 1 m intervals along the transect. One core was used to determine soil particle size distribution (pipet method; Gee and Bauder, 1986). The other soil core was passed through a 2 mm sieve to remove coarse organic fragments and gravel, dried at 60°C for 48 hours, ground in a TE-250 ring pulverizer (Angstrom, Inc., Belleville, MI), and saved for elemental and isotopic analyses.

At the same time, another set of soil cores were collected at 3 m intervals. The
Fig. 21. Locations of individual plants and along the transect. Three woody species *Prosopis glandulosa*, *Condalia hookeri*, and *Zanthoxylum fagara* were sampled along a 308m long, 12m wide (6m each side of the transect) belt. The enlarged section shows the details of a small portion of the belt.
wet weight of each core was measured and then the soil cores were dried in an oven at 105°C overnight and re-weighed. Soil gravimetric water content (GWC) was calculated by dividing soil water content by soil dry weight. Values of GWC were converted to volumetric water content (VWC). Plant-available VWC was computed from total VWC and soil texture using the equations of Cosby et al. (1984), assuming a matric potential at wilting point of -10.0 MPa. Soil water content was not quantified in August 2005 because significant rainfall occurred the day prior to plant tissue sampling; hence, soil moisture would not have reflected the antecedent soil moisture conditions that would have influenced our plant response variables in August.

Three common woody species were selected for isotopic analyses: (1) *P. glandulosa* (Fabaceae), (2) *C. hookeri* (Rhamnaceae), and (3) *Z. fagara* (Rutaceae). *P. glandulosa* is a deep-rooted deciduous tree capable of symbiotic N\(_2\)-fixation (Zitzer et al. 1996), and is the dominant overstory species in all woody assemblages at this site. *C. hookeri* and *Z. fagara* are spinescent shrubs that dominate the understory in all wooded areas. Leaf tissue was sampled from all individuals of these three species occurring within 6 m on either side of the 308 m transect in April and August, 2005. For *P. glandulosa*, approximately 10 g of fully-expanded, south-facing, sun-lit leaves in the upper canopy were sampled. For *C. hookeri* and *Z. fagara* approximately 10 g of fully-expanded, south-facing leaves located in the upper canopy approximately 1-2 m above ground level were sampled. The spatial coordinates of each plant sampled within the 12 m x 308 m area were recorded by measuring the distance along and perpendicular to the
central transect line. All sampled individuals were mapped using ArcView GIS Spatial Analyst (ESRI, 1998) (Fig. 21). Leaves were oven-dried for 48 hours at 60°C and pulverized in a TE-250 ring pulverizer (Angstrom, Inc., Belleville, MI).

$\delta^{15}N$ and nitrogen concentrations of plant tissues and soils were determined using a Carlo Erba EA-1108 (CE Elantech, Lakewood, NJ) interfaced with a Delta Plus (ThermoFinnigan, San Jose, CA) isotope ratio mass spectrometer operating in continuous flow mode. Nitrogen isotope ratios are presented in $\delta$ notation:

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

where $R_{SAMPLE}$ is the $^{15}N/^{14}N$ ratio of the sample and $R_{STD}$ is the $^{15}N/^{14}N$ ratio of the atmospheric dinitrogen (Mariotti, 1983). Precision of duplicate measurements was <0.2 ‰.

**Statistical analyses and modeling**

A proximity index (PI) was originally developed by Gustafson and Parker (1992) to measure the potential influence of surrounding patches in a neighborhood on a focal patch. Ghazoul et al. (1998) used a variation of PI to measure the relative isolation of flowering *Shorea siamensis* trees to study the effect of cross-pollination on reproductive success. We used this modified PI to evaluate the influence of *P. glandulosa* trees on leaf $\delta^{15}N$ values of associated shrub species (*C. hookeri* and *Z. fagara*):

$$PI = \sum_{i=1}^{n} 1/Z_i \quad (11)$$
where $Z_i$ is the distance in meters from the shrub to each surrounding $P. glandulosa$ tree. This index considers both the number of surrounding $P. glandulosa$ trees and their distances, weighted in favor of the nearest neighbor. PI is larger when the shrub is surrounded by closer and/or more $P. glandulosa$ and decreases as $P. glandulosa$ become more distant and/or sparser. By iterative solution of the relationship between PI and plant $\delta^{15}N$, we found that $P. glandulosa$ trees >12 m from $C. hookeri$ or >6 m from $Z. fagara$ had no influence on the $\delta^{15}N$ values of those species. Thus, PI was based on a range of 12 m for $C. hookeri$, and 6 m for $Z. fagara$.

A general linear model univariate ANOVA was used to test for the differences in leaf $\delta^{15}N$ with respect to landscape location, sampling seasons, three species and their interactions. Fisher’s LSD method was used to distinguish differences following ANOVA using SPSS 10.0 program (SPSS Inc. Chicago, IL). Regression analyses were performed using Sigma Plot 8.0 (SPSS Inc., 2002) in an effort to predict leaf $\delta^{15}N$ from leaf N, soil N, soil $\delta^{15}N$, soil available water.

**RESULTS**

**Temperature and rainfall during study period**

Average monthly temperatures during February (17.1 °C), March (19.1 °C), and April (22.8 °C) of 2005 were above the 110 year mean at Alice, TX (Fig. 22). Monthly rainfall in both February (64.3 mm) and March (62.2 mm) was approximately 60%
above the 110 year mean for those months; however, rainfall during April (2.54 mm) was only 6% of 110 year mean.

Average monthly temperatures during May, June, July and August of 2005 were 26.2°C, 29.4°C, 30.7°C, and 31.4°C respectively (Fig. 22). These temperatures were all above the 110 year mean for those months. Monthly rainfall during the same period was 41.4 mm (May), 3.3 mm (June), 64.8 mm (July) and 8.6 mm (August), which was either dramatically less than or comparable to the 110 year mean for those months. Hence, conditions prior to the April foliage sampling were comparatively warm and mesic, while those preceding the August foliage sampling were comparatively hot and dry.

Elevation, soil texture, and soil water content

The elevation of the transect ranged from 84.5 m to 86.5 m (Fig. 23A). Soil clay content increased as elevation decreased along the transect, with values of approximately 100 g kg⁻¹ in the uplands, 100-200 g kg⁻¹ in the drainage woodlands, and 250-350 g kg⁻¹ in the playas (Fig. 23B). Both soil volumetric water content (VWC) and plant available VWC were related to elevation and texture, with the highest values found in the playa (plant available VWC = 0.10 m³ m⁻³) and lowest values in the uplands (plant available VWC = 0.01 m³ m⁻³) (Fig. 23C). Plant available VWC was always significantly less than the total VWC, especially in the playa where clay content was highest.

Elemental and isotopic composition of plants and soils

Soil total N was related to both elevation along the transect and vegetation cover,
Fig. 22. Monthly mean temperature (°C) and rainfall (mm) of NOAA weather station at Alice, TX (www.ncdc.noaa.gov). Solid line and darker bars represent the monthly mean temperature and rainfall of the year of sampling (2005), while dashed line and lighter bars represent 110-year-mean.
Fig. 23. Changes in elevation (m) (A), soil particle size distribution (g kg\(^{-1}\)) (B) and soil volumetric water content and soil available water (m\(^3\) m\(^{-3}\)) (C) along the transect. Dashed lines in A indicate locations of different landscape elements along the transect.
with higher values in the lower-lying drainage woodland and playa (1-5 g N kg\(^{-1}\) soil) compared to uplands (0.6-2 g N kg\(^{-1}\) soil), and higher values under all woody vegetation types (1-5 g N kg\(^{-1}\) soil) than under grassland (0.6 g N kg\(^{-1}\) soil) (Fig. 24A). Soil \(\delta^{15}N\) ranged from 4.5‰ to 8.3‰ and was consistently higher than leaf \(\delta^{15}N\) values along the entire transect (Fig. 24).

Leaf \(\delta^{15}N\) values generally tracked those of the soil. Leaf \(\delta^{15}N\) values of \textit{P. glandulosa} ranged from -0.2‰ to 5.4‰ in April and -1.5‰ to 4.2‰ in August, and were consistently lower than those of the other two plant species. Leaf \(\delta^{15}N\) values of \textit{C. hookeri} ranged from 0.7‰ to 3.9‰ in April and 0.7‰ to 4.2‰ in August, while those of \textit{Z. fagara} ranged from 0.8‰ to 6.3‰ in April and 1.5‰ to 3.9‰ in August (Fig. 24B, 24C).

**The effects of species, landscape locations, sampling seasons and their interactions on plant \(\delta^{15}N\)**

Species and landscape location had significant main effects on leaf \(\delta^{15}N\) values of woody plants. Leaf \(\delta^{15}N\) values of \textit{P. glandulosa} averaged 1.01‰, which was significantly lower than those for \textit{C. hookeri} (1.96‰) and \textit{Z. fagara} (3.65‰). Mean leaf \(\delta^{15}N\) values in uplands were 2.01‰, and increased to 2.35‰ in drainage woodlands and 2.40‰ in playas (Table 9). While sampling season did not have a significant effect on leaf \(\delta^{15}N\), the interaction between species and season affected leaf \(\delta^{15}N\) significantly (\(p<0.001\), Table 9). This interaction reflects the fact that leaf \(\delta^{15}N\) tends to decrease in \textit{P. glandulosa} from April (1.30‰) to August (0.71‰), while increasing in \textit{C. hookeri}
Soil δ¹⁵N (‰) (0/00)

<table>
<thead>
<tr>
<th>Transect position (m)</th>
<th>Soil [N] (g N kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>10</td>
</tr>
</tbody>
</table>

Leaf δ¹⁵N (‰)

P. glandulosa
C. hookeri
Z. fagara

Upland
Grove
Grassland
Grove
Grassland
Drainage woodland
Lowland
Playa
Drainage woodland

Fig. 24. Soil total N (g N kg⁻¹) and δ¹⁵N (‰) (A), and leaf δ¹⁵N (‰) of three woody species (Prosopis glandulosa, Condalia hookeri, and Zanthoxylum fagara) along the transect in April (B) and August (C), 2005.
(1.76‰ in April to 2.16‰ in August) and Z. fagara (3.44‰ in April to 3.86‰ in August). The interaction between species and landscape locations also had a significant effect on leaf $\delta^{15}$N ($p=0.005$, Table 9), suggesting the species behaved differently in different landscape locations. Leaf $\delta^{15}$N values of P. glandulosa were not significantly different at different landscape location. Leaf $\delta^{15}$N values of C. hookeri averaged at 1.73‰ in uplands and 2.19‰ in drainage woodlands. Leaf $\delta^{15}$N values of Z. fagara also increased significantly from 3.01‰ in uplands to 3.92‰ in drainage woodlands and 4.03‰ in playas (Table 9). Therefore, follow-up regression analyses were performed separately for different species to determine the variation of leaf $\delta^{15}$N in response to environmental variables across the landscape.

**Effects of elevation on elemental and plant isotopic composition**

Both soil N ($r = -0.543$, $p<0.01$) and soil GWC ($r = -0.554$, $p<0.01$) increased as elevation decreased along the transect (Table 10). Elevation was not correlated with leaf $\delta^{15}$N for P. glandulosa (Table 10). However, for C. hookeri and Z. fagara, there was a significant negative relationship between elevation and leaf $\delta^{15}$N values in both April and August sample periods (Table 10). There was a significant negative correlation between elevation and leaf $[N]$ for Z. fagara in both April ($r = -0.578$) and August ($r = -0.514$) (Table 10). Although there was also a significant negative correlation between leaf $[N]$ and elevation for P. glandulosa ($r = -0.364$) and C. hookeri ($r = -0.420$) in April, this relationship was not significant for these two species in August (Table 10).
Table 9. Leaf $\delta^{15}$N (‰) in different species (S), landscape locations (L) and sample periods (P). Different letters indicate differences between means (Fisher’s LSD analysis; $\alpha = 0.05$). Since the interactions for L × P, and S × P × L were not significant, detailed comparisons are omitted.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Significance</th>
<th>Categories</th>
<th>Mean</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species (S)</td>
<td>0.000</td>
<td><em>Prosopis glandulosa</em></td>
<td>1.006 $^a$</td>
<td>0.112</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Condalia hookeri</em></td>
<td>1.956 $^b$</td>
<td>0.106</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Zanthoxylum fagara</em></td>
<td>3.652 $^c$</td>
<td>0.092</td>
</tr>
<tr>
<td>Landscape location (L)</td>
<td>0.001</td>
<td>Upland</td>
<td>2.014 $^a$</td>
<td>0.083</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drainage woodland</td>
<td>2.347 $^b$</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Playa</td>
<td>2.400 $^{ab}$</td>
<td>0.185</td>
</tr>
<tr>
<td>Sample period (P)</td>
<td>0.595</td>
<td>April</td>
<td>2.218 $^a$</td>
<td>0.085</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>2.253 $^a$</td>
<td>0.086</td>
</tr>
<tr>
<td>S × L</td>
<td>0.005</td>
<td><em>P. glandulosa</em> Upland</td>
<td>1.036 $^a$</td>
<td>0.141</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drainage</td>
<td>0.940 $^a$</td>
<td>0.120</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Playa</td>
<td>0.771 $^a$</td>
<td>0.279</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. hookeri</em> Upland</td>
<td>1.726 $^a$</td>
<td>0.180</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drainage</td>
<td>2.186 $^b$</td>
<td>0.111</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Playa</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Z. fagara</em> Upland</td>
<td>3.011 $^a$</td>
<td>0.098</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Drainage</td>
<td>3.916 $^b$</td>
<td>0.087</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Playa</td>
<td>4.029 $^b$</td>
<td>0.242</td>
</tr>
<tr>
<td>S × P</td>
<td>0.000</td>
<td><em>P. glandulosa</em> April</td>
<td>1.302 $^a$</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>0.709 $^b$</td>
<td>0.158</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. hookeri</em> April</td>
<td>1.757 $^a$</td>
<td>0.148</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>2.155 $^b$</td>
<td>0.151</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Z. fagara</em> April</td>
<td>3.441 $^a$</td>
<td>0.130</td>
</tr>
<tr>
<td></td>
<td></td>
<td>August</td>
<td>3.863 $^b$</td>
<td>0.130</td>
</tr>
<tr>
<td>L × P</td>
<td>0.850</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>S × L × P</td>
<td>0.771</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>
Table 10. Pearson’s correlation coefficients between elevation vs. transect properties, and elevation vs. plant properties.

<table>
<thead>
<tr>
<th></th>
<th>Transect properties</th>
<th></th>
<th>Plant properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Elevation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>April</td>
<td>August</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transect properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil GWC</td>
<td>-0.554**</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soil total N</td>
<td>-0.543**</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plant properties</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf $\delta^{15}$N</td>
<td>$\text{Prosopis glandulosa}$</td>
<td>0.224</td>
<td>0.115</td>
<td>$\text{Condalia hookeri}$</td>
</tr>
<tr>
<td></td>
<td>$\text{Zanthoxylum fagara}$</td>
<td>-0.599**</td>
<td>-0.508**</td>
<td></td>
</tr>
<tr>
<td>Leaf N</td>
<td>$\text{Prosopis glandulosa}$</td>
<td>-0.364**</td>
<td>-0.132</td>
<td>$\text{Condalia hookeri}$</td>
</tr>
<tr>
<td></td>
<td>$\text{Zanthoxylum fagara}$</td>
<td>-0.578**</td>
<td>-0.514**</td>
<td></td>
</tr>
</tbody>
</table>

** $p < 0.01$
* $p < 0.05$
Relationships between plant $\delta^{15}\text{N}$ and environmental variables

Soil gravimetric water content (GWC) showed a significantly positive relationship with leaf $\delta^{15}\text{N}$ for *C. hookeri* and *Z. fagara* ($r^2 = 0.25$ and $0.23$ respectively); however, this relationship was absent for *P. glandulosa* (Fig. 25A, Table 11). Soil total N was positively correlated with leaf $\delta^{15}\text{N}$ for *Z. fagara* and *C. hookeri* (Fig. 25B, Table 11). Although the same trend was evident for *P. glandulosa*, the relationship was not significant (Fig. 25B, Table 11). Soil $\delta^{15}\text{N}$ was positively related to leaf $\delta^{15}\text{N}$ for all three species (Fig. 25C, Table 11).

Leaf $\delta^{15}\text{N}$ was positively correlated with leaf [N] for all three species (Fig. 25D, Table 11). However this relationship was not significant for *P. glandulosa*. Leaf [N] was consistently higher in *P. glandulosa* than in the other two non-N$_2$-fixing plants (Fig. 25D).

In an effort to further explain variation in plant $\delta^{15}\text{N}$ values found in this present study, rates of nitrification and concentrations of soil NH$_4^+$-N and NO$_3^-$-N from a previous study (McCulley et al., 2004) were examined. McCulley et al. (2004) found that these parameters were all higher in wooded areas than in grassland, and were higher in drainage woodlands than in upland clusters and groves (data not shown). When mean $\delta^{15}\text{N}$ values for each plant species are plotted with respect to patch-specific rates of nitrification and concentrations of soil inorganic N, positive relationships are obtained (Fig. 26) for both April and August 2005.
Fig. 25. Relationships between leaf δ^{15}N (‰) and soil GWC (mg mg^{-1}) (A), soil N concentration (g N kg^{-1}) (B), and soil δ^{15}N (‰) (C) for *P. glandulosa*, *C. hookeri*, and *Z. fagara* in April and relationships between leaf δ^{15}N and leaf [N] of all the three species in April (solid dots and lines) and August (hollow dots and dashed lines) (D). Regression statistics are in Table 11.
Table 11. Regression parameters for changes in leaf $\delta^{15}$N with soil N concentration (g N kg$^{-1}$), soil $\delta^{15}$N (‰), leaf [N] (g N kg$^{-1}$), and soil GWC (mg mg$^{-1}$). (symbols: $b_0$ intercept, $b_i$ slope, F calculated F-ratio. Regression equations are of the form $y = b_0 + b_i(x)$.

<table>
<thead>
<tr>
<th>Regression</th>
<th>April, 2005</th>
<th>August, 2005</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species</td>
<td>$b_0$</td>
</tr>
<tr>
<td>Leaf $\delta^{15}$N × soil GWC</td>
<td>P. glandulosa</td>
<td>1.45</td>
</tr>
<tr>
<td></td>
<td>C. hookeri</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>Z. fagara</td>
<td>2.27</td>
</tr>
<tr>
<td>Leaf $\delta^{15}$N × Soil N</td>
<td>P. glandulosa</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>C. hookeri</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>Z. fagara</td>
<td>2.50</td>
</tr>
<tr>
<td>Leaf $\delta^{15}$N × Soil $\delta^{15}$N</td>
<td>P. glandulosa</td>
<td>-1.70</td>
</tr>
<tr>
<td></td>
<td>C. hookeri</td>
<td>-2.26</td>
</tr>
<tr>
<td></td>
<td>Z. fagara</td>
<td>-1.17</td>
</tr>
<tr>
<td>Leaf $\delta^{15}$N × Leaf N</td>
<td>P. glandulosa</td>
<td>-0.83</td>
</tr>
<tr>
<td></td>
<td>C. hookeri</td>
<td>-1.55</td>
</tr>
<tr>
<td></td>
<td>Z. fagara</td>
<td>-0.58</td>
</tr>
</tbody>
</table>

* p<0.01
** p<0.001
Fig. 26. Relationships between leaf $\delta^{15}$N of *P. glandulosa*, *C. hookeri*, and *Z. fagara* and soil NH$_4^+$-N (mg N kg$^{-1}$), soil NO$_3^-$-N (mg N kg$^{-1}$), and nitrification rates (mg N kg$^{-1}$d$^{-1}$). Data were measured in both April (solid symbols) and August (hollow symbols) of 2005. Soil nitrification rates, soil NH$_4^+$-N, and NO$_3^-$-N data were obtained from (McCulley et al., 2004).
The proximity index (PI) was used to evaluate the potential influence of the N$_2$-fixer *P. glandulosa* on the $\delta^{15}$N values of the other two woody species. This index accounts for both the number of surrounding *P. glandulosa* trees and their distances, weighted in favor of the nearest neighbor. Therefore, higher PI corresponds to more and/or closer neighbor *P. glandulosa* trees to the sampled species. Results showed that as PI increased, leaf $\delta^{15}$N of both *C. hookeri* and *Z. fagara* decreased significantly ($r^2=0.21$ and 0.22 respectively) (Fig. 27).

**DISCUSSION**

**Landscape variations in plant $\delta^{15}$N**

Topography exerts strong control over soil water and nitrogen (Jenny, 1980; Ruess and Seagle, 1994; Schimel et al., 1985; Turner et al., 1997), and has been reported to influence plant $\delta^{15}$N at the landscape scale due to its impacts on soil moisture, nutrient availability, and rates of N-cycling processes (Garten, 1993; Garten and van Miegroet, 1994; Schmidt and Stewart, 2003; Sutherland et al., 1993). Along a topoedaphic gradient, we found that both soil water and soil total N increased as elevation decreased from dry sandy loam uplands to more mesic clay loam and clay lowlands. In addition, elevation was negatively correlated with leaf $\delta^{15}$N values of *C. hookeri* and *Z. fagara*, and both soil water content and soil total N were positively correlated with leaf $\delta^{15}$N values of these two non-N$_2$-fixing species. Although soil water availability has been shown to be negatively correlated with leaf $\delta^{15}$N at continental and global scales (Austin and Sala, 1999; Handley et al., 1999), positive correlations between soil water
Fig. 27. Leaf $\delta^{15}$N (‰) of *C. hookeri* (upper panel) and *Z. fagara* (lower panel) along the proximity index gradient. The proximity index (PI) is a measure of isolation of the sampled plant to neighboring *P. glandulosa*. Thus, high PI means *P. glandulosa* neighbors are closer to the sampled plant and/or denser near the sampled plant.
availability and leaf $\delta^{15}$N have been found at the landscape-scale (Dijkstra et al., 2003; Farrell et al., 1996; Garten and van Miegroet, 1994). Because soil water and soil total N are major determinants of decomposition and N-transformation rates (Booth et al., 2005; Matson and Vitousek, 1981), landscape positions with higher soil water content may have more microbial activity and higher N-transformation rates, resulting in a more open N cycle with greater potential for losses of $^{15}$N-depleted N via volatilization or leaching, and therefore higher residual $\delta^{15}$N values in plants and soils (Garten and van Miegroet, 1994). Several other studies have documented a positive relationship between soil N concentrations and leaf $\delta^{15}$N. Garten (1993) found higher leaf $\delta^{15}$N was associated with greater soil N availability, higher net nitrification, and mineralization potential in valley bottoms of an eastern deciduous forest in Tennessee. Garten and Van Miegroet (1994) found leaf $\delta^{15}$N values were positive correlated with soil N availability, net mineralization and net nitrification rate in evergreens, maples and ferns. Sah and Brumme (2003) found soil N poor sites were associated with more negative soil and leaf $\delta^{15}$N in a pine forest in Nepal. Pardo et al. (2006) found a significantly positive relationship between leaf $\delta^{15}$N of many forest tree species and nitrification rates. They also found site-averaged leaf $\delta^{15}$N was strongly related to nitrification, mineralization rates and forest floor $\delta^{15}$N of many sites in northeastern USA.

In our study area, McCulley et al. (2004) found that potential nitrifications rates and soil pool sizes of $\text{NH}_4^+$-N and $\text{NO}_3^-$-N increased in the sequence clusters $<$ groves $<$ drainage woodlands. The process of nitrification results in losses of N as $\text{N}_2\text{O}$ and $\text{NO}$, and $\text{NH}_4^+$ and $\text{NO}_3^-$ represent forms of N that could potentially be lost from the soil via
ammonia volatilization, nitrification, and/or denitrification (Firestone and Davidson, 1989). Thus, we might infer that the potential for N losses and the δ^{15}N of residual soil N would also increase in the sequence clusters < groves < drainage woodlands. When we plot leaf δ^{15}N values against potential nitrification and soil NH_{4}^{+} and NO_{3}^{-} measured by McCulley et al. (2004), it is evident that δ^{15}N values of all three plant species increase as these proxies for N-loss potential also increase (Fig. 26). Thus, our results appear to be consistent with prior assessments of landscape-scale variation in plant δ^{15}N.

Small-scale variation in plant δ^{15}N: Influence of the N_{2}-fixer P. glandulosa on neighboring plants

In our study area, P. glandulosa develops active root nodules capable of symbiotic N_{2}-fixation (Zitzer et al., 1996). In the present study, P. glandulosa had consistently lower leaf δ^{15}N values and higher leaf [N] than that the non-N_{2} fixing species C. hookeri and Z. fagara (Table 9, Fig. 25D). Lower leaf δ^{15}N and higher leaf [N] are relatively consistent and well-documented characteristics of N_{2}-fixing plants in many ecosystems around the world (Dijkstra et al., 2003; Salas et al., 2001; Schmidt and Stewart, 2003; Virginia and Jarrell, 1983).

By fixing atmospheric N_{2} with a δ^{15}N ≈ 0‰, P. glandulosa could influence the availability and δ^{15}N of soil N near its canopy in a manner that should be reflected in the δ^{15}N values of adjacent non-N_{2}-fixing plants. Indeed, results showed that the proximity index (a reflection of the density and distance of surrounding P. glandulosa trees) was negatively correlated with leaf δ^{15}N values in both C. hookeri (r^2=0.21) and Z. fagara
(r²=0.22) (Fig. 27). That is, plants that were near one or more *P. glandulosa* trees generally had lower δ¹⁵N values than those located farther away. These data indicate that soil N derived from N₂-fixing *P. glandulosa* trees may make important contributions to the N nutrition of associated non-N₂-fixing plant species. Few studies have examined the potential influences of N₂-fixing plants on adjacent non-N₂-fixing plants in natural ecosystems. Handley et al. (1994) found that leaf δ¹⁵N values of non-N₂ fixing plants were not related to distance from putative N₂-fixing *Acacia* plants. Belsky et al. (1989) also found that N₂-fixing *Acacia tortilis* did not influence adjacent plants differently than non-N₂-fixing baobab trees in Kenya.

Previous studies at this site have shown that open grassland was once dominant across the entire landscape, and that woody plants have only recently encroached into this former grassland during the past 100 years (Archer et al., 2001; Archer et al., 1988; Boutton et al., 1998). Woody encroachment into grassland is initiated by *P. glandulosa*, which then appears to facilitate the establishment of other woody species beneath its canopy. The exact mechanisms by which *P. glandulosa* facilitates woody patch development are not clear, but may include enhanced soil moisture via hydraulic lift (Zou et al., 2005) and accelerated rates of N cycling processes (Hibbard et al., 2001; McCulley et al., 2004). Results of the present study suggest that N derived from N₂-fixation by *P. glandulosa* is important to neighboring non-N₂-fixing woody plants, and may represent an important mechanism by which *P. glandulosa* facilitates the formation of woody patches in grasslands in this region.
Variation in $\delta^{15}N$ at the individual plant scale

Leaf $\delta^{15}N$ values were lower than those of soils (Fig. 24), which is consistent with numerous other studies (Garten, 1993; Koba et al., 2003; Miller and Bowman, 2002). Although there is isotopic fractionation during nitrogen uptake by plants (Hogberg, 1997), leaf $\delta^{15}N$ values of non-N$_2$-fixing species _C. hookeri_ and _Z. fagara_ had positive relationships with their soil sources. Handley et al. (1999) found a similar correlation between globally-derived site-averaged leaf $\delta^{15}N$ and the $\delta^{15}N$ of whole surface mineral soil. Because _P. glandulosa_ trees obtain their nitrogen partially from the atmospheric N$_2$, their leaf $\delta^{15}N$ values were unrelated to those of the soil in which they grew.

A positive relationship between leaf [N] and leaf $\delta^{15}N$ was observed in all three plant species in April (Fig. 25D), and similar relationships have been documented by others (Garten, 1993; Hobbie et al., 2000; Pardo et al., 2006; Sah and Brumme, 2003; Vitousek et al., 1989). We suggest that this relationship between leaf [N] and leaf $\delta^{15}N$ is a function of the fact that plant-available N (as reflected in pool sizes of NH$_4^+$ and NO$_3^-$) is greatest on those portions of the landscape where N-transformations are occurring most rapidly, and therefore where N-losses and $\delta^{15}N$ of residual N are also greatest. Additional mechanistic studies are required to evaluate this hypothesis.

Seasonal variation in plant $\delta^{15}N$

We found leaf $\delta^{15}N$ values of _P. glandulosa_ were lower in August than in April;
however, those of *C. hookeri* and *Z. fagara* were higher in August than April (Table 9). The difference in temporal responses between *P. glandulosa* and the other two species may be related to the fact that *P. glandulosa* is an N$_2$-fixing plant and therefore has access to a source of N that is unavailable to non-N$_2$-fixing species. We currently lack direct information that can explain these temporal differences. However, in previous studies, seasonal variation in leaf $\delta^{15}$N values has been attributed to changes in the openness of the ecosystem N cycle (Filella and Penuelas, 2003; Peñuelas et al., 2000; Schulze et al., 1994) and to internal plant N mobilization (Chang and Handley, 2000; Garten, 1993; Handley and Scimgeour, 1997). For example, Filella and Penuelas (2003) reported leaf $\delta^{15}$N was higher in spring than summer in Mediterranean woody shrubs in NE Spain. They suggested that spring rains and increasing temperatures accelerated N cycle processes, resulting in a more open N cycle and higher leaf $\delta^{15}$N at that time. Chang and Handley (2000) found foliar $\delta^{15}$N was lower in August than in May for western redcedar trees and shrub salal on Vancouver Island, British Columbia. They suggested that higher leaf $\delta^{15}$N values in spring were strongly influenced by the mobilization of internally stored N, while lower leaf $\delta^{15}$N values in summer were the result of uptake of $^{15}$N-depleted soil N. Basipetal translocation of N in deciduous plants prior to autumn leaf fall may cause higher leaf $\delta^{15}$N in older leaves (Bustamante et al., 2004; Chang and Handley, 2000; Garten, 1993).

**CONCLUSIONS**

At the landscape-scale, spatial patterns of leaf $\delta^{15}$N values of non-N$_2$-fixing
plants are controlled by topography which influences soil water availability and the concentration and $\delta^{15}N$ of soil total N in this subtropical savanna parkland ecosystem. Greater soil N content and soil water availability are usually associated with higher rates of N transformations (e.g. mineralization, nitrification, and denitrification) which result in N-losses from the ecosystem, a more open N cycle, and $^{15}$N-enrichment of the residual N. Thus, plants in lower-lying portions of the landscape have higher leaf $\delta^{15}N$ values than those in upland areas. In general, the N$_2$-fixing plant *P. glandulosa* had leaf $\delta^{15}N$ values that were closer to values for atmospheric N$_2$ and lower than leaf values for the two non-N$_2$-fixing shrub species. At smaller scales, spatial patterns of leaf $\delta^{15}N$ values from non-N$_2$-fixing plants appear to be influenced by proximity to the N$_2$-fixing *P. glandulosa*, suggesting that encroachment of N$_2$-fixing *P. glandulosa* may influence the N cycle and facilitate N acquisition of other woody plants. This result gives us insights into the influences of N$_2$-fixation on N cycling, plant interactions and successional processes. Our study also indicates plant $\delta^{15}N$ values can reflect the extent of N retention within an ecosystem and help us better understand N dynamics and plant-soil interaction.
CHAPTER VI

VARIATION IN $\delta^{13}C$ VALUES OF WOODY PLANTS ALONG A
TOPOEDAPHIC GRADIENT IN A SUBTROPICAL SAVANNA PARKLAND
LANDSCAPE

SYNOPSIS

Variation in the carbon isotope composition of plants affords an opportunity to quantify the spatial and temporal distribution of sources and sinks in the global carbon cycle, and assess ecosystem-scale responses to environmental change. We determined landscape-level variation in leaf $\delta^{13}C$, leaf nitrogen concentration [N], and specific leaf area (SLA; cm$^2$ g$^{-1}$) in April and August 2005 for all individuals of three common woody species with different growth forms within a 308 m x 12 m belt transect spanning an upland-lowland topoedaphic gradient in a subtropical savanna in southern Texas, USA. Clay content, available soil moisture, and soil total N were all negatively correlated with elevation along the transect. $\delta^{13}C$ of Prosopis glandulosa (deeply-rooted summergreen legume), Condalia hookeri (facultative wintergreen non-legume), and Zanthoxylum fagara (shallow-rooted wintergreen non-legume) leaves increased 1-4‰ with decreasing elevation during both sample periods. Contrary to theory and previous studies, $\delta^{13}C$ values were highest where soil water was most available, suggesting that some other variable was overriding or interacting with water availability to influence plant water use. Leaf [N] was positively correlated with leaf $\delta^{13}C$ of all species (p<0.001), and appeared to exert the strongest control over carbon isotope discrimination.
along the topoedaphic gradient. *P. glandulosa* (N₂ fixer) leaf δ¹³C was 1-3 ‰ higher than that of the other two non-N₂ fixing species. Since leaf [N] is positively related to photosynthetic capacity, plants with high leaf [N] are likely to have low $p_i/p_a$ ratios and therefore higher δ¹³C values. SLA was not correlated significantly with leaf δ¹³C.

Following progressive growing season drought in July/August, leaf δ¹³C, leaf [N], and SLA all decreased. Lower δ¹³C in August may reflect the lower leaf [N] at that time, or the accumulation of ¹³C-depleted epicuticular leaf wax. We suggest that the control of leaf δ¹³C along this topoedaphic gradient is mediated by leaf [N] rather than by stomatal limitation caused by water availability and leaf conductance.

**INTRODUCTION**

Carbon isotopes can be helpful in estimating the pools and fluxes of the global carbon cycle because they give information about both sources and processes of CO₂ exchange (Randerson, 2005; Schulze, 2006; Suits et al., 2005). Quantification of spatial variability in leaf carbon isotope composition (δ¹³C) affords an opportunity to evaluate plant-environment interactions and plant responses to environmental change. Many studies have quantified leaf δ¹³C variation along environmental gradients, particularly altitudinal gradients in mountainous regions (Cordell et al., 1999; Hultine and Marshall, 2000; Korner et al., 1988). An increase in δ¹³C with altitude, which is a combined result of precipitation (Van de Water et al., 2002), temperature (Panek and Waring, 1995), and nutrient availability (Schulze et al., 1998), has been observed in many species. However, less is known about variations in leaf δ¹³C along local topoedaphic gradients where the
macroclimate is the same, but where changes in soil moisture, texture and nutrients may interact to influence leaf carbon isotopic composition.

In C₃ plants, the carboxylation enzyme Rubisco (ribulose 1·5-bisphosphate carboxylase / oxygenase) discriminates against ¹³C during photosynthesis. This effect is moderated by the ratio of CO₂ partial pressure at the carboxylation sites ($p_i$) to that in ambient air ($p_a$), which is strongly determined by stomatal aperture and photosynthetic capacity (Farquhar et al., 1982; Farquhar et al., 1989). Plants with lower $p_i/p_a$ ratios usually have higher leaf δ¹³C values (Farquhar et al., 1982). Therefore, a wide range of intrinsic plant characteristics and environmental variables play roles in determining leaf δ¹³C through effects on $p_i/p_a$. For example, leaf [N] can be positively related to leaf δ¹³C by elevating photosynthetic capacity and lowering $p_i$ (Morecroft and Woodward, 1996; Sparks and Ehleringer, 1997). Similarly, irradiance (Arthur et al., 2001; Zimmerman and Ehleringer, 1990) and air temperature have been found positively correlated to leaf δ¹³C (Panek and Waring, 1995); and precipitation negatively correlated to leaf δ¹³C (Ferrio and Voltas, 2005). Although process studies have revealed how leaf δ¹³C responds to specific abiotic factors, we know little of how these interact to influence the δ¹³C of the foliage in plant canopies along landscape-scale gradients during a growing season.

Since the $p_i/p_a$ ratio is also related to plant water-use efficiency (WUE), it has often been noted that leaf δ¹³C and WUE are positively related via $p_i/p_a$ (Ehleringer and Cooper, 1988; Farquhar et al., 1982; Mooney et al., 1989; Saurer et al., 2004; Stewart et
al., 1995). In some cases, plants located on drier portions of a landscape have higher leaf $\delta^{13}C$, suggesting higher WUE (Anderson et al., 1996; Comstock and Ehleringer, 1993). In other cases, the reverse is true. For example, Read and Farquhar (1991) found a positive relationship between water availability and leaf $\delta^{13}C$ of *Nothofagus* species in New Guinea. They speculated that because soil moisture was seldom limiting in their study area, that other factors such as soil nutrients may be dictating leaf $\delta^{13}C$.

Similarly, Schulze et al. (1998) found that variation in leaf [N] rather than variation in annual rainfall dictates community-averaged leaf $\delta^{13}C$ in northern Australia. Zhang and Marshall (1995) reported lower leaf $\delta^{13}C$ from dry environments, and speculated that there could be differential sensitivity of stomatal conductance to water availability among different species. Therefore, for natural vegetation, which is influenced by a wide range of factors, caution should be taken while interpreting the link between leaf $\delta^{13}C$ and WUE (Griffiths et al., 2000; Vitousek et al., 1990).

There has also been some debate about whether higher $\delta^{13}C$ values are a result of larger carboxylation capacity or higher internal resistance to CO$_2$ diffusion. While both reflect increased stomatal limitation on photosynthesis, higher values in leaf $\delta^{13}C$ can arise from either lower conductance of CO$_2$ into leaf or higher photosynthetic capacity (Cordell et al., 1999; Vitousek et al., 1990; Williams and Ehleringer, 2000). Vitousek et al. (1990) suggested that internal resistance to CO$_2$ diffusion in *Metrosideros polymorpha* might have been the major cause of the observed $\delta^{13}C$ trends. Cordell et al. (1999) concluded differently and suggested carboxylation efficiency and N content dominated influences on $\delta^{13}C$ of *M. polymorpha*. However, Williams and Ehleringer
(2000) found tissue [N] can have a negative relationship with $\delta^{13}$C due to the greater internal resistance as reflected by higher leaf mass per area.

The objectives of our study were to: (1) quantify landscape-scale variations of leaf $\delta^{13}$C along a topoedaphic gradient varying in soil texture, soil moisture, and soil fertility in a subtropical savanna parkland landscape; and (2) evaluate potential controls over these isotopic variations. To examine inter- and intraspecific variability in $\delta^{13}$C, we focused on three woody plant growth forms ubiquitous along the gradient: a deeply rooted, summergreen arborescent legume [Prosopis glandulosa Torr. var. glandulosa (honey mesquite)], a facultative wintergreen non-legume [Condalia hookeri M.C. Johnst. (bluewood)], and a shallow-rooted, evergreen non-legume [Zanthoxylum fagara (L.) Sarg. (lime prickly ash)]. Leaf $\delta^{13}$C, specific leaf area (SLA) and leaf [N] were measured to test the following hypotheses: (1) Leaf $\delta^{13}$C is related to the availability of soil moisture along the topoedaphic gradient, with lower values in wetter area; (2) Leaf [N] is positively correlated with leaf $\delta^{13}$C due to its influence on photosynthetic capacity and SLA is negatively correlated with leaf $\delta^{13}$C due to its influence on leaf internal resistance to CO$_2$ diffusion; (3) Leaf $\delta^{13}$C of P. glandulosa is lower than that of the other two species due to its N$_2$-fixing ability; and (4) Seasonal drought will increase leaf $\delta^{13}$C values of all species.

**MATERIALS AND METHODS**

**Study area**

Research was conducted at the Texas Agricultural Experiment Station La Copita
Research Area (27° 40’ N; 98° 12’ W; elevation 80m) in Jim Wells County, 15 km SW of Alice, TX in the eastern Rio Grande Plains of the Tamaulipan Biotic Province. The climate of the region 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; July and August are typically the warmest, driest months of the year.

Landscapes at La Copita typically grade (1-3% slopes) from sandy loam uplands to clay loam and clay lowlands which receive runoff from the uplands, and may have standing water after large rainfall events. Upland 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). Upland vegetation is savanna parkland consisting of discrete woody clusters (comprised of a single *P. glandulosa* trees with up to 15 understory tree/shrub species, 3–10 m diameter) and larger groves (comprised of several woody clusters that have fused together, 10 to >20 m diameter) embedded within a matrix of grassland vegetation (Fig. 28). Upland grasslands consist mainly of rhizomatous and weakly caespitose C4 grasses, and C3 forbs. Clusters and groves are dominated by *P. glandulosa* and *Z. fagara*. *P. glandulosa* and several other leguminous trees and shrubs present in wooded areas are capable of symbiotic N₂-fixation (Zitzer et al., 1996).

Soils in lower-lying drainage woodlands are generally Pachic Argiustolls and are covered by closed-canopy woodlands (Fig. 28) similar in composition to upland clusters and groves. Playas are closed-basin depressions that occur within the drainage
Fig. 28. Color IR aerial view (2006) of the 308 m transect at the savanna parkland study site showing clusters (C) and groves (G) embedded within an herbaceous grassland matrix (H) in upland areas, and drainage woodland (W) and playa (P) located in lower portions of the landscape. Red tones indicate woody plant cover, while gray and white tones indicate herbaceous grassland vegetation (largely senescent) and bare ground, respectively. Green dots (10 m apart) indicate the location of the transect.
woodlands, and occupy the lowest portions of the landscape. Playa soils are Ustic Epiaquerts and Vertic Argiaquolls, and vegetation there consists of a continuous grass layer dominated by *Paspalum pubiflorum* and *Bothriochloa ischaemum*, with *P. glandulosa* and *Acacia farnesiana* trees of variable density (Farley, 2000).

Woody plant encroachment has occurred throughout the study area over the past 75-100 years due to the interaction of heavy livestock grazing and reduced fire frequency (Archer, 1995). Archer (1995), and Boutton et al. (1998) have additional details on soils, vegetation, and climate.

**Transect description**

A 308 m transect spanning a hillslope gradient from an upland through a drainage woodland and into a playa was established (Fig. 28). All five of the major landscape elements at the study site (grasslands, clusters, groves, drainage woodlands, and playa) occurred along the transect. The transect was marked with steel posts at 1 m intervals, and georeferenced by Trimble GPS pathfinder Pro XRS system (Trimble Navigation Limited, Sunnyvale, CA) at 5 m intervals. Elevations were determined by surveying, and ranged from 86.5 m in the upland to 84.5 m in the playa.

**Plant and soil sampling and analysis**

Soils (0-15 cm) along the transect were sampled in April, 2005. Two soil cores were collected at 1 m intervals along the transect. One core was used to determine soil particle size distribution (pipet method; (Gee and Bauder, 1986)); the other was used to
determine soil total N by combustion/gas chromatography using a Carlo Erba EA-1108 elemental analyzer (CE Elantech, Lakewood, NJ) following procedures outlined in Harris et al. (2001). Another set of soil cores were collected at 3 m intervals. The wet weight of each core was measured and then the soil cores were dried in an oven at 105°C overnight and re-weighed. Soil gravimetric water content (GWC) was calculated by dividing soil water content by soil dry weight. Values of GWC were converted to volumetric water content (VWC). Plant-available VWC was computed from total VWC and soil texture using the equations of Cosby et al. (1984), assuming a matric potential at wilting point of -10.0 MPa. Soil water content was not quantified in August 2005 because significant rainfall occurred the day prior to plant tissue sampling; hence, soil moisture would not have reflected the antecedent soil moisture conditions that would have influenced our plant response variables in August.

Three common woody species were selected for isotopic analyses: (1) *P. glandulosa* (Fabaceae), (2) *C. hookeri* (Rhamnaceae), and (3) *Z. fagara* (Rutaceae). *P. glandulosa* is a deep-rooted deciduous tree capable of symbiotic N$_2$-fixation (Zitzer et al. 1996), and is the dominant overstory species in all woody assemblages at this site. *C. hookeri* and *Z. fagara* are spinescent shrubs that dominate the understory in all wooded areas. Leaf tissue was sampled from all individuals of these three species occurring within 6 m on either side of the 308 m transect in April and August, 2005. For *P. glandulosa*, approximately 10g of fully-expanded, south-facing, sun-lit leaves in the upper canopy were sampled. For *C. hookeri* and *Z. fagara* approximately 10g of fully-expanded, south-facing, leaves located in the upper canopy approximately 1-2m above
ground level were sampled. The spatial coordinates of each plant sampled within the 12 m x 308 m area were recorded by measuring the distance along and perpendicular to the central transect line.

Leaf area was measured on fresh tissue using a leaf area meter (CI-202, CID Inc., Vancouver, WA, USA). Leaves were then oven-dried for 48 hours at 60°C and weighed. Specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry weight. Leaves were then pulverized in a centrifugal mill (Angstrom, Inc., Belleville, MI, USA).

\[ \delta^{13} \text{C} \] and leaf [N] were determined using a Carlo Erba EA-1108 elemental analyzer (CE Elantech, Lakewood, NJ) interfaced with a Delta Plus (Thermo Electron Corp., Waltham, MA) isotope ratio mass spectrometer operating in continuous flow mode. Carbon isotope ratios are presented in \( \delta \) notation:

\[
\delta = \left[ \frac{R_{\text{SAMPLE}} - R_{\text{STD}}}{R_{\text{STD}}} \right] \times 10^3
\]

(12)

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

Temperature and rainfall data from Dec. 2004 to Aug. 2005 were obtained from the Alice, TX weather station (available at www.ncdc.noaa.gov.), with some missing data obtained from the Alice International Airport weather station.

**Statistical analyses**

A univariate general linear model ANOVA was used to test for the differences in leaf \( \delta^{13}\text{C} \) with respect to landscape locations, sampling seasons, species, and their
interactions. Fisher’s LSD method was used to distinguish differences following ANOVA. Regression analyses were performed to determine the significance of relationships between leaf [N], SLA, plant available water and leaf \( \delta^{13}C \). Stepwise multiple linear regression was performed to determine if certain combinations of plant and/or environmental variables could be used to predict leaf \( \delta^{13}C \). Analysis of covariance (ANCOVA) was used to compare the slopes of the relation for leaf [N] versus leaf \( \delta^{13}C \) between the two sampling seasons. All the statistical analyses were performed using the SPSS 10.0 program (SPSS Inc. Chicago, IL).

RESULTS

Temperature and rainfall during study period

Average monthly temperatures during February (17.1 °C), March (19.1 °C), and April (22.8 °C) of 2005 were above the 110 year mean at Alice, TX (Fig. 22). Monthly rainfall in both February (64.3 mm) and March (62.2 mm) was approximately 60% above the 110 year mean for those months; however, rainfall during April (2.54 mm) was only 6% of 110 year mean.

Average monthly temperatures during May, June, July and August of 2005 were 26.2°C, 29.4°C, 30.7°C, and 31.4°C respectively (Fig. 22). These temperatures were all above the 110 year mean for those months. Monthly rainfall during the same period was 41.4mm (May), 3.3mm (June), 64.8mm (July) and 8.6mm (August), which was either dramatically less than or comparable to the 110 year mean for those months. Hence,
conditions prior to the April foliage sampling were comparatively warm and mesic, while those preceding the August foliage sampling were comparatively hot and dry.

**Transect characteristics**

Elevation along the transect ranged from 84.5 m to 86.5 m (Fig. 29A). However, these small variations in elevation have induced significant variation in soil particle size distribution (Fig. 29B), soil water content (Fig. 29C), and soil total N (Fig. 29D). Soil clay content, plant available water and soil total N were all negatively correlated with elevation (Table 12). Soil clay content increased as elevation decreased along the transect, with values of approximately 100 g kg⁻¹ in the uplands, 100-200 100 g kg⁻¹ in the drainage woodlands, and 250-350 100 g kg⁻¹ in the playas (Fig. 29B). Both soil VWC and plant available VWC were related to elevation and texture, with the highest values found in the playas (plant available VWC =0.10 m³ m⁻³) and lowest values in the uplands (plant available VWC=0.01 m³ m⁻³) (Fig. 29C). Plant-available VWC was always significantly less than the total VWC, especially in the playas where clay content was highest. Soil total N was related to both elevation along the transect and vegetation cover, with higher values in the lower-lying drainage woodlands and playas (1-5 g N kg⁻¹ soil) compared to uplands (0.6-2 g N kg⁻¹ soil), and higher values in woody vegetation types (1-5 g N kg⁻¹ soil in grove, cluster and woodland communities) than in grassland (0.6 g N kg⁻¹ soil) (Fig. 29D).
Fig. 29. Changes in elevation (m) (A), soil particle size distribution (g kg$^{-1}$) (B), soil volumetric water content (total and available) (m$^3$ m$^{-3}$) (C), soil total nitrogen (g N kg$^{-1}$) (D) and \textit{P. glandulosa}, \textit{C. hookeri} and \textit{Z. fagara} leaf $\delta^{13}$C (‰ vs. V-PDB) (E-G) along the transect.
Leaf $\delta^{13}C$ values

All three variables (species (S), landscape locations (L) and sample periods (P)) had significant main effects on leaf $\delta^{13}C$ values (Table 12). Mean leaf $\delta^{13}C$ values of *P. glandulosa* (-25.7‰) were significantly higher than those of *C. hookeri* (-27.8‰) and *Z. fagara* (-27.4‰) (Table 12, Fig. 29E-G). Mean leaf $\delta^{13}C$ values of all shrub samples in uplands (-27.2‰) was significantly lower than in woodlands (-26.5‰) and playas (-26.3‰). Leaf $\delta^{13}C$ values of all shrub samples averaged -26.4‰ in April, which was significantly higher than in August (-27.0‰) (Table 12). There was an interaction between species and sample period, indicating for different species, the effect of sampling time on leaf $\delta^{13}C$ values was different. More specifically, leaf $\delta^{13}C$ of *P. glandulosa* was -24.9 ± 0.13 ‰ in April, which was significantly higher than that in August (-26.2 ± 0.13 ‰) (Table 12, Fig. 29E). However, leaf $\delta^{13}C$ values of *C. hookeri* and *Z. fagara* in April (-27.5 ± 0.12 ‰ and -27.2 ± 0.11 ‰, respectively) and August (-27.8 ± 0.13 ‰ and -27.2 ± 0.11 ‰, respectively) were not significantly different (Table 12, Fig. 29F, 29G). *C. hookeri* was not present in the playa.

Correlations between plant $\delta^{13}C$ and biological and environmental variables

There was a significant negative correlation between leaf [N] and elevation for *Z. fagara* in both the April and August sample periods (p<0.001) (Table 13). Significant negative correlations between leaf [N] and elevation were also observed for *P. glandulosa* and *C. hookeri* in April (p<0.01), but not in August. There was a significant positive correlation between leaf [N] and leaf $\delta^{13}C$ for all the three species in April: *P. glandulosa*...
Table 12. Leaf δ¹³C (‰) in different species (S), landscape locations (L) and sample periods (P). Different letters indicate differences between means (Fisher’s LSD analysis; α = 0.05). Since the interactions between S × L, L × P, and S × P × L were not significant, detailed comparisons were omitted.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Significance</th>
<th>Categories</th>
<th>Mean</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Species (S)</strong></td>
<td>0.000</td>
<td><em>Prosopis glandulosa</em></td>
<td>-25.7</td>
<td>0.126</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Condalia hookeri</em></td>
<td>-27.8</td>
<td>0.110</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Zanthoxylum fagara</em></td>
<td>-27.4</td>
<td>0.079</td>
</tr>
<tr>
<td><strong>Landscape location (L)</strong></td>
<td>0.000</td>
<td><em>Upland</em></td>
<td>-27.2</td>
<td>0.070</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Drainage woodland</em></td>
<td>-26.5</td>
<td>0.053</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Playa</em></td>
<td>-26.3</td>
<td>0.158</td>
</tr>
<tr>
<td><strong>Sample period (P)</strong></td>
<td>0.000</td>
<td><em>April</em></td>
<td>-26.4</td>
<td>0.072</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>August</em></td>
<td>-27.0</td>
<td>0.073</td>
</tr>
<tr>
<td><strong>S × P</strong></td>
<td>0.000</td>
<td><em>P. glandulosa</em></td>
<td>April</td>
<td>-24.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>August</td>
<td>-26.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>C. hookeri</em></td>
<td>April</td>
<td>-27.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>August</td>
<td>-27.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Z. fagara</em></td>
<td>April</td>
<td>-27.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>August</td>
<td>-27.2</td>
</tr>
<tr>
<td><strong>S × L</strong></td>
<td>0.368</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>L × P</strong></td>
<td>0.316</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>S × P × L</strong></td>
<td>0.994</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 13. Pearson’s correlation coefficients for elevation vs. soil properties, and elevation vs. plant properties along a hillslope gradient.

<table>
<thead>
<tr>
<th></th>
<th>Elevation</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>April</td>
<td>August</td>
<td></td>
</tr>
<tr>
<td><strong>Soil properties</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clay</td>
<td>-0.617**</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Plant available VWC</td>
<td>-0.575**</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Soil total N</td>
<td>-0.543**</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td><strong>Plant properties</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leaf $\delta^{13}$C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Prosopis glandulosa</em></td>
<td>-0.260*</td>
<td>-0.306*</td>
<td></td>
</tr>
<tr>
<td><em>Condalia hookeri</em></td>
<td>-0.430**</td>
<td>-0.464**</td>
<td></td>
</tr>
<tr>
<td><em>Zanthoxylum fagara</em></td>
<td>-0.357**</td>
<td>-0.346**</td>
<td></td>
</tr>
<tr>
<td>Leaf [N]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Prosopis glandulosa</em></td>
<td>-0.364**</td>
<td>-0.132</td>
<td></td>
</tr>
<tr>
<td><em>Condalia hookeri</em></td>
<td>-0.420**</td>
<td>-0.192</td>
<td></td>
</tr>
<tr>
<td><em>Zanthoxylum fagara</em></td>
<td>-0.578**</td>
<td>-0.514**</td>
<td></td>
</tr>
<tr>
<td>SLA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Prosopis glandulosa</em></td>
<td>-0.565**</td>
<td>-0.613**</td>
<td></td>
</tr>
<tr>
<td><em>Condalia hookeri</em></td>
<td>-0.552**</td>
<td>-0.488**</td>
<td></td>
</tr>
<tr>
<td><em>Zanthoxylum fagara</em></td>
<td>-0.541**</td>
<td>-0.308**</td>
<td></td>
</tr>
</tbody>
</table>

** p < 0.01  
*   p < 0.05
glandulosa (R² =0.16, p=0.001), C. hookeri (R² =0.46, p<0.001) and Z. fagara (R² =0.42, p<0.001). This relationship was also significant for August samples: P. glandulosa (R² =0.34, p<0.001), C. hookeri (R² =0.13, p<0.01) and Z. fagara (R² =0.29, p<0.001). At any given leaf [N], leaf δ¹³C of P. glandulosa was higher than that of C. hookeri and Z. fagara (Fig. 30).

There was also a significant negative correlation between specific leaf area (SLA) and elevation for all three species during both sample periods (p<0.001) (Table 13). Leaf δ¹³C tended to increase with SLA for all the three species; however, this relationship was not significant (Fig. 30). Data of both April and August sample periods of all the three species was consistent.

Plant available water was not related to leaf δ¹³C for either C. hookeri or Z. fagara in April, 2005. However, water did have a significant positive relationship with leaf δ¹³C for P. glandulosa (R²=0.11, p<0.01) (Fig. 30).

Stepwise multiple linear regression analysis was performed to test the effects of soil available water, SLA and leaf [N] on leaf δ¹³C of each species. Leaf [N] was the only variable that accounted for a significant proportion (p < 0.01) of the variation in leaf δ¹³C; no other variables were able to improve landscape-level prediction of leaf δ¹³C values at this site.

**Seasonal variation in plant δ¹³C**

Leaf δ¹³C values of P. glandulosa were significantly higher than those of the
Fig. 30. Relationships between leaf $\delta^{13}C$ (‰) and plant available volumetric water content (VWC; m$^3$ m$^-3$) (a), specific leaf area (SLA; m$^2$ kg$^-1$) (b), and leaf [N] (g kg$^-1$) (c) for *Prosopis glandulosa*, *Condalia hookeri*, and *Zanthoxylum fagara* in April (---●---) and August (----○----) of 2005.
other two species (Table 12). Highest leaf [N] was found in *P. glandulosa* in April (mean=35.9 g/kg), when leaf δ¹³C value was also highest. Leaf δ¹³C of *P. glandulosa* became more negative in August (mean = -26.2‰) than in April (mean = -24.9‰); this decrease was not significant for the other two species (Table 12).

Comparing the linear regression lines of leaf [N] versus leaf δ¹³C in April and August, ANCOVA analysis indicated that there was no significant difference between the slopes for both *P. glandulosa* and *Z. fagara* at $\alpha = 0.05$. The regression slopes were significantly different for *C. hookeri* between the two seasons.

**DISCUSSION**

**Spatial variability**

Given the semiarid conditions in this subtropical savanna parkland landscape, we hypothesized that soil water availability would have a large and significant impact on spatial and temporal patterns of plant leaf δ¹³C values. According to Farquhar et al. (1989), any factor that increases leaf conductance and photosynthesis tends to increase $p_i/p_a$ and therefore decreases leaf δ¹³C. Hence, greater soil water availability should cause higher $p_i$ and more negative leaf δ¹³C. Many previous studies have observed this negative correlation between water availability and leaf δ¹³C (Anderson et al., 1996; Comstock and Ehleringer, 1993). In contrast, our studies showed that overall mean leaf δ¹³C values of all three shrub species were more negative in the upland (-27.2 ‰), where near-surface (0-15 cm) soil moisture was less available (Fig. 29C), than in drainage
woodland (-26.5 ‰) and playa (-26.3 ‰), where near-surface soil moisture was relatively more available (Fig. 29C, Table 12). For each species, regression analyses indicated leaf δ¹³C was negatively correlated with elevation and positively related to plant-available VWC (Fig. 30, Table 13). This implied that plants may be relatively de-coupled from near-surface soil moisture conditions or that some other variables could be overriding or interacting with water availability to influence the observed spatial pattern of plant δ¹³C values.

It is important to note that the assessment of soil water in this study was based on a single point in time, and was confined to the surface soil (0-15 cm). This assessment is probably inadequate because leaf δ¹³C values integrate the isotopic composition of plant carbon acquired over a longer period of time, and because *P. glandulosa* and *C. hookeri* have roots that extend much deeper than 15 cm below the soil surface (Boutton et al., 1999; Midwood et al., 1998; Zou et al., 2005). *Z. fagara* is highly responsive to near-surface moisture conditions (Zou et al., 2005). Although the absolute magnitude of water stored in the soil would have fluctuated over the duration of this study, it seems likely that the overall relative pattern of water availability along the upland-lowland transect in this landscape would be comparable to that described in this study both over time and in deeper portions of the profile.

The robust positive correlation between leaf [N] and leaf δ¹³C could account for the observed spatial pattern. Leaf [N] is highly correlated with photosynthetic capacity (Field and Mooney, 1986; Hikosaka et al., 1998; Santiago et al., 2004). Plants with
higher leaf [N] are likely to have lower $p/p_a$ ratios due to rapid assimilation of CO$_2$
during photosynthesis, resulting in higher $\delta^{13}$C values. In our study area, leaf [N]
increased as elevation decreased (Table 13), potentially contributing to the increase of
leaf $\delta^{13}$C along the transect. Comparing the linear regression lines of leaf [N] versus leaf
$\delta^{13}$C in April and August, ANCOVA analysis indicated that there was no significant
difference between the slopes for both *P. glandulosa* and *Z. fagara*. This result revealed
the effects of leaf [N] on leaf $\delta^{13}$C in August did not differ from that in April after a
period of drought, indicating seasonal water availability had no effect on the positive
relationship between leaf [N] and leaf $\delta^{13}$C. Therefore, we speculate that the major
factor contributing to the spatial variation of leaf $\delta^{13}$C may be increased photosynthetic
capacity as indicated by leaf [N] instead of soil moisture availability.

Why was the spatial pattern of plant $\delta^{13}$C uncoupled from the spatial pattern of
soil moisture availability? One reason might be that soil moisture availability did not
change enough across this transect to influence leaf $\delta^{13}$C values. A more plausible
explanation would be that under semiarid conditions, plants have multiple adaptations
for coping with water limitation such as high root-shoot ratios and osmotic adjustment.
These mechanisms may dampen stomatal response to variations in apparent soil
moisture availability across the landscape. In previous studies, Read and Farquhar (1991)
found positive relationship between water availability and leaf $\delta^{13}$C of *Nothofagus*
species. Schulze et al. (1998) confirmed that annual rainfall had minor influences on
community-averaged leaf $\delta^{13}$C. They concluded that the maintenance of leaf isotope
discrimination with increasing aridity was because leaf [N] remained constant within
each plant functional type. Therefore, in dry habitats, those species well adapted to water limitation may have little stomatal response to changes in soil moisture availability. Hence, soil moisture availability may have little impact on leaf $\delta^{13}C$ values in semiarid habitats.

Alternatively, soil moisture could be influencing foliar $\delta^{13}C$ by affecting N-availability along the topoedaphic gradient. Water and nitrogen are often co-limiting in dryland ecosystems (Hooper and Johnson, 1999). Both soil water content and soil total N increase as elevation decreases along the transect, and both of these variables are strongly correlated with rates of N mineralization (Booth et al., 2005). Thus, water may be influencing foliar $\delta^{13}C$ by regulating soil N availability and leaf [N] along this gradient.

Leaf $\delta^{13}C$ may also be influenced by leaf internal resistance to CO$_2$ diffusion as indicated by SLA. Vitousek et al. (1990) found that leaf $\delta^{13}C$ of *Metrosideros polymorpha* did not reflect $p_i/p_a$, but was strongly correlated with leaf mass per unit area; they concluded that internal resistance to CO$_2$ diffusion was the main cause of the observed trend. They speculated that lower SLA (thicker leaves) imposed greater resistance to CO$_2$ diffusion; therefore, CO$_2$ partial pressure at the fixation site should be lower, and leaf $\delta^{13}C$ would be negatively correlated with SLA. However, Cordell et al. (1999) found that internal resistance could not entirely explain the spatial variation of *M. polymorpha* leaf $\delta^{13}C$, and that its impact was secondary to the influence of photosynthetic capacity. In our study, leaf $\delta^{13}C$ tended to increase as SLA increased, but
the relationship was not statistically significant (Fig. 30). This trend is opposite to that observed by Vitousek et al. (1990) and Sparks and Ehleringer (1997), but consistent with Anderson et al. (1996) and Schulze et al. (1998). We speculate that the positive correlation between leaf [N] and SLA may account for the observation. Leaves with higher SLA had higher [N], leading to higher photosynthetic capacity and therefore, higher leaf $\delta^{13}C$. Apparently, high leaf [N] offset the impact of low leaf thickness which would result in high conductance and lower leaf $\delta^{13}C$.

Hence, in summary, plants in the lower portions of the transect had higher SLA, higher leaf [N], and higher leaf $\delta^{13}C$. We suggest that the controlling factor of the spatial pattern of leaf $\delta^{13}C$ was leaf [N].

**Interspecific variability**

Interspecific variations of leaf $\delta^{13}C$ of C$_3$ plants are usually within the range of 2-5‰ (O'Leary, 1981). We found that leaf $\delta^{13}C$ of *P. glandulosa* was up to 3‰ higher than that of *C. hookeri* and *Z. fagara* (Table 12, Fig. 29E-G). This difference may be related to the fact that *P. glandulosa* is a N$_2$ fixer (Zitzer et al., 1996). Consistent with other studies on this site (e.g. Nelson et al. 2002), leaf [N] of *P. glandulosa* was higher than that of *C. hookeri* and *Z. fagara*, perhaps because *P. glandulosa* has access to both atmospheric and soil sources of N while the other two species are limited to the soil for their N source. The higher leaf [N] in *P. glandulosa* potentially confers a higher photosynthetic capacity (Barnes and Archer, 1999; Zou et al., 2005) and, therefore,
higher leaf $\delta^{13}C$. This is consistent with Schulze et al. (1998) who found that N$_2$-fixers had 1.2‰ to 2.4‰ higher $\delta^{13}C$ than non-N$_2$ fixing species.

Tree height may also influence interspecific variation of leaf $\delta^{13}C$. Lower leaf $\delta^{13}C$ of understory plants in forest ecosystems has been reported (Berry et al., 1997; Ometto et al., 2002). Two explanations for this vertical variation are possible: (a) reduction in light intensity, and/or (b) fixation of respired CO$_2$ which has much lower $\delta^{13}C$ values than tropospheric CO$_2$. In our study, the canopy of the overstory $P$. glandulosa is generally fully illuminated, whereas the canopies of $C$. hookeri and Z. fagara often receive light that has been attenuated by the overstory $P$. glandulosa canopy. Thus, variations in light intensity and the isotopic composition of assimilated atmospheric CO$_2$ may both contribute to the higher leaf $\delta^{13}C$ in $P$. glandulosa.

**Seasonal variability**

Many previous studies have shown an increase in leaf $\delta^{13}C$ during the growing season associated with progressive seasonal drought (Baldocchi and Bowling, 2003; Ehleringer et al., 1992; Smedley et al., 1991). Because $\delta^{13}C$ provides a relative index of instantaneous water use efficiencies (WUE) and long-term transpiration efficiencies (Farquhar et al., 1989), the increase of WUE after a period of drought will be reflected by the increase of leaf $\delta^{13}C$. Therefore, we hypothesized that leaf $\delta^{13}C$ would be higher in August than in April. However, the results were contrary to the hypothesis; mean of leaf $\delta^{13}C$ values of all the three species was lower in August. More specifically, leaf $\delta^{13}C$ remained constant across time for $C$. hookeri and Z. fagara, and were lower in
August than in April for *P. glandulosa* (Table 12). Studies from hanging gardens in southern Utah found similar seasonal patterns (Flanagan et al., 1997), but these observations were unexplained. One potential mechanism for the decrease in leaf $\delta^{13}C$ in *P. glandulosa* during the growing season is the accumulation of epicuticular wax. Jacoby et al. (1990) found epicuticular wax on the leaves of *P. glandulosa* accumulated rapidly from May through July. Leaf wax of *P. glandulosa* consists mostly of esters and alkanes (Mayeux and Wilkinson, 1990). Conte et al. (2003) found that n-alcohol, n-acid and n-alkane wax compounds were on average depleted in $^{13}C$ by approximately 6.0±1‰ relative to total plant carbon. Thus, the accumulation of $^{13}C$-depleted leaf wax could account for decreased leaf $\delta^{13}C$ in August vs. April in *P. glandulosa*.

In addition, we found leaf [N] decreased from April to August, especially in *P. glandulosa* and *C. hookeri* (Fig. 30). The positive correlation between leaf $\delta^{13}C$ and leaf [N] through time was consistent with the spatial correlation observed along the topoedaphic gradient, and provided further evidence that leaf [N] appeared to exert the strongest control on leaf $\delta^{13}C$ in our study.

**CONCLUSIONS**

In this semi-arid subtropical savanna, $\delta^{13}C$ dominant C₃ tree species foliage was negatively correlated with elevation and soil moisture, suggesting soil water availability was not the controlling factor driving leaf $\delta^{13}C$ values. On the contrary, leaf $\delta^{13}C$ values along this topoedaphic gradient appeared to be most strongly influenced by leaf [N]. Higher leaf $\delta^{13}C$ values at lower elevation appeared to be the result of larger
carboxylation capacity resulting from higher leaf [N]. Interspecific and seasonal patterns of leaf δ¹³C values also appeared to be most strongly related to leaf [N]. This study suggests that factors other than plant water availability can play an important role in influencing spatial patterns of leaf δ¹³C values at the landscape scale.
CHAPTER VI

SUMMARY AND CONCLUSIONS

Geostatistical analyses showed that soil $\delta^{13}$C values in upland areas of the Rio Grande Plains were heterogeneous and autocorrelated within a range of 12.6m. Spatial variation of soil $\delta^{13}$C values within woody patches indicated woody plant encroachment was initiated by honey mesquite. As mesquite grew, subordinate woody species entered the system and formed multispecies clusters. As clusters grew, discrete clusters coalesced and formed larger groves. Groves could keep growing around the boundary recruiting new mesquite trees and other understory shrubs. A contour map of soil $\delta^{13}$C revealed that some clusters are currently growing rapidly towards each other and might coalesce into groves in the near future. This pattern of woody patch development was substantiated by sequential aerial photographs taken during the past 80 years. Kriged maps of soil $\delta^{13}$C allowed us to accurately reconstruct landscape-scale spatial pattern and temporal dynamics of woody plant encroachment into grassland, suggesting this approach can be reliably applied to reconstruct large-scale changes in woody cover in areas where historical aerial photography or satellite imagery are not available. Kriged maps of soil $\delta^{13}$C can also provide a strong spatial context for future studies aimed at understanding the functional consequences of this change in landscape structure.

On lower-lying portions of the landscape, grassland-to-woodland conversion has gone to completion and formed closed-canopy drainage woodlands. Unlike the uplands, spatial patterns of soil $\delta^{13}$C values in these lower-lying drainage woodlands were
unrelated to the presence and distribution of mesquite trees. Instead, spatial patterns of soil $\delta^{13}C$ were most strongly affected by SOC concentration and by soil particle size distribution. Soil silt+clay content and SOC together accounted for 73% of the variance in soil $\delta^{13}C$ values in this area. The positive correlation between silt+clay and soil $\delta^{13}C$ indicates that the formation of organomineral complexes and microaggregates may slow SOC turnover rates and favor the persistence of C$_4$-derived SOC from the grassland that dominated this site approximately 100 yrs ago. The negative correlation between SOC and soil $\delta^{13}C$ indicates that on those portions of the landscape with high SOC, a significant proportion of that SOC is derived from the invading C$_3$ woody plants.

Spatial patterns of leaf $\delta^{15}N$ values of non-N$_2$-fixing plants were controlled by topography which influences soil water availability and the concentration and $\delta^{15}N$ of soil total N in this subtropical savanna parkland ecosystem. Greater soil N content and soil water availability are usually associated with higher rates of N transformations (e.g. mineralization, nitrification, and denitrification) which result in N-losses from the ecosystem, a more open N cycle, and $^{15}N$-enrichment of the residual N. Thus, plants in lower-lying portions of the landscape have higher leaf $\delta^{15}N$ values than those in upland areas. In general, the N$_2$-fixing plant mesquite had leaf $\delta^{15}N$ values that were closer to values for atmospheric N$_2$ and lower than leaf values for the two non-N$_2$-fixing shrub species. At smaller scales, spatial patterns of leaf $\delta^{15}N$ values from non-N$_2$-fixing plants appear to be influenced by proximity to the N$_2$-fixing mesquite, suggesting that encroachment of N$_2$-fixing mesquite may influence the N cycle and facilitate N acquisition by other woody plants. We hypothesize that mesquite may alter the cycling
and availability of soil nitrogen in a manner that favors the recruitment and establishment of other tree/shrub species that occur beneath its canopy, and that this may be an important mechanisms that promotes woody encroachment in the Rio Grande Plains of southern Texas.

$\delta^{13}C$ values of leaves of dominant C$_3$ tree species were negatively correlated with elevation and soil moisture, suggesting soil water availability was not controlling leaf $\delta^{13}C$ values. This result is unique in that most other studies show that water availability strongly controls plant $\delta^{13}C$ values at the landscape scale. Instead, leaf $\delta^{13}C$ values along this topoedaphic gradient appeared to be most strongly influenced by leaf [N]. Higher leaf $\delta^{13}C$ values at lower elevation appeared to be the result of larger carboxylation capacity resulting from higher leaf [N]. Interspecific and seasonal patterns of leaf $\delta^{13}C$ values also appeared to be most strongly related to leaf [N]. These results emphasize that factors other than plant water availability can play an important role in influencing spatial patterns of leaf $\delta^{13}C$ values at the landscape scale.

Collectively, the information on factors controlling spatial variation of soil and leaf isotopic values in this study enhances our current understanding of the natural abundance of stable isotopes at the landscape scale and their use in studying vegetation changes and biogeochemical cycles. Stable isotopes in conjunction with geostatistics can provide a powerful tool to study dynamics of vegetation change and nutrient cycling processes. Furthermore, grassland to woodland conversion has been a globally widespread phenomenon that continues to threaten grassland biodiversity, ecosystem
services, and land use options in grass-dominated ecosystems around the world. Results from this study may enhance our understanding of the potential patterns, causes and consequences of this land cover change and the dynamics of carbon and nitrogen cycles that accompany this transformation.
REFERENCES


ERDAS. 1998. Using Arcview Image Analysis ERDAS Inc., Atlanta, GA.


Farley, A.L. 2000. Vegetation, soils and surface hydrology of playa landforms in the Rio Grande Plains, Texas, Texas A&M University, College Station, TX.


Rosenberg, M.S. 2001. PASSAGE: pattern analysis, spatial statistics, and geographic exegesis. 1.0 ed. Department of Biology, Arizona State University, Tempe, AZ.


Soil Survey Staff. 1996. Soil survey laboratory methods manual No. 42. USDA-NRCS, Lincoln, NE.


Stokes, C.J. 1999. Woody plant dynamics in a south Texas savanna: pattern and process, Texas A&M University, College Station, TX.


Watts, S.E. 1993. Rooting patterns of co-occurring woody plants on contrasting soils in a subtropical savanna, Texas A&M University, College Station, TX.


VITA

E Bai

EDUCATION:

2001 – 2007 **Texas A&M University**, College Station, TX
Ph.D. in Rangeland Ecology and Management, Aug. 2007

1996 – 2000 **Nankai University**, Tianjin, China
B.Sc. in Environmental Science, June 2000

HONORS & AWARDS:

- Tom Slick Fellow, Texas A&M University (2005-2006)
- Wasko Fellow, Texas A&M University (2005-2006)
- Regents Fellow, Texas A&M University (2001-2002)
- Association of Former Students Fellowship, Texas A&M University (2001-2002)
- Research and Presentation Grant, Office of Graduate Studies and Association of Former Students, Texas A&M University. (2004)
- Excellent Student in Academic, Moral and Health Award of Nankai University (1999)

SELECTED PRESENTATIONS AT PROFESSIONAL MEETINGS: