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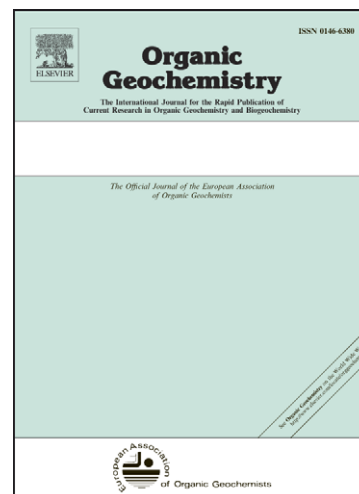
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1 **Spatial patterns of soil $\delta^{13}\text{C}$ reveal grassland-to-woodland successional processes**

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20 **Abstract**

21 Many grasslands and savannas around the world have experienced woody plant
22 encroachment in recent history. In the Rio Grande Plains of southern Texas, subtropical
23 woodlands dominated by C₃ trees and shrubs have become significant components of
24 landscapes once dominated almost exclusively by C₄ grasslands. In this study, spatial
25 variation of soil $\delta^{13}\text{C}$ was used to investigate patterns of transformation. Previous
26 research has shown that grassland-to-shrubland transitions are initiated when discrete,
27 multi-species shrub clusters organized around a honey mesquite (*Prosopis glandulosa*)
28 tree nucleus established in grassland. It is inferred from space-for-time substitution and
29 modeling studies that as new shrub clusters are initiated and existing clusters enlarge,
30 coalescence will occur, leading to the formation of groves; and that groves will
31 eventually merge to form woodlands. The hypothesis that present-day mesquite groves
32 represent areas where individual discrete shrub clusters have proliferated and coalesced
33 was evaluated by comparing patterns of soil $\delta^{13}\text{C}$ within isolated shrub clusters (n=6) to
34 those in nearby groves (n=3). Mean soil $\delta^{13}\text{C}$ within discrete clusters was lowest in the
35 center (-23.3 ‰), increased exponentially toward the dripline (-20.1 ‰), and stabilized at
36 a relatively high value approximately 15 cm beyond the dripline (-18.9 ‰). The spatial
37 structure of soil $\delta^{13}\text{C}$ in groves was consistent with that which would be expected to
38 occur if present-day grove communities were a collection of what once were individual
39 discrete clusters that had fused. As such, it provides direct evidence in support of
40 conceptual and mathematical models derived from indirect assessments. However,
41 spatial patterns of soil $\delta^{13}\text{C}$ suggest that groves are not simply a collection of clusters

42 with respect to primary production and SOC turnover. This study illustrates how soil
43 $\delta^{13}\text{C}$ values can be used to reconstruct successional processes accompanying vegetation
44 compositional change, and its consequences for ecosystem function.

45 **Introduction**

46 In recent history, many grasslands and savannas in Africa, Asia, Australia, Europe,
47 South America, and North America have experienced woody plant encroachment (Archer,
48 1995; Archer *et al.*, 2001; Arnold, 1995; Bai *et al.*, 2009; Boutton *et al.*, 1998; Maestre *et*
49 *al.*, 2009; Mast *et al.*, 1997; Menaut *et al.*, 1990; van Auken, 2000). This dramatic
50 change in tree-shrub-grass composition appears to be driven by human manipulation of
51 fire frequency and livestock grazing (Archer, 1995; Asner *et al.*, 2004; Boutton *et al.*,
52 1998; Jeltsch *et al.*, 2000; Midgley & Bond, 2001), but may also be influenced by
53 changes in climate, atmospheric composition, and atmospheric deposition (Archer *et al.*,
54 2001; Bond, 2008; Bond & Midgley, 2000; Tape *et al.*, 2006; Wigley *et al.*, 2009).
55 Increased abundance of woody plants has the potential to profoundly influence grassland
56 biodiversity, hydrology, biogeochemistry, and landscape evolution (Boutton *et al.*, 1998;
57 Breshears, 2006; Nobel, 1997; Rappole *et al.*, 1986; Schlesinger *et al.*, 1990; Shachak *et*
58 *al.*, 2008). Due to its impacts on commercial livestock grazing and other land uses, this
59 phenomenon has long been a concern to natural resource managers (Fisher, 1950; Fisher,
60 1977; Rappole *et al.*, 1986). Although this vegetation change has been widely recognized,
61 relatively little is known of the rates, dynamics, and patterns of woody plant proliferation
62 and its impact on ecosystem processes.

63 Subtropical woodlands dominated by C₃ trees and shrubs in the Rio Grande Plains
64 of southern Texas have become significant components of landscapes once dominated
65 almost exclusively by C₄ grasslands (Boutton *et al.*, 1998). Reports from early settlers
66 indicated that much of southern Texas was open grassland or savanna in the mid-1800s
67 (Inglis, 1964), but today the vegetation in this region is savanna parkland in which
68 subtropical woodlands cover large portions of the landscape (Archer *et al.*, 2001; Bai *et*
69 *al.*, 2009). Previous studies in this region have shown that woody encroachment is
70 initiated by the establishment of *Prosopis glandulosa* (honey mesquite – an N₂-fixing tree
71 legume) in the grassland. As these mesquite trees grow, they serve as recruitment foci,
72 facilitating the establishment of other woody species beneath their canopies (Archer,
73 1995; Archer *et al.*, 1988). This process has led to the formation of multi-species woody
74 clusters that may range up to ~10 m in diameter. It has been hypothesized that as new
75 clusters form and existing clusters expand, coalescence occurs, leading to the formation
76 of mesquite groves that range from ~20-100 m in diameter in size (Archer, 1995; Stokes,
77 1999). Woodlands would eventually develop as shrub clusters and groves continue to
78 form and expand. This hypothetical successional scenario is based on “space-for-time”
79 and modeling studies of vegetation structure, wherein shrub clusters of different sizes and
80 compositional attributes were inferred to represent the stages of successional
81 development. Though widely used in studies of plant succession such inferences can be
82 misleading (Austin, 1977; Johnson & Miyanishi, 2008; Shugart *et al.*, 1981).
83 Confirmation with direct measurements is thus desirable.

84 The stable carbon isotopic composition ($\delta^{13}\text{C}$) of soil organic carbon (SOC) is a
85 powerful tool for making direct assessments of vegetation changes where C₃ ($\delta^{13}\text{C} \approx -27$

86 ‰) and C₄ ($\delta^{13}\text{C} \approx -13\text{‰}$) plants coexist. Spatial variation in $\delta^{13}\text{C}$ of SOC provides
87 direct evidence of patterns and processes of woodland development in bioclimatic
88 regions where C₃ woody plants occur with C₄ grasses (Bai *et al.*, 2009; Biedenbender *et*
89 *al.*, 2004; Boutton *et al.*, 1999; Jessup *et al.*, 2003; Krull *et al.*, 2005; McPherson *et al.*,
90 1993). While some studies have reported that there can be carbon isotopic fractionation
91 during decomposition of soil organic matter (Wynn 1997; Novak *et al.* 2009) and
92 selective preservation of C₃-derived SOC over C₄-derived SOC (Wynn & Bird 2007),
93 prior studies in our study area showed no significant isotopic fractionation during soil
94 organic carbon formation (Boutton, 1996; Boutton *et al.*, 1998; 1999). In the Rio Grande
95 Plains of southern Texas, all woody plants use the C₃ photosynthetic pathway ($\delta^{13}\text{C} \approx -$
96 27‰) and all grasses use the C₄ pathway ($\delta^{13}\text{C} \approx -13\text{‰}$) (Boutton *et al.*, 1999). Thus,
97 soil $\delta^{13}\text{C}$ values can provide information regarding both the shift from C₄ to C₃
98 dominance, and the relative contribution of the invading C₃ woody plants to SOC. Since
99 vegetation change from C₄ grassland to C₃ shrubland has been relatively recent in this
100 region, the soil organic carbon pool beneath wooded areas is comprised of carbon derived
101 from both the original C₄-dominated grassland and from the more recent C₃ woody plants,
102 with $\delta^{13}\text{C}$ values varying as a function of time of woody plant occupation (e.g., Boutton
103 *et al.*, 2009; Liao *et al.*, 2006b;).

104 The purpose of this study was to quantify within-patch variability of the $\delta^{13}\text{C}$ of
105 soils associated with discrete shrub cluster and grove communities as a direct test of
106 hypothesized grassland-to-woodland successional processes. We examine the following
107 possible scenarios of woody cluster and grove formation within a grassland matrix (see
108 legend of Fig. 1 for additional details): (1) If woody clusters form via recruitment of

109 shrubs beneath and around mesquite canopies, then soil $\delta^{13}\text{C}$ should increase along
110 transects from the bole of mesquite plants in cluster centers (the point of longest woody
111 plant occupancy) out into the adjacent grasslands (Fig. 1a); and (2) If groves develop via
112 the establishment, expansion and coalescence of discrete mesquite clusters, then spatial
113 patterns of soil $\delta^{13}\text{C}$ within groves should mimic those observed along bole-to-dripline
114 transects within discrete clusters; and should vary as a function of the size (age) of the
115 mesquite plants within the groves (Fig. 1b).

116 **Materials and methods**

117 **Study area**

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

123 The landscape grades (1-3% slopes) from sandy loam uplands to clay loam and
124 clay lowlands, and elevations range from 75-90 m. Soils are primarily Typic Argiustolls
125 with a subsurface argillic horizon; however, patches of Typic Haplustepts lacking an
126 argillic horizon are also found in the uplands (Archer, 1995). The vegetation is
127 subtropical savanna parkland comprised of discrete shrub clusters (consisting of a single
128 honey mesquite tree with up to 15 understory shrub species) and larger groves (consisting
129 of multiple mesquite tree with associated understory shrubs) embedded within a

130 grassland matrix. The grasslands consist mainly of rhizomatous and weakly caespitose
131 C₄ grasses, and C₃ forbs. Clusters and groves are dominated by honey mesquite and
132 *Zanthoxylum fagara* (lime prickly ash). Honey mesquite and several other leguminous
133 trees and shrubs present in wooded areas are capable of symbiotic N₂-fixation (Zitzer *et*
134 *al.*, 1996). See Archer (1995) and Boutton *et al.* (1998) for additional details on soils,
135 vegetation, and climate.

136 **Field sampling procedures**

137 Six discrete shrub clusters and three groves were randomly selected in the sandy
138 loam upland portion of a landscape. Three transects radiating from the center to the
139 perimeter of each cluster and into the adjoining grassland matrix were established in
140 random directions at approximately 120° apart. A total of 130 spatially-explicit soil
141 samples (0-15cm) were collected in the six cluster plots as shown in Fig. 2. Groves (n=3)
142 were sampled using tree-to-tree transects originating from the approximate center of the
143 grove and zig-zagging from one mesquite tree to the next mesquite tree and towards the
144 grove perimeter and into the grasslands. These transect samples were supplemented with
145 additional random samples. The total numbers of sampling points were: (a) 45 transect
146 points and 17 random points in Grove 1; (b) 19 transect points and 4 random points in
147 Grove 2; and (c) 29 transect points and 7 random points in Grove 3. Points along the
148 grove transects were classified into one of four categories based on their positions
149 relative to mesquite trees: (1) base: next to the bole of a mesquite tree, (2) mid: ca. half-
150 way between the canopy edge and a bole, (3) edge: within 15 cm of a mesquite canopy
151 edge and (4) grass: in grassland matrix (Fig. 3b).

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

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

164 **Soil analyses**

165 Soils were sieved through a 2 mm screen to remove coarse organic fragments and
166 gravel, dried at 60°C for 48 hours and ground in a centrifugal mill (Angstrom, Inc.,
167 Belleville, MI). Subsamples were weighed into silver capsules using a microbalance,
168 treated with HCl vapor in a desiccator (Harris *et al.*, 2001) to volatilize inorganic C
169 present as CaCO₃, then analyzed for $\delta^{13}\text{C}$ of SOC using a Carlo Erba EA-1108 (CE
170 Elantech, Lakewood, NJ) elemental analyzer interfaced with a Delta Plus isotope ratio
171 mass spectrometer operating in continuous flow mode (ThermoFinnigan, San Jose, CA).

172 Carbon isotope ratios are presented in standard δ notation:

173
$$\delta = [(R_{SAMPLE} - R_{STD}) / R_{STD}] \times 10^3 \text{‰} \quad (1)$$

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

176 **Statistical analyses**

177 One-way ANOVA was used to test for the differences in soil $\delta^{13}\text{C}$ with respect to
178 vegetation type (grassland, discrete shrub cluster, and grove) and transect locations in
179 discrete shrub clusters and groves. Fisher's LSD was used to distinguish differences
180 among means. Regression analyses were performed using Sigma Plot 8.0 (SPSS Inc.,
181 2002) to determine the relationship between mesquite tree GPI (see definition below)
182 values and soil $\delta^{13}\text{C}$.

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

194
$$\text{GPI} = \sum_{i=1}^n D_i / Z_i \quad (2)$$

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

203 **Results**

204 The mean $\delta^{13}\text{C}$ of grassland soils ($-19.0 \pm 0.1 \text{‰}$) was significantly greater than
205 that of soils within discrete shrub clusters ($-21.0 \pm 0.2 \text{‰}$) and groves ($-21.6 \pm 0.2 \text{‰}$);
206 and values for groves were significantly lower than those for clusters ($F=106.0$; $p<0.001$;
207 Table 1). The coefficient of variation of soil $\delta^{13}\text{C}$ was highest in clusters (9.5%) and
208 lowest in grasslands (5.3%). There was no evidence of past shrub occupation in the
209 grassland grids because soil $\delta^{13}\text{C}$ values were relatively homogeneous and higher than -
210 20.9‰.

211 Soil $\delta^{13}\text{C}$ increased exponentially from the center of the cluster (-23.3‰) to the
212 edge of the canopy (-20.1‰), and then reached a value typical of grassland (-19.3‰) at
213 a distance approximately 1/3 of the cluster diameter beyond the canopy (Fig. 3a). Within
214 groves, mean $\delta^{13}\text{C}$ values were lowest at the base of mesquite trees, increasing towards
215 the perimeters of their canopies (Fig. 3b). This was the case for both interior and
216 periphery mesquite trees. However, with the exception of soils near the base of the

217 mesquite trees, interior soils had consistently lower soil $\delta^{13}\text{C}$ values than periphery soils
218 (Fig. 3b). $\delta^{13}\text{C}$ values for soils at the base of mesquite trees in groves were an inverse
219 function of mesquite basal diameter (Fig. 4a). The GPI, which accounts for the number,
220 distance, and size of mesquite trees in the neighborhood of non-mesquite bole soil
221 sampling points, was also inversely related to soil $\delta^{13}\text{C}$ (Fig. 4b),

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

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

231 Discussion

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

233 Present grasslands in the Rio Grande Plains of southern Texas are comprised of a
234 mixture of C_3 forbs and C_4 grasses. $\delta^{13}\text{C}$ values of soils in the grassland matrix at our
235 study site averaged $-19.0 \pm 1.0 \text{‰}$ (Table 1), similar to values reported previously for
236 grassland litter (-19‰) and roots (-20.5‰) in this and many other C_4 -dominated
237 grasslands around the world (Boutton *et al.*, 1998). Following woody plant invasion into
238 the grassland, mean soil $\delta^{13}\text{C}$ values decreased to -21.0‰ in shrub clusters and -21.6‰
239 in groves (Table 1), reflecting the simultaneous loss of SOC derived from the original C_4

240 grassland and the accumulation of SOC derived from the invading C₃ woody vegetation.
241 Soil $\delta^{13}\text{C}$ values indicated that a substantial proportion of the SOC presently in both
242 clusters and groves was derived from C₄ plants (grasses). This is consistent with previous
243 isotopic studies in this region (Boutton *et al.*, 1998; 1999; Gill & Burke, 1999; Liao *et al.*,
244 2006a) and supports the conclusion that C₄ grass communities have been replaced by C₃
245 woody plant communities. Analyses of repeat aerial photography at this site (Archer *et al.*
246 1988) and others (Browning *et al.*, 2008) suggests that while there may be net increases
247 in woody plant cover within grasslands at decadal time scales, shrubs and shrub patches
248 do turnover and revert back to grass domination. However, we found no isotopic
249 evidence for this in our detailed grid-sampling within grasslands. This may simply
250 indicate insufficient sampling ($n = 3\text{-}6 \times 10\text{ m}$ areas); or it may indicate that shrub to
251 grass transitions most often occur where shrubs have not occupied the site long enough to
252 have substantively influenced soil $\delta^{13}\text{C}$.

253 **Spatial patterns of soil $\delta^{13}\text{C}$ in discrete shrub clusters**

254 Soil $\delta^{13}\text{C}$ was lowest in shrub cluster centers and increased sigmoidally towards
255 the cluster/grassland boundary (Fig. 3a), consistent with the hypothesis that honey
256 mesquite initially colonizes the grassland, and then other shrub species subsequently
257 establish beneath its canopy (Fig. 1a) (Archer, 1995; Archer *et al.*, 1988). Patterns of soil
258 $\delta^{13}\text{C}$ have also been shown to be strongly coupled to the distribution of mesquite
259 canopies in semiarid grassland in southeastern Arizona (Biggs *et al.*, 2002) and to vary
260 with time since mesquite occupation (McClaran *et al.*, 2008; Throop & Archer, 2008;
261 Wheeler *et al.*, 2007).

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

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

269 Soil $\delta^{13}\text{C}$ was heterogeneous in groves and strongly affected by the presence of
270 mesquite. The inverse relationship between soil $\delta^{13}\text{C}$ and mesquite basal diameter (Fig.
271 4a) indicates points with lower soil $\delta^{13}\text{C}$ have a longer history of C_3 organic matter inputs
272 from mesquite. This is consistent with other studies showing a negative relationship
273 between the size/age of mesquite trees and soil $\delta^{13}\text{C}$ (Liao *et al.*, 2006a; Tieszen &
274 Archer, 1990; Wheeler *et al.*, 2007). The negative relationship between soil $\delta^{13}\text{C}$ and the
275 mesquite GPI (Fig. 4b) further reflects the fact that areas within groves with larger/denser
276 mesquite plants have a longer history of shrub occupation, a higher C_3 shrub net primary
277 productivity, lower C_4 grass production or a combination of these. Therefore, we
278 conclude that the present-day pattern of soil $\delta^{13}\text{C}$ values reflects successional processes
279 leading to the formation of shrub clusters and groves on this landscape.

280 If present-day groves were formed by coalescence of discrete clusters as inferred
281 from space-for-time and modeling reconstructions (Archer, 1995; Stokes, 1999), patterns
282 of soil $\delta^{13}\text{C}$ would mimic those in Fig. 3a, be repeated for each mesquite tree in the grove,
283 and thus vary as depicted in Fig. 1b, with bole-to-mesquite dripline gradients being
284 attenuated with decreasing mesquite plant size. Results support the proposed mechanism

285 of woody plant community development in that soil $\delta^{13}\text{C}$ in groves was lowest next to the
286 boles of mesquite plants, and increased steadily toward the grassland (Fig. 3b), similar to
287 the isotopic pattern in discrete clusters (Fig. 3a). As with discrete clusters, soils at the
288 base of mesquite plants in groves typically had the lowest $\delta^{13}\text{C}$ values relative to sample
289 points around them, indicating the specific locations mesquite plants initially colonized
290 within the grassland matrix; with spatial variation in mesquite bole $\delta^{13}\text{C}$ (Fig. 5)
291 reflecting variation in time of occupation (as inferred from plant size; Fig. 4a). Direct
292 evidence from isotopic patterns therefore supports the hypothesis that mesquite groves
293 are comprised of shrub cluster age-states, and that they have developed via the
294 coalescence of discrete woody clusters.

295 Do ecosystem processes change as discrete clusters develop into groves? Or put
296 another way, are groves merely a collection of clusters from an ecosystem function
297 standpoint? Figure 3b suggests ‘yes’ and ‘no’, respectively, to these two questions. The
298 bole points generally had lower soil $\delta^{13}\text{C}$ compared to other points along the transects,
299 showing the locations of the pioneer clusters. However, the amplification of the C_3 signal
300 amongst samples associated with interior shrub clusters relative to those at the grove
301 perimeter suggests that processes and environmental factors influencing SOC pools and
302 fluxes (e.g., primary production, decomposition, microclimate) are likely to differ
303 between discrete clusters and groves. The occurrence of lower-than-expected soil $\delta^{13}\text{C}$
304 values in locations without mesquite trees may also be indicative of divergence in
305 ecological function. For example, the 4th point on transect 2 of grove 1 (an “edge” point),
306 the 11th point on transect 3 of grove 1 (a “mid” point), points 2 and 3 on transect 2 in
307 grove 3, and points 2-5 on transect 2 in grove 3 had comparable or lower soil $\delta^{13}\text{C}$ values

308 than their nearest bole point (Fig. 5). It is also possible that there were mesquite trees
309 located at these points sometime in the past, but they have since died. Previous studies
310 indicated that shallow-rooted understory shrubs may accelerate the mortality of mesquite
311 trees (Archer, 1995; Archer *et al.*, 1988; Barnes & Archer, 1999). We speculate these
312 “anomalous” soil $\delta^{13}\text{C}$ values within groves may represent locations where founding
313 mesquite plants have died and are no longer evident aboveground. This is analogous to
314 “ghost trees” in burned grasslands of Arizona where aboveground evidence for trees once
315 present in grasslands had been obliterated by fire (Biggs *et al.*, 2002). Thus, while it
316 appears that groves are an amalgamation of discrete cluster age-states that have fused,
317 they appear to have emergent properties that distinguish them from a simple collection of
318 isolated discrete clusters.

319 **Synthesis and conceptual model of woody patch development in grasslands**

320 Spatial patterns of soil $\delta^{13}\text{C}$ values provided direct evidence for successional
321 processes resulting in the formation of woody patches in grasslands of the Rio Grande
322 Plains of Texas, and supported prior inferences based on plant community analyses and
323 space-for-time studies of vegetation dynamics (Archer *et al.*, 1988; Bai *et al.*, 2009).
324 Based on the results of this study and previous findings (Stokes, 1999), we propose a
325 conceptual model (Fig. 6) that appears to account for the successional processes of
326 woody encroachment into upland grasslands. Woody clusters are initiated when
327 mesquite trees establish in the grassland (Fig. 6a); as these mesquite grow, other woody
328 species establish beneath their canopies, and multispecies woody clusters develop (Fig.
329 6b). Discrete clusters then grow in size as the canopies of the mesquite plant and its
330 associated shrubs develop and as new individuals are recruited around the canopy

331 perimeter. As new clusters are initiated and existing clusters grow, coalesce occurs and
332 leads to the formation of groves (Fig. 6c). Based on ages of mesquite trees, historical
333 aerial photographs, turnover rates of SOC, and natural ^{14}C measurements on SOC, it
334 appears that that mature woody clusters and groves can form within 150 years or less
335 (Boutton et al. 1998, Archer et al. 2001).

336 Spatial patterns of soil $\delta^{13}\text{C}$ provide not only chemical evidence of vegetation
337 successional patterns, but also a strong spatial context for future studies aimed at
338 understanding the factors controlling vegetation dynamics and predicting the future
339 dynamics of the landscape. These methods and approaches should be applicable in any
340 ecosystem or landscape where successional processes have led to shifts in the distribution
341 and abundance of $\text{C}_3\text{-C}_4$ functional types.

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505 **Tables**

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

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

509

510 **Figure legends:**

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

520 Fig. 2. The sampling regime in upland discrete shrub clusters. Soil samples were taken
521 along transects extending from the center (location 1) and into the surrounding grassland
522 (location 7). Locations 2 and 3 were 1/3 and 2/3 of distance from center to drip line.
523 Locations 4 and 5 were ~15 cm inside and beyond the canopy dripline, respectively.
524 Locations 6 and 7 were beyond the cluster drip line at distances corresponding to 1/3 and
525 2/3 the cluster center to drip line distance.

526 Fig. 3. Mean (\pm SE) soil $\delta^{13}\text{C}$ (‰) along transects within discrete shrub cluster (a) and
527 grove (b) patches. Transects within the clusters extend from their centers into the
528 surrounding grasslands (numbers on the x-axis correspond to sampling locations
529 described in Fig. 2). The dashed vertical line and the dotted horizontal line in (a)
530 represent the location of the shrub cluster margin and the mean soil $\delta^{13}\text{C}$ value in the

531 grassland plots respectively. Within groves (b), “base” represents sampling points at the
532 boles of the mesquite trees; “mid” represents points midway between the bole and the
533 edge of the mesquite canopy; “edge” represents points at the mesquite canopy drip line;
534 and “grass” represents samples in the adjoining grassland community. “Periphery” points
535 (open circles) are from mesquite plants along the perimeter of the grove (n=36);
536 “Interior” points are those from mesquite plants within the groves (n=51). “All”
537 represents all points sampled on transects (n=87). Different letters indicate differences
538 between means (test results were the same for the three categories in groves) (Fisher’s
539 LSD; $\alpha = 0.05$).

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

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

548 Fig. 6. Conceptual model of successional processes in the conversion of grassland to
549 woodland (based on Archer 1995, Stokes 1999 and data from this study). Black dots
550 represent pioneer mesquite trees within a C_4 grassland matrix (white background, highest
551 soil $\delta^{13}\text{C}$ values). Cross-hatched areas of various sizes represent shrub cluster age-states
552 organized around mesquite plants (lowest soil $\delta^{13}\text{C}$ values). Grey areas represent groves

553 of coalesced clusters (intermediate soil $\delta^{13}\text{C}$ values). Some newly established mesquite
554 tree may get into the system during the grove development.

ACCEPTED MANUSCRIPT

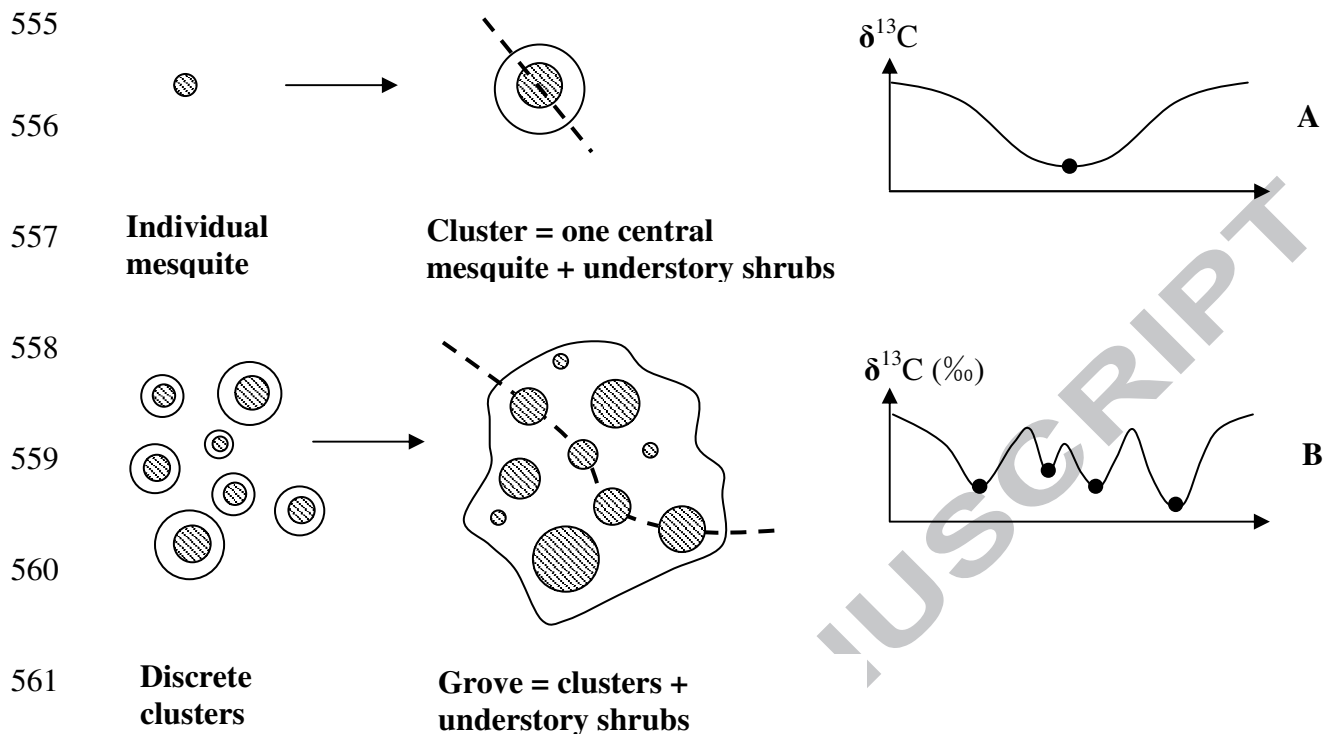


Figure 1

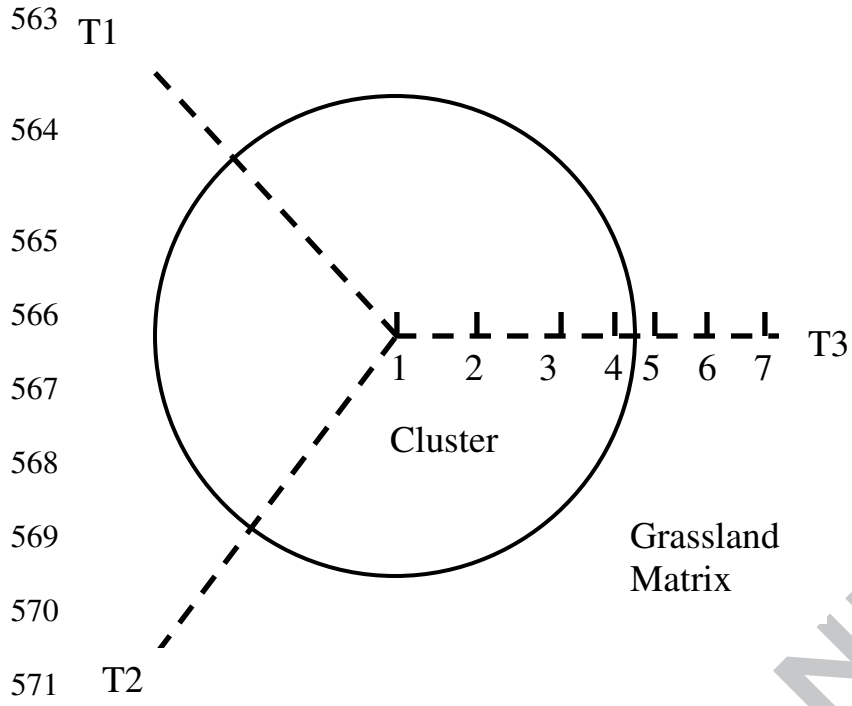
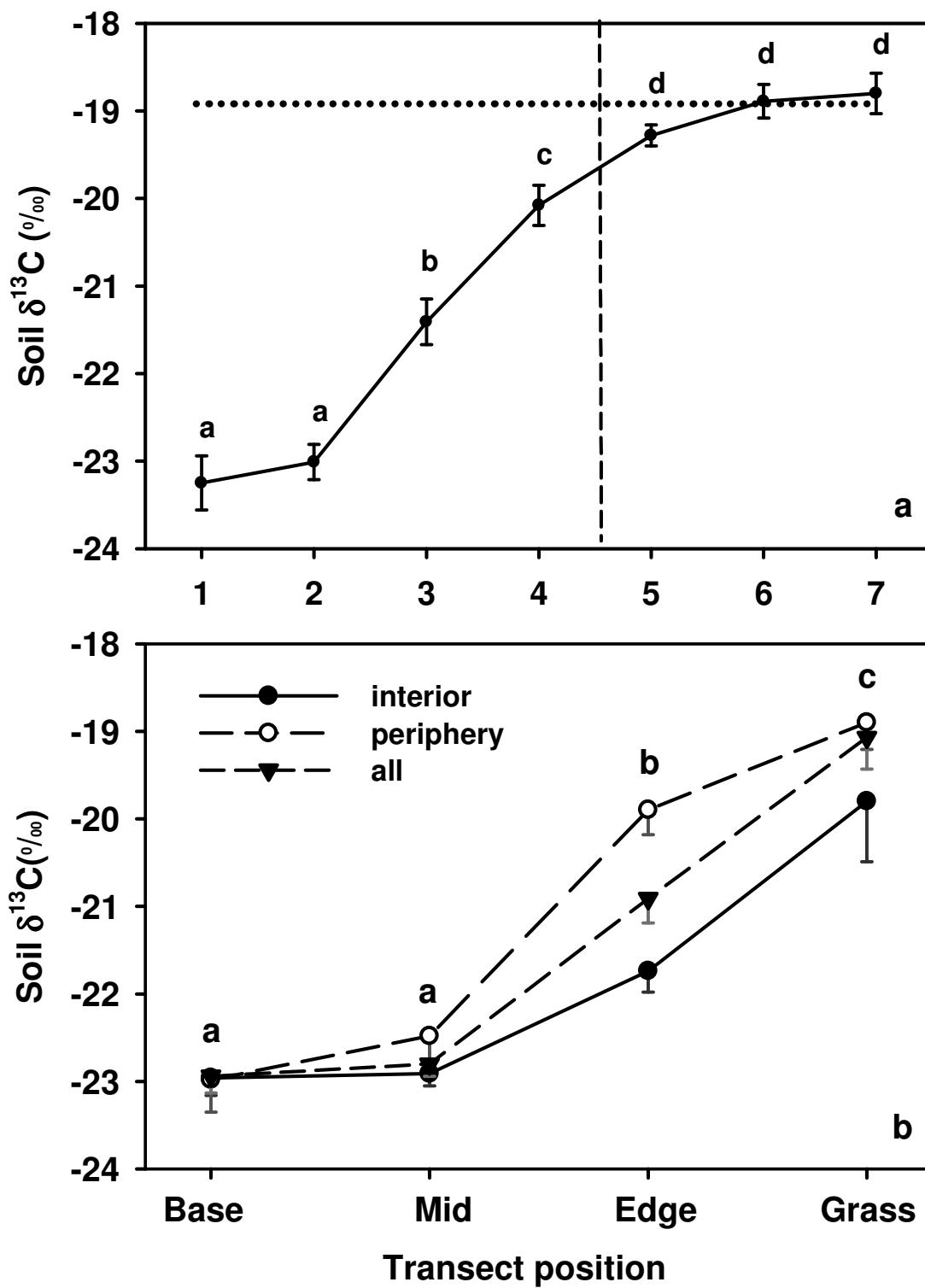
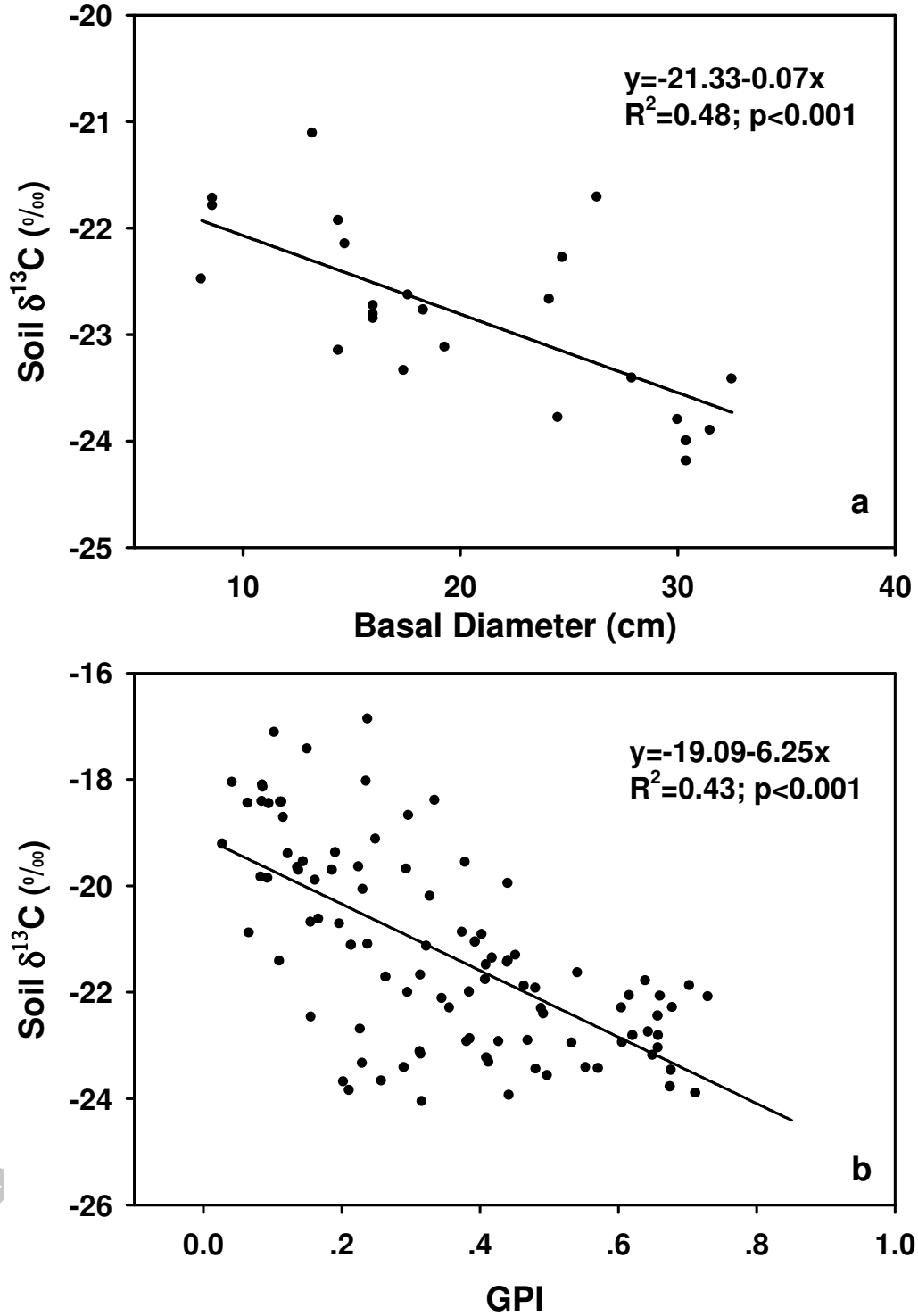


Figure 2

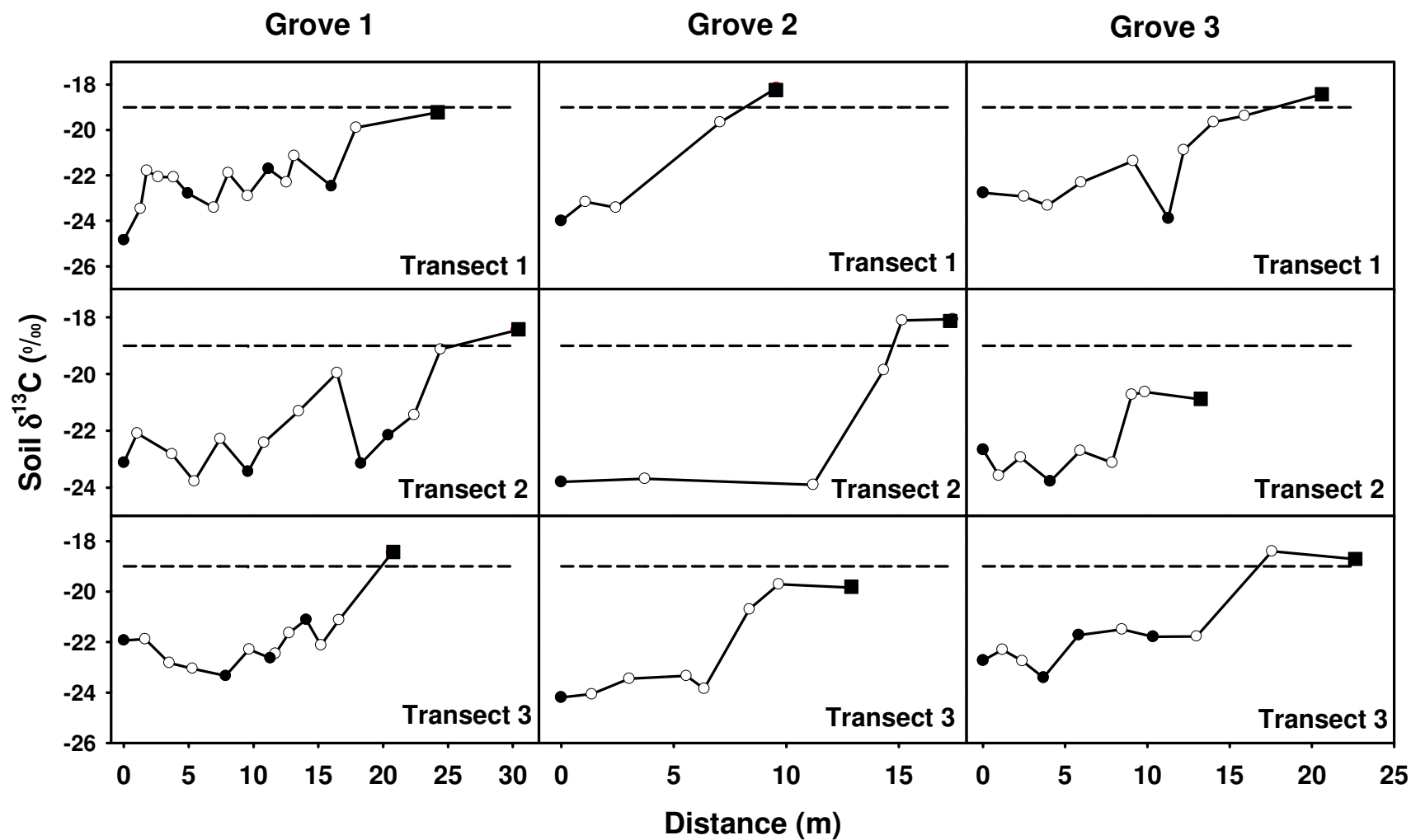


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575 Figure 3



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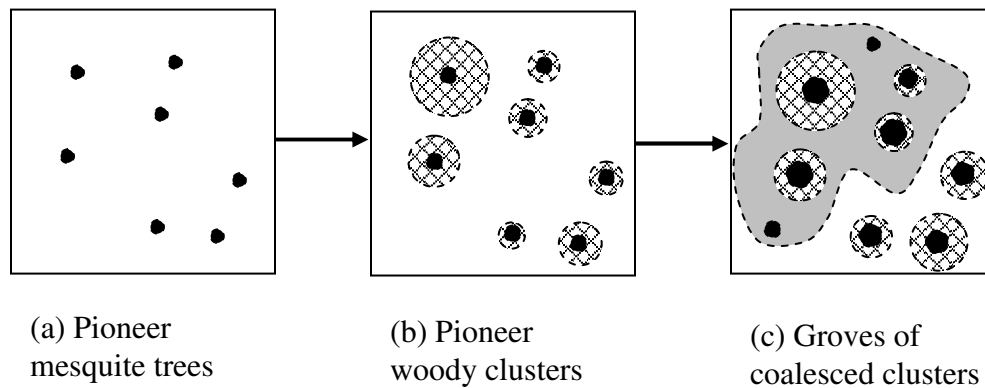


Figure 6