

1 **Title:** Root density distribution and biomass allocation of co-occurring woody plants on
2 contrasting soils in a subtropical savanna parkland

3 **Running title:** Root distribution on contrasting soils in savanna

4 **Authors:** Yong Zhou^{1,4} *, Stephen E. Watts², Thomas W. Boutton¹, Steven R. Archer³

5 **Author affiliations:**

6 ¹ Department of Ecosystem Science and Management, Texas A&M University, College Station,
7 TX, 77843-2126, USA

8 ² 412th Civil Engineer Group, Environmental Management Division, Edwards Air Force Base, CA
9 93524-8600

10 ³ School of Natural Resources and the Environment, University of Arizona, Tucson, AZ, 85721-
11 0043, USA

12 ⁴ Present address: Department of Ecology and Evolutionary Biology, Yale University, New
13 Haven, CT 06520-8106, USA

14 *** Corresponding author**

15 E-mail: yong.zhou@yale.edu

16 **Abstract**

17 **Background and aims:** Root niche partitioning among trees/shrubs and grasses facilitates their
18 coexistence in savannas, but little is known regarding root distribution patterns of co-occurring
19 woody plants, and how they might differ on contrasting soils.

20 **Methods:** We quantified root distributions of co-occurring shrubs to 2 m on argillic and non-
21 argillic soils.

22 **Results:** Root biomass in the two shrub communities was 3- to 5- fold greater than that in the
23 grassland community. *Prosopis glandulosa*, the dominant overstory species was deep-rooted,
24 while the dominant understory shrub, *Zanthoxylum fagara*, was shallow-rooted (47% vs. 25 % of
25 root density at depths > 0.4 m). Shrubs on argillic soils had less aboveground and greater
26 belowground mass than those on non-argillic soils. Root biomass and density on argillic soils was
27 elevated at shallow (< 0.4 m) depths, whereas root density of the same species on non-argillic soils
28 were skewed to depths > 0.4 m. Root density decreased exponentially with increasing distance
29 from woody patch perimeters.

30 **Conclusions:** Belowground biomass (carbon) pools increased markedly with grassland-to-
31 shrubland state change. The presence/absence of a restrictive barrier had substantial effects on root
32 distributions and above- vs. belowground biomass allocation. Differences in root distribution
33 patterns of co-occurring woody species would facilitate their co-existence.

34 **Key words:** root distribution, soil profile, *Prosopis glandulosa*, argillic horizon, woody patches,
35 interspecific competition, subtropical savanna parkland, woody plant encroachment.

36 **Introduction**

37 The proliferation of woody species in grass-dominated ecosystems has occurred in many
38 arid and semi-arid regions around the world during the past century (van Auken 2000; Archer et
39 al. 2001; Stevens et al. 2017). This geographically extensive land cover change appears to be
40 driven largely by chronic livestock grazing, fire suppression, rising atmospheric CO₂
41 concentrations, and their interactions (Wigley et al. 2010; Archer et al. 2017; Brunsell et al. 2017;
42 Devine et al. 2017; Venter et al. 2018), and has the potential to alter grassland biodiversity,
43 hydrology, biogeochemistry, and landscape evolution (Boutton et al. 1999; Huxman et al. 2005;
44 Ratajczak et al., 2012; Zhou et al. 2017a, 2018a, b).

45 In the Rio Grande Plains of southern Texas, C₃ trees and shrubs have formed multispecies
46 patches within the original C₄ grassland matrix, resulting in a subtropical savanna parkland
47 landscape (Archer 1995; Boutton 1996; Archer et al. 2001). Upland soils of this area are sandy
48 loams characterized by a laterally continuous argillic (*B_t*) horizon interspersed with patches where
49 the argillic horizon is absent (Archer 1995; Zhou et al. 2017a). Previous studies in this region
50 showed that the development of woody patches is initiated by the colonization of N₂-fixing
51 *Prosopis glandulosa*, which then facilitate the recruitment of a diverse assemblage of other shrub
52 species under their canopies to form small discrete clusters (< 100 m²) (Archer et al. 1988, 2001;
53 Archer 1995). Where non-argillic inclusions are present, discrete clusters expand laterally and
54 coalesce to form large groves (> 100 m²) (Archer 1995; Bai et al. 2012; Zhou et al. 2017a).
55 However, where the argillic horizon is present, woody clusters remain relatively small and isolated
56 within the grassland matrix and do not fuse with other clusters. This suggests that the
57 presence/absence of the argillic horizon is influencing vegetation dynamics and landscape
58 evolution, perhaps by affecting root distribution patterns of the encroaching woody species.

59 Root architecture and distribution are, in part, genetically determined, but are also
60 influenced by soil properties (e.g. texture and bulk density) and soil resources (e.g. water and
61 nutrients) (Valverde-Barrantes et al. 2013; Wachsman et al. 2015; Morris et al. 2017; Fan et al.
62 2017). The impact of temporally and spatially heterogeneous soil resources on root distribution
63 patterns of individual or coexisting species has long been studied (Walter 1971; Parrish and Bazzaz
64 1976) and reviewed recently (Hutching et al. 2003; Hodge 2004; de Kroon et al. 2012; Ward et al.
65 2013). Site-specific soil physical attributes such as texture can also significantly influence root
66 distribution (e.g., Schenk and Jackson 2005; Xu and Li 2009). Several studies have reported
67 negative correlations between soil clay content and root density (Strong and Roi 1985; Plante et
68 al. 2014), likely a result of reduced soil porosity, hydraulic conductivity, shrink/swell shearing
69 forces and/or increased soil resistance, all of which can inhibit root growth and elongation
70 (Lodovici 2004). In addition, the formation of clay-rich subsurface soil horizons (e.g. argillic
71 horizons) has been shown to affect vertical root distribution (Macinnis-Ng et al. 2010). For
72 example, Sudmeyer et al. (2004) found that where argillic horizons were present, tree root densities
73 were high in the coarse-textured surface soil, but decreased sharply below the subsurface clay-rich
74 argillic horizon.

75 Root distribution patterns of coexisting species affect vegetation dynamics and landscape
76 evolution by influencing interspecific competition for resources (Parrish and Bazzaz 1976; Tilman
77 1985; Schenk 2006; Ratajczak et al. 2011; Zhou et al. 2018c). During stand development, for
78 example, early successional species often have a significantly greater proportion of roots occurring
79 deeper in the soil profile than late successional species (Gale and Grigal 1987), ostensibly enabling
80 them to exploit larger volumes of soil with limited water and nutrients. Shallow-rooted species
81 appearing at later successional stages are better adapted to exploit resources that have accumulated

82 over time in the surface soil, thereby facilitating their establishment and coexistence with earlier
83 successional species via reduced interspecific competition. Numerous studies in savanna
84 ecosystems have used differences in root distribution between trees and grasses to explain their
85 coexistence via niche partitioning of water uptake (e.g. Walter 1971; Sankaran et al. 2004;
86 February and Higgins 2010; Holdo 2013; Zhou et al. 2018c). However, few studies have quantified
87 root distribution patterns of co-occurring trees and shrubs in multispecies woody patches in
88 savanna ecosystems, especially in response to contrasting soils.

89 The purpose of this study was to quantify root distribution patterns of co-occurring woody
90 plants in small, discrete clusters on soils with a well-developed argillic horizon (hereafter argillic
91 soils), and in groves that occur on soils lacking an argillic horizon (hereafter non-argillic soils) in
92 a subtropical savanna parkland where the abundance of woody species has increased substantially
93 during the past century (Archer 1995; Boutton et al. 1998; Bai et al. 2009; Zhou et al. 2017a).
94 Specific objectives were to quantify: (1) the influence of the presence or absence of an argillic
95 horizon on root distribution; (2) the vertical root distributions of co-occurring woody plant species;
96 (3) patterns of belowground vs. aboveground biomass allocation of woody plants on argillic and
97 non-argillic soils; and (4) the extension of woody plant lateral roots beyond cluster perimeters.
98 Results from these investigations were then used to explain observed vegetation dynamics and
99 patterns of landscape development in this subtropical savanna parkland.

100 **Methods and materials**

101 **Study site**

102 This study was conducted at the Texas A&M AgriLife La Copita Research Area (27°40' N,
103 98°12' W) located in the Rio Grande Plains of southern Texas, approximately 65 km west of Corpus

104 Christi. Climate of this region is subtropical, with mean annual temperature and precipitation of
105 22.4 °C and 680 mm, respectively. Annual rainfall is bimodal with peaks in May and September.
106 Soil moisture to a depth of 1.2 m ranges from 2 to 10 % (gravimetric soil water content) during
107 the growing season (unpublished data). Elevation ranges from 75 to 90 m above sea level.
108 Topography consists of uplands with slopes of 1-3% surrounded by lower-lying intermittent
109 drainages. Upland portions of the landscape where this study was conducted classify as fine-loamy,
110 siliceous, hyperthermic, Typic Argiustolls (Boutton et al. 1998) and are characterized by a well-
111 developed, nearly continuous clay-rich argillic (B_t) horizon. However, on some portions of the
112 upland landscape, there are patches where the argillic horizon is absent, and these soils classify as
113 coarse-loamy, siliceous, hyperthermic, Pachic Haplustolls (Boutton et al. 1998). Soil pH was
114 neutral at the surface, increased slightly (max = 7.8) with depth to 1.2 m, and did not differ between
115 argillic and non-argillic soils (Zhou et al. 2017b). Prior to the colonization of woody plants,
116 nitrogen and phosphorus content of argillic and on non-argillic soils to a depth of 1.2 m were
117 comparable (Zhou et al. 2018b). The presence/absence of the argillic horizon in upland soils is a
118 strong determinant of vegetation cover, as discussed below. The region, including the La Copita
119 Research Area, has been grazed continuously by domestic livestock since the mid- to late-1800s.
120 Grazing pressure at the La Copita has been light to moderate since it was designated as a research
121 area in 1981, and no fires have been documented for at least the past 50 yrs.

122 Evidence from sequential aerial photography, tree ring analyses, vegetation dynamics
123 modeling, and the isotopic composition of soils all indicate that this region was once relatively
124 open grassland and that woody plant encroachment into those grasslands has occurred over the
125 past 150 yrs, apparently in response to livestock grazing and fire suppression (Archer et al., 1988,
126 2001, 2004; Boutton et al., 1998, 1999). Our prior research at this site shows that woody

127 encroachment into grassland is initiated by the establishment of *Prosopis glandulosa* (honey
128 mesquite, hereafter *Prosopis*), a N₂-fixing legume (Zitzer et al. 1996; Soper et al. 2015) with an
129 arborescent growth form. *P. glandulosa* then serves as a nurse plant facilitating the recruitment of
130 other fruticose shrub species beneath its canopy, resulting in the formation of small (< 100 m²)
131 discrete woody clusters (Archer et al. 1988). Where the argillic horizon is absent, these shrub
132 clusters expand laterally and fuse to form larger (> 100 m²) groves (Archer 1995; Bai et al. 2012;
133 Zhou et al. 2017a). Therefore, upland portions of this landscape currently consist of discrete
134 clusters and groves scattered within a matrix of remnant C₄ grassland (Figure 1), which is similar
135 to the two-phase vegetation pattern described by Whittaker et al. (1979) for this region. Discrete
136 clusters and groves have similar plant species composition, with an overstory dominated by
137 *Prosopis* and an understory dominated by *Zanthoxylum fagara* (lime prickly ash, hereafter
138 *Zanthoxylum*) (Liu et al. 2013). See Appendix S1 for a listing of other common understory woody
139 species. Perennial grasses, including *Paspalum setaceum*, *Setaria geniculata*, *Bouteloua*
140 *rigidiseta*, and *Chloris cucullata*, dominate the remnant grassland matrix. Species names follow
141 Hatch et al (1990).

142 **Field sampling**

143 Two discrete clusters and two groves located within a 260 x 200 m upland grazing enclosure
144 established in 1984 were selected (Figure 1). A vegetation inventory was conducted in May of
145 1991 prior to excavating trenches for quantifying root distribution patterns (Table S1). Location,
146 species, height, and canopy diameter of each woody plant were recorded for each discrete cluster,
147 and in a 4 m wide belt immediately overlying the planned trench locations in each grove.
148 Aboveground biomass was quantified in plots (0.5 m × 0.5 m) spaced evenly along the center-line
149 of the planned trenches in each discrete cluster (n=3/cluster) and grove (n=5/grove). Shrubs were

150 cut at ground level and biomass sorted by species, oven-dried (60 °C) to constant weight, and
151 weighed. Age of *Prosopis* occurring within the harvested plots was estimated by counting annual
152 rings (Flinn et al. 1994).

153 Four main trenches (1 m wide × 2 m deep) were carefully excavated in June 1991, using a
154 backhoe to quantify vertical and horizontal root distribution patterns. The trench through discrete
155 cluster 1 was oriented north-south, while that for discrete cluster 2 was oriented east-west.
156 Trenches for the two groves were oriented the same as those for clusters and extended from their
157 canopy perimeters to their centers.

158 Short secondary trenches perpendicular to main trenches of each discrete cluster were
159 excavated to determine the magnitude of lateral root extension beyond woody canopy boundaries
160 (Figure S1). One of the secondary trenches was placed with the inner face 1 m from the edge of
161 the cluster canopy; the other was excavated at the opposite end of the main trench with the inner
162 face 3 m from the edge of cluster canopy edge. Data on shrub root density at 1, 2, 3, and 4 m
163 beyond the edge of cluster canopies were thus generated (Figure S1).

164 Roots were exposed by removing 3-5 mm of soil from trench faces with a small-toothed
165 scraper after wetting with a garden sprayer. This procedure ensured that roots obscured by backhoe
166 excavation were accounted for. Root density was quantified during June through August 1991 by
167 placing a gridded frame (0.2 x 0.2 m cells) against the trench face and counting the number of
168 roots in three diameter classes (1-3 mm, 3-10 mm, and >10 mm) in each grid cell. These root
169 diameter classes were chosen to facilitate parameterization of CENTURY model (Parton et al.
170 1992). Root density was quantified continuously from the soil surface to a depth of 2 m along the
171 entirety of all trench faces in clusters and groves. Shrub roots were visually distinct from grass and

172 herbaceous dicot roots and were identified to species using a key based on physical characteristics,
173 such as color, texture and morphology (Appendix S1). Roots of some species had black or brown
174 sheaths that distinguished them from other species, but no features that allowed us to distinguish
175 them from each other. Accordingly, we lumped species with ‘black’ or ‘brown’ roots that could
176 not identified to species. We made no attempt to distinguish between live and dead roots.

177 Root biomass was quantified within 0.2 m × 0.2 m × 0.2 m soil blocks arrayed continuously
178 from the soil surface to a depth of 2 m (Figure S2). This sampling was conducted at 1 m horizontal
179 intervals along the trench faces in discrete clusters, and at 2 m intervals in groves. Coarse woody
180 plant roots were removed by hand from each soil block, sorted by species and diameter class (1-3
181 mm, 3-10 mm and > 10 mm), and washed gently in water to remove adhering soil particles. Roots
182 not identified to species were recorded as black or brown. Then, 300 cm³ subsamples from each
183 soil block were used to isolate fine roots (< 1 mm) using a hydropneumatic elutriation system
184 (Gillison’s Variety Fabrication, Inc., Benzonia, MI) with primary (760 μm) and secondary (410
185 μm) sieves (Smucker et al., 1982). Non-root organic matter was separated manually from fine roots
186 following elutriation. Fine roots could not be identified to species. All root samples were oven-
187 dried (60 °C) to a constant weight for biomass determination.

188 Subsamples from each soil block were analyzed to determine both wet and dry Munsel color
189 (Pendleton and Nickerson 1951; Owens and Rutledge 2005). Munsel colors were used in
190 conjunction with other soil attributes to map soil horizons (Table S2). In addition, subsamples of
191 soil from selected blocks (Block C and E for both discrete clusters, Block A, I and Q for grove 1,
192 and Block A, K, and U for grove 2, see Figure S2) were analyzed for soil texture using the
193 hydrometer method (Sheldrick and Wang 1993) (Table S3).

194 We were also interested in determining the extent to which root biomass in the shrub
195 communities on argillic and non-argillic sites differed from that of the herbaceous matrix within
196 which they were embedded. To quantify root biomass in the herbaceous area surrounding groves
197 and clusters, we drove 5 cm diameter cores to a depth of 1.2 m using a pneumatic hammer. Cores
198 were then partitioned into depth increments (0-20, 20-40, 40-60, 60-80, 80-100, and 100-120 cm).
199 Root biomass within soil cores was determined as described above. There were no remnant
200 grassland patches on non-argillic soils.

201 **Data analysis**

202 Root density and root biomass for cluster and grove data sets were standardized as the
203 number of roots counted per m² of trench face (roots m⁻²), and as grams per m² (g m⁻²), respectively.
204 Both root numbers and root biomass were summed over 1 m horizontal by 0.4 m vertical
205 increments to a depth of 2 m to achieve a degree of spatial integration. Preliminary data analysis
206 indicated correlations between means and standard deviations, so a natural logarithm
207 transformation was applied to all data for subsequent statistical analyses (McDonald 2014).

208 Root density was analyzed using the General Linear Models procedure for multivariate
209 analysis of variance (ANOVA). The dependent variable was root density, and the multivariate was
210 depth (0.0-0.4, 0.4-0.8, 0.8-1.2, 1.2-1.6, and 1.6-2.0 m). Independent variables were soil type
211 (argillic vs. non-argillic soil), root diameter class (1-3, 3-10 and > 10 mm), replication (1 or 2),
212 trench face (left or right), relative horizontal position (exterior = outside cluster canopy perimeters,
213 edge = within 1 m (discrete clusters) or 4 m (groves) of the canopy perimeter, and interior = all
214 other data under canopy), and plant species. For lateral root extension analysis, independent
215 variable distance (1, 2, 3, or 4 m from the canopy perimeter) and block (relative horizontal position

216 along perpendicular trench (1-4)) were also included. Significance was tested at $\alpha = 0.05$. The
217 Bonferroni correction ($\alpha = 0.05/n$) was used for each of n pairwise comparisons, to achieve an
218 experiment wide error rate of $\alpha = 0.05$. All analyses were performed on SAS 6.07 software
219 (SAS Institute, Cary, NC, USA).

220 **Results**

221 **Shrub cluster and grove vegetation and soil properties**

222 A total of 403 plants representing 18 woody species were inventoried in the two discrete
223 clusters and the two 4 m wide belt transects in groves (Table S1). Both discrete clusters and groves
224 contained 10-15 woody species, and plant densities were greater in discrete clusters (5.4 m^{-2}) than
225 in groves (1.7 m^{-2}) (Table 1). *Prosopis* was the largest plant with respect to aboveground biomass,
226 height, and canopy area. Based on annual ring counts, the largest *Prosopis* in groves (74.0 years)
227 were older than the largest *Prosopis* in discrete clusters (61.5 years). *Prosopis* and *Zanthoxylum*
228 dominated the overstory-understory aboveground biomass, respectively, of both discrete clusters
229 and groves, accounting for ~ 85% of the total.

230 Soils in discrete clusters and the perimeter of groves were similar and were characterized by the
231 presence of an argillic horizon (B_t) and distinct structure and horizon boundaries (Figure S2).
232 Toward the interior of groves, the argillic horizon gradually disappeared, and horizon boundaries
233 became less distinct. In both clusters and groves, sand content decreased from the surface to a
234 depth of 2 m, while silt content remained relatively constant and clay content gradually increased
235 (Table 1). However, clay content in the 0.8-1.2 m depth increment in the interior of both groves
236 (20-28%) was sharply reduced compared to values near the canopy perimeter (34-38%), reflecting
237 the absence of the argillic horizon beneath groves (Table S3).

238 **Root distribution patterns in shrub clusters and groves**

239 Results from multivariate ANOVA of root density showed a significant depth \times soil type
240 interaction, indicating that the presence or absence of the argillic horizon significantly influenced
241 root densities in the vertical plane (Table S4). Compared to non-argillic soils, root densities on
242 argillic soils were significantly greater above 0.4 m depths, but similar or significantly lower at
243 depths > 0.4 m (with the exception of 1-3 mm diameter roots at 0.4 - 0.8 m depth) (Figure 2). There
244 was also a significant depth \times root diameter interaction, reflecting the fact that small roots (1-3 and
245 3-10 mm diam.) decreased exponentially with depth, whereas declines in large roots (> 10 mm
246 diam.) were more monotonic with depth (Table S4, Figure 2).

247 When plant species were included in the multivariate ANOVA, significant depth \times species
248 interactions were obtained for both argillic and non-argillic soils, indicating significant differences
249 in root vertical distribution among species (Table S5). More specifically, *Prosopis* was the deepest-
250 rooted species on each soil type, with root densities below 0.4 m accounting for > 47 % and 59 %
251 of the total root density on argillic vs. non-argillic soils, respectively (Figure 3). Only *Prosopis*
252 had roots > 10 mm diameter at depths > 0.4 m (Figure 3). Compared to *Prosopis* plants on argillic
253 soils, those on non-argillic soils had higher root densities in all size classes (Figure 3).
254 *Zanthoxylum*, a relatively shallow-rooted species with > 75 % of its total roots occurring above
255 0.4 m on both soil types, dominated the density of small-to-medium root diameter classes on
256 argillic soils, and was second only to *Prosopis* on non-argillic soils (Figure 3). In contrast to
257 *Prosopis*, *Zanthoxylum* had higher root densities on argillic soils than on non-argillic soils (Figure
258 3).

259 Excluding black and brown roots that we could not identify to species, *Prosopis* and
260 *Zanthoxylum* together accounted for ~ 80 % of total root density in non-argillic soils compared to
261 49 % in argillic soils. This was especially true for 1-3 mm diameter roots on argillic soils where

262 other woody genera (*Lycium*, *Schaefferia*, *Celtis*, and *Opuntia*) comprised ~ 60 % of the total
263 (Figure 3). Differences between *Prosopis* and other understory species in root biomass distribution
264 patterns were also evident (Figure S3).

265 **Root biomass and plant biomass allocation in shrub clusters and groves**

266 Mean total root biomass to a depth of 2 m among shrubs on argillic soils (2712 g m⁻²) was
267 higher than that of shrubs on non-argillic soils (1740 g m⁻²) (Figure 4 and Table 2). Root biomass
268 in the upper 0.4 m accounted for 69% of the total root biomass on argillic soils, compared to 38%
269 on non-argillic soils (Figure 4). Root biomass for all diameter classes on argillic soils was
270 significantly higher than that on non-argillic soils at depths < 0.4 m. However, at depths > 1.2 m,
271 root biomass on non-argillic soils was higher than that on argillic soils, especially for roots > 3
272 mm diameter.

273 Biomass allocation above- and belowground was strongly affected by the presence/absence
274 of an argillic horizon. The mean belowground to aboveground biomass ratio (root: shoot ratio) on
275 argillic soils was ~3X greater than that on non-argillic soils (0.57 vs. 0.20) (Table 2). This was
276 also true for the dominant overstory (*Prosopis glandulosa*) and understory (*Zanthoxylum fagara*)
277 species (Table 2).

278 **Root extension beyond discrete cluster perimeters**

279 Contour maps of root density along trench faces within the discrete clusters showed an
280 inverted cone-shape distribution (Figure 5). Root densities were highest near the center and
281 gradually decreased towards the cluster canopy/grassland interface. Root penetration was deepest
282 near discrete cluster centers and decreased toward cluster perimeters. Multivariate ANOVA

283 showed a significant interaction between horizontal position and soil depth (Tables S4 and S5),
284 confirming the root distribution patterns visually apparent in Figure 5.

285 Moving beyond the discrete cluster boundary into the surrounding grasslands, root density
286 typically decreased exponentially with increasing distance from cluster canopy perimeters (Figure
287 6). The amount of variation in root density explained by distance from discrete cluster perimeters
288 generally declined with increasing soil depth and root diameter. Results from the multivariate
289 ANOVA revealed a significant depth \times distance interaction which was a consequence of
290 decreasing rooting depth with increasing distance from the cluster perimeter (Table S6). The root
291 diameter \times distance interaction term was also significant, reflecting the fact that distance-from-
292 cluster effects diminished with increases in root size (Figure 6).

293 **Root biomass in grasslands, shrub clusters and groves**

294 Total root biomass in each of these patch types was highest in the upper 40 cm and declined
295 markedly with depth to 1.2 m (Figure 7). The root biomass in cluster and grove communities at
296 each depth was substantially greater than that in the grassland community. Summarized over all
297 depths, root biomass in the two shrub communities was 3 - to 5- fold greater than that of the
298 grassland community (ranging from 1.4 - to 7.3- fold greater, depending on depth).

299 **Discussion**

300 **Edaphic influences on root distribution and biomass partitioning**

301 The ability to identify roots to species for the predominant and co-occurring woody plants
302 in this system afforded unique and novel perspectives on plant-plant and plant-soil relationships
303 in this savanna parkland. Differences in root distribution with depth on soils with and without a

304 well-developed argillic horizon (Figure 2 and 4) ostensibly reflect the combined effect of physical
305 restrictions to root penetration, resource availability and plant genetic potential. Soil clay content
306 is often correlated negatively with root density and root growth (Strong and Roi 1985; Plante et al.
307 2014). This may result from increased soil strength and reduction in soil aeration which inhibit
308 root growth and metabolism (Gerard et al. 1982; Clark et al. 2003; Bengough et al., 2006; Haling
309 et al., 2011). Bulk density $> 1.5 \text{ g cm}^3$ [similar to those of the argillic horizon on this site (Zhou et
310 al. 2017b)], can generally reduce root growth through reduced pore spaces (Clark et al. 2003;
311 Lodovici 2004; Xu and Li 2009; Plante et al. 2014). Accordingly, we observed an abrupt decrease
312 in root density and biomass below 0.4 m in shrub clusters where the argillic horizon begins (Figure
313 2 and 4). This is consistent with several other studies reporting that the presence of a concentrated
314 clay hardpan in subsoils had strong influences on root vertical distribution patterns (Dracup et al.
315 1992; Sudmeyer et al. 2004; Macinnis-Ng et al. 2010).

316 Except where physical restrictions occur, roots are known to proliferate in zones of high
317 moisture availability (Eissenstat and Caldwell 1988; Schenk and Jackson, 2002; Metcalfe et al.
318 2008; Fan et al. 2017). Thus, perching of water above and retention of water within the argillic
319 horizon, coupled with limited recharge of subsoil moisture (Archer 1995), likely contributed to
320 enhanced root production in surficial soils while simultaneously inhibiting root penetration and
321 retarding growth in deeper soil. However, it should be noted that the proportion of *Zanthoxylum*
322 root biomass and density in the upper 0.4 m of soil was comparable on argillic and non-argillic
323 soils ($> 68\%$ for both variables). Rooting patterns of *Prosopis* were also relatively consistent on
324 both soils ($> 47\%$ and $> 59\%$ of roots below 0.4 m when the argillic horizon was present and
325 absent, respectively). These data suggest that *Zanthoxylum* and *Prosopis* are intrinsically and

326 genetically shallow- and deep-rooted, respectively, and that soil physical properties are of
327 secondary importance in influencing their vertical root distribution patterns.

328 Root: shoot ratios are an indicator of photosynthate allocation priorities. The ratios observed
329 at this site (Table 2) generally fell within global range for subtropical dry woodlands (0.26-0.72,
330 Mokany et al. 2006). Root: shoot ratios are affected by a variety of factors related to stand
331 development. As noted in Mokany et al. (2006), root: shoot ratios generally decrease with a variety
332 of factors all of which are consistent with our data: increasing stand age (61.5 vs. 74.0 years for
333 largest *Prosopis* in discrete clusters and groves), height (3.5 vs. 4.7 m), and mean basal diameter
334 (11.1 vs. 15.9 cm), and decreasing plant density (5.4 vs 1.7 m⁻²). In addition, edaphic
335 characterizations in this study compliment those of Loomis (1989), Stokes (1999), and Zhou et al
336 (2017b) on this site and further substantiate that patches of grassland and discrete clusters of woody
337 plants occur where there is a well-developed argillic horizon and that large groves of woody plants
338 have developed where soils are coarse-textured throughout the profile. Our data illustrating the
339 larger size of *Prosopis* in groves relative to discrete clusters (Table 1) agree with more extensive
340 surveys (Archer 1995; Boutton et al. 1998) and indicate that upland soil inclusions lacking an
341 argillic horizon are more productive sites for woody plants. We hypothesize that a greater
342 abundance of soil moisture, associated with receipt of surface runoff and subsurface interflow from
343 portions of landscape with a continuous argillic horizon, has enabled woody plants in groves to
344 support greater aboveground biomass with substantially less investment in root biomass.

345 Depth distribution of root density and biomass revealed that small roots (< 10 mm) on both
346 argillic and non-argillic soils decreased exponentially with depth, whereas large roots (> 10 mm)
347 on argillic soils presented different vertical patterns compared to those on non-argillic soils
348 (Figures 2 and 4). Accordingly, multivariate ANOVA of root density showed a significant soil

349 type \times root diameter interaction (Table S4) at depths > 0.4 m. As discussed above, non-argillic
350 soils are more productive for woody plants (Archer 1995; Boutton et al. 1998) and support larger
351 woody plants compared to argillic soils (Table1), potentially resulting in more large roots (> 10
352 mm) in non-argillic soils, especially at depths > 0.4 m. More large roots in non-argillic soils then
353 contributed to the lower root: shoot ratios in grove communities (Table 1).

354 **Species rooting patterns and plant succession**

355 Root systems of co-occurring plant species often overlap substantially, but differences in
356 distribution, turnover rates, activity, phenology and size may be sufficient to affect competitive
357 interactions and resource partitioning (Walker 1971; Seghieri 1995; Hipondoka et al. 2003;
358 Sankaran et al. 2004; Schenk 2006; February and Higgins 2010; Stokes and Archer 2010; Zhou et
359 al. 2018c). Extensive intermingling of species root systems was observed in this subtropical
360 savanna ecosystem, but contrasting patterns were evident for key co-occurring species (Figure 3).
361 *Prosopis*, the pioneer species in discrete cluster development that facilitates the subsequent ingress
362 and establishment of subordinate shrub species (Archer 1990 and 1995), was deep-rooted with 47
363 % and 59 % of roots present below 0.4 m in discrete clusters and groves, respectively. This deep
364 rooting system not only enhances the survival of *Prosopis* during the critical seedling
365 establishment phase by reducing competition with grasses for water (Brown and Archer 1990;
366 Ansley et al. 2014), but also facilitates subsequent recruitment of subordinate shrub species by
367 providing nutrient-enriched soil conditions (Hibbard et al. 2001; Zhou et al. 2018a and 2018b).
368 *Prosopis* is known to form symbiotic associations with N-fixing bacteria, and plants at this site do
369 nodulate and fix N (Zitzer et al. 1996; Soper et al. 2015). As a result, soil N in the upper 30 cm of
370 the profile is greater under *Prosopis* canopies than adjacent herbaceous zones (Archer 1995;
371 Hibbard et al. 2001; Boutton and Liao 2010; Zhou et al. 2018a). In addition, deep-rooted *Prosopis*

372 transfers phosphorus (P) from deeper soil layers and enlarges the P pool in the upper soil layers
373 where it is more actively cycled (Zhou et al. 2018b).

374 *Zanthoxylum* is among the first shrub species to colonize beneath *Prosopis* canopies, and
375 typically dominates the understory layer (Archer et al.1988; Archer1990). Although the precise
376 mechanisms of this facilitation have not yet been fully addressed experimentally, the shallow root
377 systems of these early arrivals (Figure 3) would make them well-suited to take advantage of (1)
378 N- and P-enriched surface soils resulting from the establishment and growth of *Prosopis* (Hibbard
379 et al. 2001; Zhou et al. 2018a and 2018b); and (2) redistribution of soil moisture from deep to
380 surface horizons that might occur as the result of hydraulic redistribution, as has been shown on
381 this site (Zou et al. 2005). In addition, the dense shallow root systems of these early arrivals
382 underneath *Prosopis* canopies may contribute to the observed, significant declines in shallow-
383 rooted grasses and forbs by intensifying competition for soil resources while simultaneously
384 reducing light levels. This, in turn, may create opportunities for the establishment of shade-tolerant
385 understory woody species appearing later during woody patch development.

386 Different root distribution patterns among shrub species would diffuse competition and
387 enable the dynamic co-existence of woody species in discrete clusters and groves. For example,
388 the natural abundance of ^2H and ^{18}O in stem water indicates that shallow-rooted understory species
389 such as *Zanthoxylum* are closely coupled to availability of surface soil moisture, while deep-rooted
390 *Prosopis* acquires water from deeper soil layers (Boutton et al. 1999). However, as the size and
391 density of understory species increases over time, co-existence appears to give way to asymmetric
392 competition (Archer 1989, 1995), as field observations indicate that the overstory *Prosopis* in
393 about 25 % of discrete clusters have died. One explanation supported by experimental evidence is
394 that roots of *Zanthoxylum* and other shallow-rooted shrubs sequester water and nutrients that might

395 otherwise percolate to depths where *Prosopis* roots dominate and adversely impact *Prosopis*
396 growth (Barnes and Archer 1999). Since the demise of the central *Prosopis* is less common in
397 groves on non-argillic soils, we hypothesize that these competitive effects would be most
398 pronounced in discrete clusters on soils with a well-developed argillic horizon, where near-surface
399 understory root densities and biomass are higher (Figure 2 and 4) and where the clay-rich argillic
400 horizon restricts percolation of water and nutrients to deeper soil layers (Archer et al. 1988 and
401 Archer 1995).

402 **Root extension and landscape development**

403 The current two-phase landscape (woody clusters and groves interspaced within a grassland
404 matrix, Figure 1) on the upland portions of this study site has been hypothesized to represent an
405 intermediate stage in landscape development from open grassland to monophasic woodland
406 (Archer et al. 1988; Archer 1995; Boutton et al. 1996, 1998; Zhou et al. 2017b). Within this two-
407 phase landscape, 95% of woody clusters are within 15 m of another, and seedlings of *Prosopis*
408 occur in high densities in the grassland matrix (Archer 1988), suggesting the potential for cluster
409 formation through the aforementioned vegetation succession. This hypothesis presumes that
410 interactions between woody clusters are minimal and that as new clusters form and grow,
411 coalescence will occur.

412 However, cluster coalescence may not occur if intense competition from laterally extensive
413 root systems of existing clusters significantly reduces the survival probability of *Prosopis*
414 seedlings in the grassland matrix or limits subsequent cluster formation and development. Our data
415 showed that root density decreased exponentially with increasing distance from cluster perimeters
416 and that few roots extended more than 1 m beyond cluster perimeters, especially at soil depths

417 below 0.4 m (Figure 5 and 6). These results indicate that *Prosopis* seedlings in the grassland matrix
418 likely receive no or limited competition from existing woody plants and have the potential to grow
419 and recruit understory species to form discrete clusters. Indeed, a chronological sequence of aerial
420 photos at this site spanning the period 1930 to 2016 documents the active formation of discrete
421 clusters and their expansion and coalescence (Archer et al. 2001; Bai et al. 2009; Zhou et al.
422 2017b), providing direct evidence to support this hypothesis. Therefore, our results suggest that
423 succession to monophasic woodland may occur on upland portions of this study site, with the rate
424 being mediated by edaphic heterogeneity and the nature of future climate and disturbance (e.g.
425 grazing and fire) regimes.

426 **Belowground consequences of woody plant proliferation in grassland**

427 Shrub clusters and groves in this subtropical system have developed on former grasslands
428 (Archer 1995, Bouton et al 1998). The consequences for this change with respect to aboveground
429 biomass, primary production and near-surface (upper 20 cm) soil carbon pools and fluxes have
430 been summarized in earlier work (e.g., Archer et al 2001, Hibbard et al. 2003, McCulley et al.
431 2004, Boutton and Liao 2010). Here, we show that the change in community structure from grass
432 to woody plant dominance has profoundly increased the magnitude of the carbon pool associated
433 with plant roots to depths of 1.2 m (3- to 5-fold, Figure 7). Our ability to predict how root biomass
434 might change with changes in growth form/life form composition presently relies on a ‘space-for-
435 time substitution’ approach. For example, if a tropical savanna transitioned to a tropical evergreen
436 forest or a tropical deciduous forest, we could use data such as that presented in Jackson et al.
437 (1996) to predict the consequences for below ground biomass with depth. The veracity of this
438 prediction would be predicated on the strength of the assumption that such a space-for-time
439 substitution is accurate and robust. Although our data is also based on a space-for-time approach,

440 it is at more local scale which might make the results more germane to developing accurate
441 predictions. In any case, global budgets for root biomass, surface area and nutrient content (e.g.
442 Jackson et al. 1997) will be challenged to take into account shifts from grass to woody plant
443 dominance and how such shifts will play out on various soil types. To further complicate things,
444 root longevity, turnover and decay likely vary with species, size (diameter) and depth to determine
445 the dynamics of this substantial belowground carbon pool.

446 **Conclusions and Implications**

447 Edaphic characteristics strongly influenced root distribution patterns and biomass allocation
448 of co-occurring woody plants in this subtropical savanna parkland. Root density and biomass in
449 soils with a well-developed argillic horizon were generally greater above a depth of 0.4 m and
450 lower below 0.8 m of the profile compared to similar depths in soils where the argillic horizon was
451 absent. Woody plants maintained greater aboveground biomass with substantially less root
452 biomass on soils lacking an argillic horizon. The dominant overstory species *Prosopis* was deep-
453 rooted with a high proportion of its total roots below 0.4 m. In contrast, the dominant understory
454 shrub *Zanthoxylum* and other subordinate species were relatively shallow-rooted, facilitating their
455 early co-existence with *Prosopis* -- by minimizing interspecies competition during the formation
456 of woody clusters and groves -- but later forming the basis for asymmetric competition that may
457 hasten the demise of the *Prosopis* overstory. Root density decreased exponentially with increasing
458 distance from cluster perimeters, and few woody plant roots extended > 1 m beyond cluster
459 canopies, thus minimizing the potential for density dependent constraints on future cluster
460 development. The ability to identify roots to species for the predominant and co-occurring woody
461 plants in this system afforded unique and novel perspectives on plant-plant and plant-soil

462 relationships and provide valuable insights for explaining vegetation dynamics and landscape
463 development of this subtropical savanna parkland.

464 Root distribution datasets of co-occurring woody species, such as those presented in this
465 study, are comparatively rare and novel, as the savanna literature has focused primarily on rooting
466 differences between woody plants and grasses. The patterns of species-specific root distribution
467 revealed in this study and have relevance to understanding plant succession and landscape
468 development. Our data provide potential insights into the structure-function relationships of grass-
469 woody, shrub-shrub and shrub-tree growth forms and life forms in savanna, parkland and
470 woodland settings. Our results show the broad range of rooting patterns occurring in dryland
471 woody plants and challenge prevailing generalization that they deep rooted and/or that they have
472 extensive shallow lateral root systems. We clearly demonstrate how edaphic factors affecting water
473 regimes are critical in shaping biomass partitioning (Tomlinson et al. 2012) and hence global
474 patterns of rooting depth distribution (Fan et al. 2017). Furthermore, root biomass in shrub cluster
475 and grove communities to a depth of 1.2 m were 3- to 5-times greater than that of the grasslands
476 they ostensibly replaced (Figure 7), suggesting belowground carbon inputs can increase
477 dramatically when woody plants proliferate in grasslands. This substantive change in the
478 magnitude of belowground C pools would markedly influence assessments of C-sequestration
479 potential and source-sink relationships (Barger et al. 2011). Accordingly, improvements in our
480 understanding rooting patterns of dryland woody species is paramount to advancing our ability to
481 predict species interactions, vegetation dynamics, and responses of mixed growth form/life form
482 systems to future environmental conditions.

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695 **Figure legends**

696 **Figure 1** Aerial photograph of the grazing enclosure at the Texas A&M AgriLife La Copita
697 Research Area. This upland landscape consisted of discrete woody clusters and groves embedded
698 within a grassland matrix. Green areas represent woody vegetation, while light gray areas indicate
699 the grassland matrix.

700 **Figure 2** Mean root densities (roots m⁻²) for root diameter classes 1-3 mm (A), 3-10 mm (B), and
701 > 10 mm (C) throughout the soil profile. Asterisks indicate significant ($p < 0.05$) differences in
702 root density between argillic soils and non-argillic soils within a depth interval. Values are mean
703 \pm SE.

704 **Figure 3** Relative root densities (mean percentage of root density in each depth interval to total
705 root density from the ground surface to a depth of 2 m) by species and root diameter classes on
706 argillic (A, B, and C) and non-argillic soils (D, E, and F). Values to right of bars are mean (\pm SE)
707 total root densities (roots m⁻²) for each plant species.

708 **Figure 4** Mean (\pm SE) root biomass (g m⁻²) for root diameter classes < 1 mm (A), 1-3 mm (B), 3-
709 10 mm (C), and > 10 mm (D) to a depth of 2 m. Asterisks indicate significant ($p < 0.05$) differences
710 between argillic soils and non-argillic soils within a depth interval.

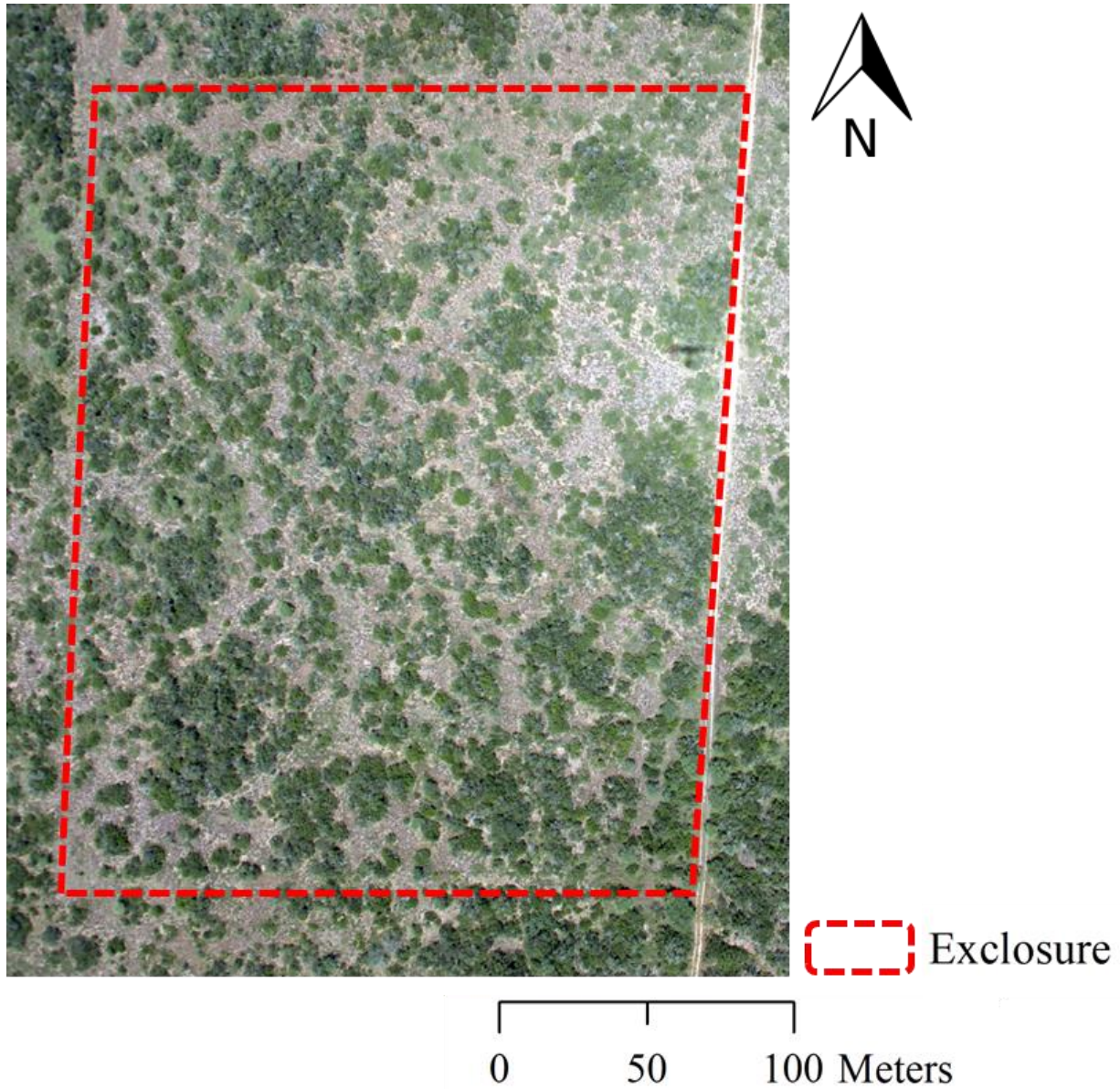
711 **Figure 5** Contour maps of root densities (roots m⁻²) along trench faces for discrete clusters 1 (A)
712 and 2 (B). Values are the mean of both trench faces. Solid black horizontal line denotes top of
713 argillic horizon (Fig. S2); dashed vertical lines denote location of shrub cluster canopy perimeters.

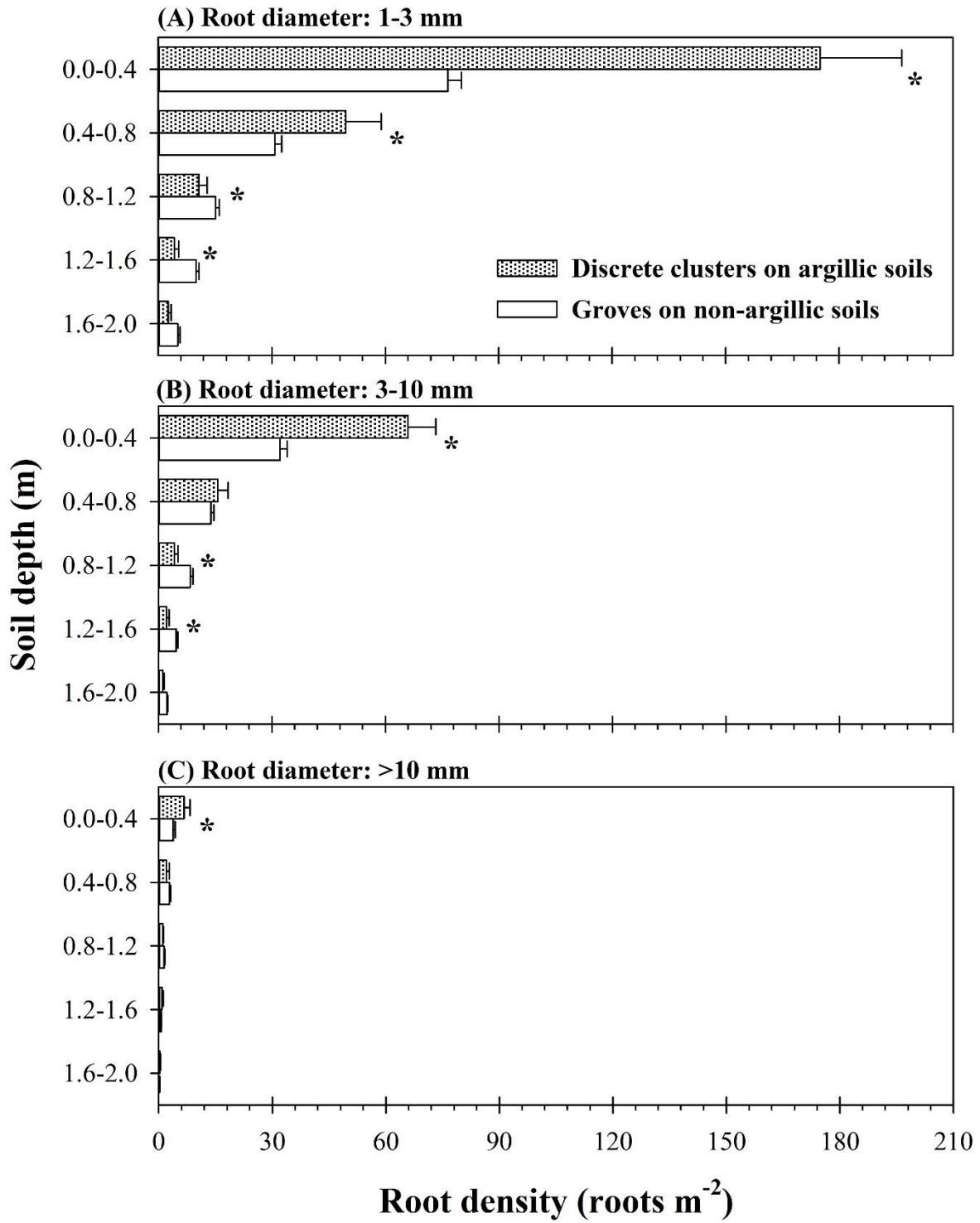
714 **Figure 6** Mean (\pm SE) root densities (roots m⁻²) at 0, 1, 2, 3 and 4 m beyond discrete cluster canopy
715 perimeters for root diameter classes 1-3 mm, 3-10 mm, and > 10 mm. Bars with different letters
716 are significantly different ($p < 0.05$). Exponential decay functions were fitted to the means of root
717 density vs. the distances from cluster canopy perimeters for each root diameter class. Panels

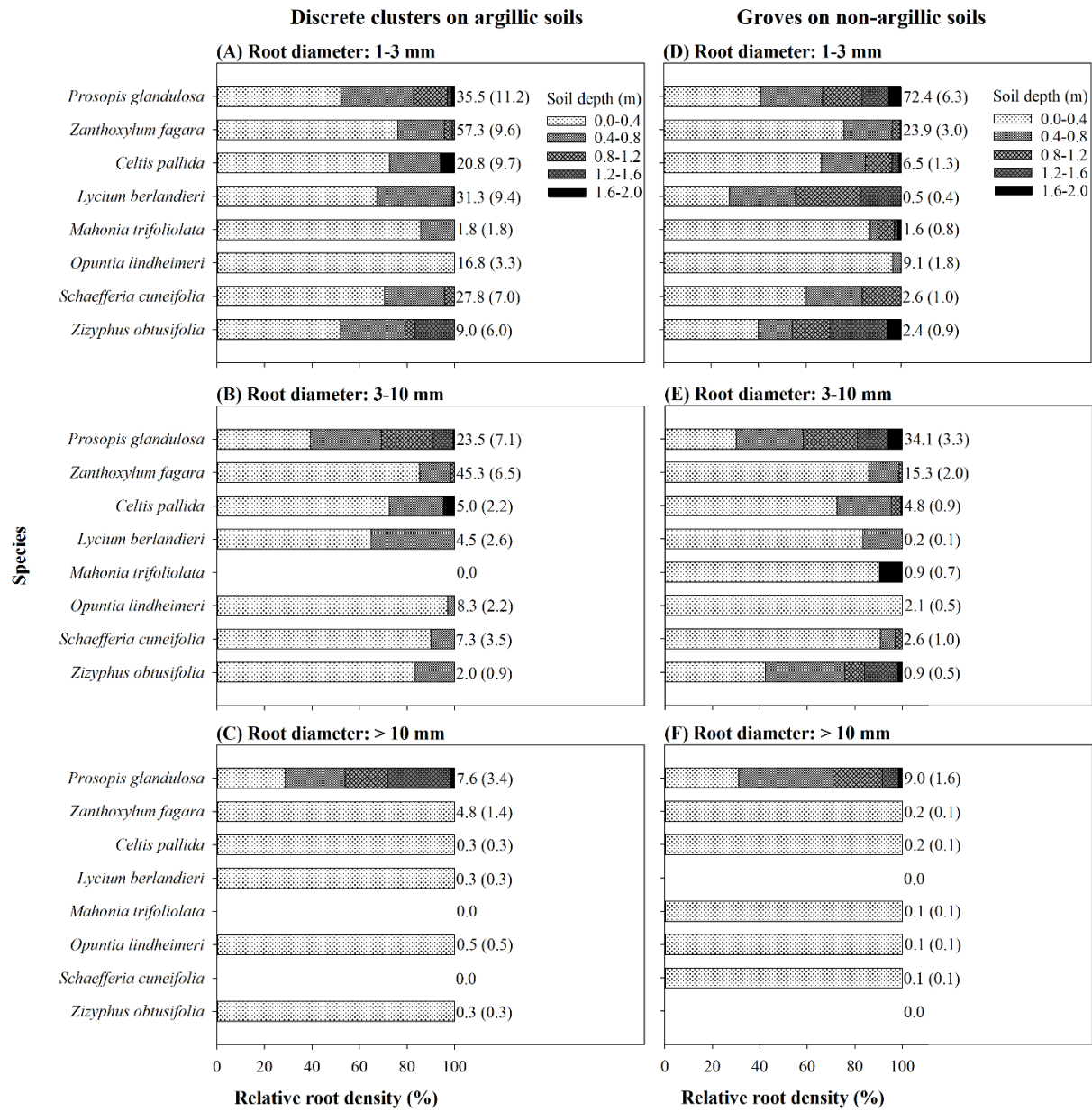
718 without equations indicate failed fit with exponential decay functions. Results from depths > 1.2
719 m in the soil profile are not presented, as there were no significant differences in root densities
720 with distance from cluster canopy perimeters, but can be found in Table S8.

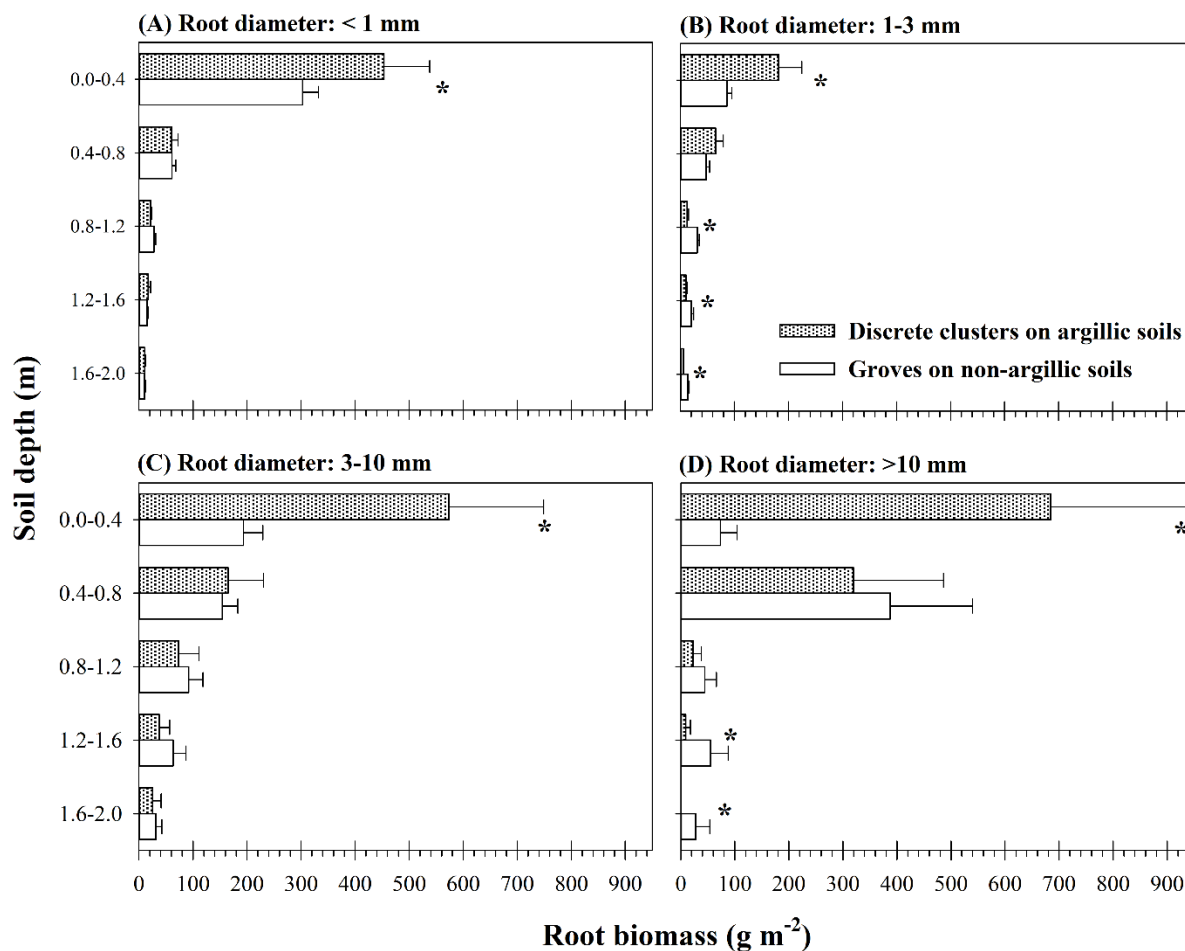
721 **Figure 7** Mean (\pm SE) total root biomass with depth (to 1.2 m) for clusters on argillic soils (n =13),
722 groves on non-argillic soils (n = 20), and grassland on argillic soils (n = 10) (data not available for
723 grassland on non-argillic soil).

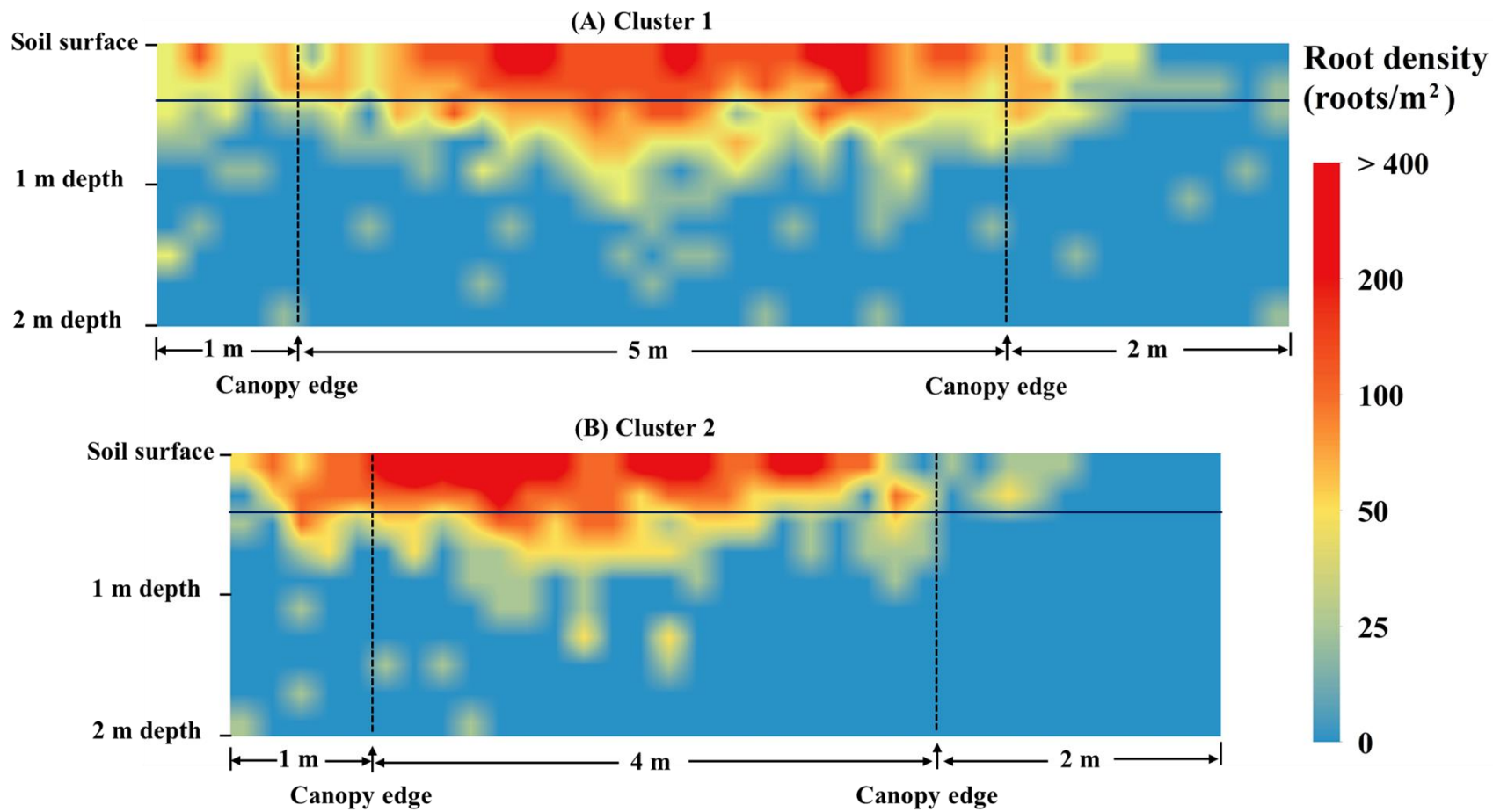
724 Figure 1

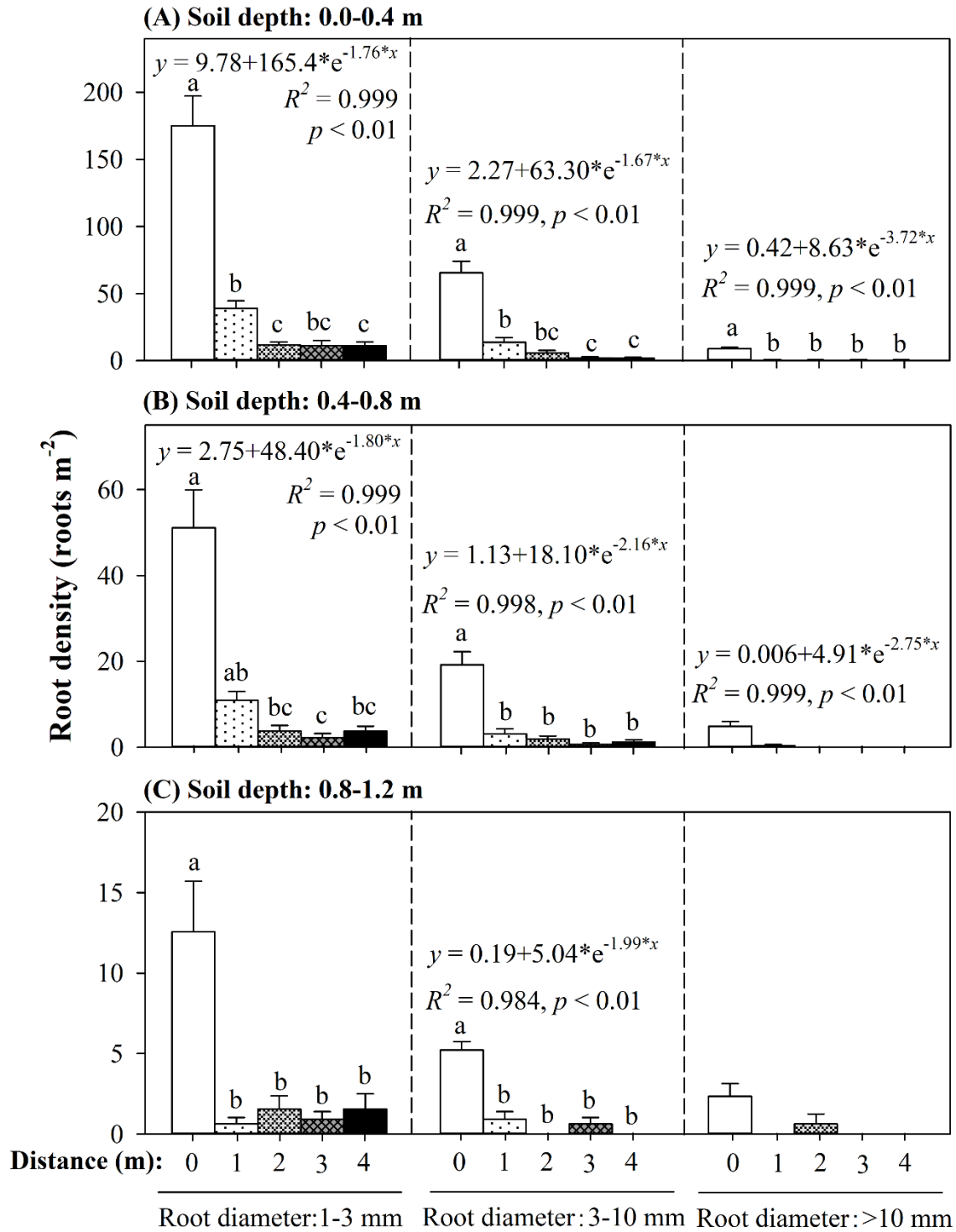


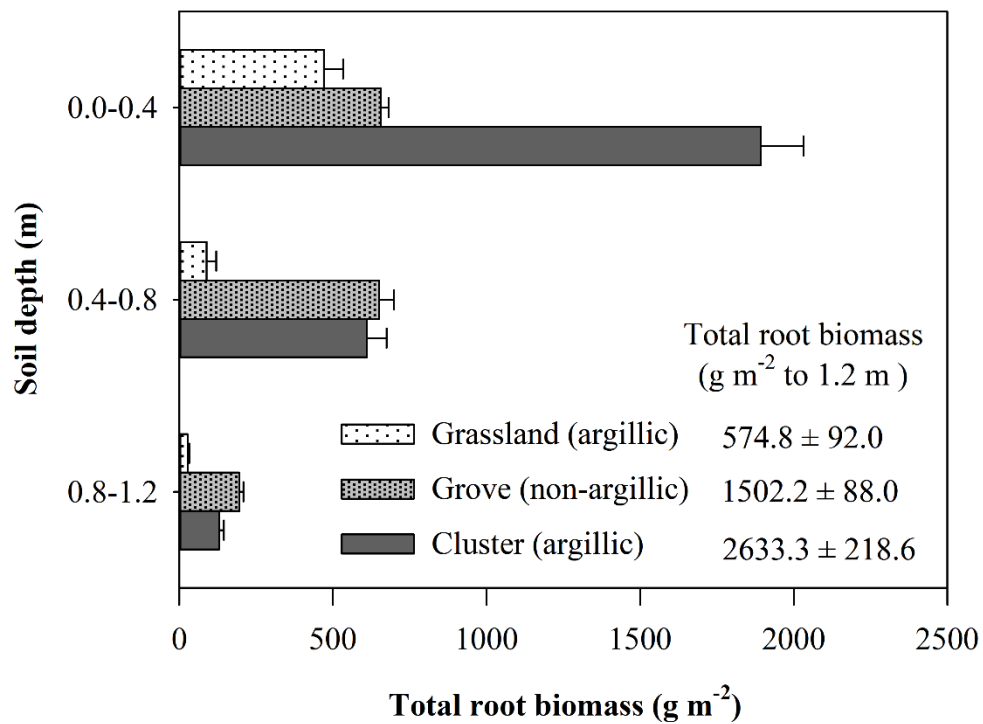












738 Table 1

739 Summary of the aboveground inventory of plants and soil texture [mean (SE); n=2] for discrete
 740 cluster and grove communities.

	Discrete clusters			Groves		
Number of plants	82.0 (2.0)			119.5 (4.5)		
Number of species	13.0 (2.0)			11.5 (1.5)		
Largest <i>Prosopis</i> stem age (years)	61.5 (4.5)			74.0 (9.0)		
Largest <i>Prosopis</i> basal diameter (cm)	11.1 (1.8)			15.9 (3.4)		
Plant density (plants m ⁻²)						
<i>Prosopis</i>	0.17 (0.07)			0.06 (0.01)		
<i>Zanthoxylum</i>	2.0 (0.8)			0.8 (0.2)		
Total (all species)	5.4 (1.3)			1.7 (0.2)		
Aboveground biomass (g m ⁻²)						
<i>Prosopis</i>	2490 (1682)			7362 (2717)		
<i>Zanthoxylum</i>	1554 (587)			494 (25)		
Total (all species)	4750 (2660)			8584 (2299)		
Plant height (m)						
<i>Prosopis</i>	3.5 (0.5)			4.7 (0.3)		
<i>Zanthoxylum</i>	0.8 (0.0)			1.1 (0.2)		
All other species	0.7 (0.1)			0.9 (0.1)		
Canopy Diameter (m)						
<i>Prosopis</i>	5.6 (0.2)			5.2 (0.6)		
<i>Zanthoxylum</i>	0.5 (0.0)			0.7 (0.2)		
All other species	0.7 (0.1)			0.7 (0.1)		
Soil texture (%)						
Depth (m)	Sand	Silt	Clay	Sand	Silt	Clay
0.0-0.4	67 (1)	12 (0)	21 (1)	69 (2)	10 (2)	21 (0)
0.4-0.8	60 (1)	13 (1)	28 (1)	64 (0)	11 (0)	25 (0)
0.8-1.2	50 (1)	14 (2)	37 (1)	55 (4)	13 (1)	32 (3)
1.2-1.6	46 (4)	16 (2)	39 (3)	51 (5)	14 (2)	35 (4)
1.6-2.0	44 (4)	16 (2)	40 (2)	50 (5)	15 (1)	35 (4)

741

742 Table 2

743 Mean belowground and aboveground biomass and associated ratios of belowground to
744 aboveground biomass (root: shoot ratios) by soil types and species. Values in parentheses are
745 standard errors.

Soil type	Species	Belowground biomass (g m ⁻²)			Aboveground biomass (g m ⁻²)	Root: shoot
		≥ 1 mm	< 1mm	total		
Argillic soil	<i>Prosopis</i>	696 (249)	NA	NA	2490 (1682)	0.28†
	<i>Zanthoxylum</i>	627 (225)	NA	NA	1554 (587)	0.40†
	All	2149 (621)	563 (100)	2712 (697)	4750 (2660)	0.57
Non-argillic soil	<i>Prosopis</i>	942 (187)	NA	NA	7362 (2717)	0.13†
	<i>Zanthoxylum</i>	134 (37)	NA	NA	494 (25)	0.27†
	All	1322 (291)	418 (33)	1740 (205)	8583 (2299)	0.20

746 NA indicates that data is not available

747 †The ratios of belowground to aboveground biomass were based on coarse (diameter ≥ 1mm) roots
748 only