1   Running head: Plant dynamics in a Chihuahuan ecotone

2

3   Title: Seed-bank structure and plant-recruitment conditions regulate the dynamics of a

4   grassland-shrubland Chihuahuan ecotone

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26 *Abstract*. Large areas of desert grasslands in southwestern USA have shifted to sparse

27 shrublands dominated by drought-tolerant woody species over the last 150 years,

28 accompanied by accelerated soil erosion. An important step towards the understanding of

29 patterns in species dominance and vegetation change at desert grassland-shrubland transitions

30 is the study of environmental limitations imposed by the shrub-encroachment phenomenon

31 on plant establishment. Here, we analyze the structure of soil seed-banks, environmental

32 limitations for seed germination (i.e., soil-water availability and temperature) and simulated

33 seedling emergence and early establishment of dominant species (black grama, *Bouteloua*

34 *eriopoda*, and creosotebush, *Larrea tridentata*) across a Chihuahuan grassland-shrubland

35 ecotone (Sevilleta National Wildlife Refuge, New Mexico, USA). Average viable seed

36 density in soils across the ecotone is generally low (200-400 seeds m^{-2}), although is largely

37 concentrated in densely vegetated areas (with peaks up to 800-1200 seeds m^{-2} in vegetated

38 patches). Species composition in the seed-bank is strongly affected by shrub encroachment,

39 with seed densities of grass species sharply decreasing in shrub-dominated sites.

40 Environmental conditions for seed germination and seedling emergence are synchronized

41 with the summer monsoon. Soil-moisture conditions for seedling establishment of *B.*

42 *eriopoda* take place with a recurrence interval ranging between 5 and 8 years for grassland

43 and shrubland sites, respectively, and are favored by strong monsoonal precipitation. Limited

44 *L. tridentata* seed dispersal and a narrow range of rainfall conditions for early seedling

45 establishment (50-100 mm for 5-6 consecutive weeks) constrain shrub-recruitment pulses to

46 localized and episodic decadal events (9-25 years recurrence intervals) generally associated

47 with late-summer rainfall. Re-establishment of *B. eriopoda* in areas now dominated by *L.*

48 *tridentata* is strongly limited by the lack of seeds and decreased plant-available soil moisture

49 for seedling establishment.

INTRODUCTION

The encroachment of native woody species has affected several hundred million

hectares of arid and semi-arid grasslands throughout central Australia, southern Africa,

southwestern United States of America, South America and the Mediterranean Basin over the

last 150 years (Van Auken 2000, D'Odorico et al. 2012, Turnbull et al. 2014). This process is

well documented in the Chihuahuan Desert, southwestern USA, where drought-tolerant C_3

shrub species (mainly honey mesquite, *Prosopis glandulosa*, and creosotebush, *Larrea*

tridentata) have colonized large areas of C_4 semi-arid grasslands since 1850 (Schlesinger et

al. 1990, Van Auken 2000, D'Odorico et al. 2012, Turnbull et al. 2014). Shrub encroachment

in this region has caused largely irreversible changes in ecosystem function (e.g., alterations

in landscape net primary production patterns and reductions in biodiversity) accompanied by

accelerated wind and water erosion, which is perceived to be symptomatic of land

degradation and desertification (Baez and Collins 2008, Turnbull et al. 2010a, Moreno-de las

Heras et al. 2015, Peters et al. 2015).

A range of mechanisms has been suggested to explain the encroachment of desert

shrubs in north Chihuahuan grasslands, including overgrazing, drought, changes in the

frequency of rainfall, climate variability, fire suppression, and increased atmospheric CO_2

concentrations (Van Auken 2000, D'Odorico et al. 2012, Turnbull et al. 2014, Peters et al.

2015). Directional changes in these environmental conditions can cause either gradual or

sudden alterations in ecosystem structure, promoting the shrub-encroachment phenomenon

(D'Odorico et al. 2012, Collins and Xia 2015); e.g., the severe multiyear drought between

1950 and 1960 caused rapid regional changes in Chihuahuan landscapes across southwestern

75   USA (Peters et al. 2015). In the Sevilleta National Wildlife Refuge (SNWR, central New

76   Mexico) the 1950s drought led to the conversion of large areas of grasslands dominated by *B.*

77   *eriopoda* into sparse desert shrublands dominated by *L. tridentata* (Gosz 1992). Remote

78   sensing explorations of vegetation and field study of both shrub architecture and vegetation

79   dynamics indicates that the SNWR grassland-shrubland ecotone (i.e., the transition between

80   the *B. eriopoda-* and *L. tridentata*-dominated communities) has remained stable for long

81   periods after 1960-70, showing little changes in either shrub progression or grass recovery

82   (Allen et al. 2008, Peters and Yao 2012, Moreno-de las Heras et al. 2015).

83   An important step towards understanding patterns in plant-species dominance and

84   vegetation change at desert grassland-shrubland ecotones is determining plant-recruitment

85   limitations imposed by the shrub-encroachment process. Seed availability and the dynamics

86   of seed germination and early seedling establishment are critical plant recruitment processes

87   that can condition species dominance, plant-regeneration patterns and the rates of vegetation

88   change in drylands (Coffin and Lauenroth 1989, Espigares and Peco 1993, Kigel 1995,

89   Bowers et al. 2004, Koontz and Simpson 2010, Peters 2014, Bochet 2015). Previous studies

90   of *L. tridentata* plant-age structure in a variety of North American desert sites suggests that

91   recruitment of seedlings occurs primarily as rare episodic events that may influence shrub-

92   expansion rates and the frequency of active encroachment pulses for the region (Chew and

93   Chew 1965, Barbour 1969, Allen et al. 2008). Polyploid *L. tridentata* shows vegetative shrub

94   reproduction by clonal growth in the Mojave Desert (Vasek 1980). However, there is no

95   evidence that Chihuahuan (diploid) *L. tridentata* spreads clonally, and consequently,

96   colonization in the Chihuahuan Desert of this long-lived (approx. 400 years life-span) shrub

97   appears to depend exclusively on seed reproduction (Miller and Huenneke 2000, Pendleton et

98   al. 2008, Peters and Yao 2012). Seedling recruitment has been shown to occur infrequently

99   for *B. eriopoda*, a perennial and relatively short-lived (20-40 years life-span) C_4 grass species

100   that mostly regenerates through stoloniferous expansion (Nelson 1934, Neilson 1986, Collins

101   and Xia 2015). However, analysis at large-scale biome transitions indicates that recruitment

102   of *B. eriopoda* from seed accounts for localized patterns in the dominance of grass species

103   across Chihuahuan landscapes and the Shortgrass Steppe, and may also impact plant-

104   colonization patterns in areas where *B. eriopoda* patches are locally extinct, which prevents

105   vegetative grass recovery (Minnick and Coffin 1999, Peters 2000, 2002a). Shrub

106   encroachment promotes alterations in soil properties, soil-moisture patterns and the

107   availability of plant propagules, which may constrain the re-establishment of *B. eriopoda* in

108   areas now dominated by desert shrubs (Schlesinger et al. 1990, Turnbull et al. 2010b, Peters

109   and Yao 2012).

110   The aim of this study is to analyze how seedling recruitment for *B. eriopoda* and *L.*

111   *tridentata* can impact shrub encroachment in a Chihuahuan grassland-shrubland ecotone

112   (SNWR, New Mexico). To achieve this aim we study the soil seed-bank structure and

113   environmental limitations for seed germination, and simulate plant emergence and early

114   establishment of *B. eriopoda* and *L. tridentata* over the ecotone.

115

116   MATERIALS AND METHODS

117   *Site description*

118   This study was carried out in the SNWR, central New Mexico (Socorro County),

119   USA, the location of a National Science Foundation Long Term Ecological Research (LTER)

120   site (Fig. 1a). The SNWR is a biome transition zone between the Chihuahuan Desert, the

121   Great Plains grasslands, the Colorado Plateau steppe, and the Mogollon coniferous woodland.

122   Annual precipitation is 230 mm, with approx. 60% falling in the form of high-intensity

123   convective storms in the summer monsoon (from late-June to September). Average annual air

124   temperature is 14°C (2°C in January and 26°C in July). Peak vegetation growth in the area is

125 concentrated in July-September, coinciding with the summer monsoon (Moreno-de las Heras

126 et al. 2015)

127   We selected for analysis four study sites within a 5 km^2 grassland-shrubland ecotone

128 (*B. eriopoda* dominated grassland and *L. tridentata* dominated shrubland) in the SNWR

129 McKenzie Flats (Five Points experimental area), a zone of gently sloping (<5°) terrain (Fig.

130 1b). Analysis of soil-carbon isotopes, shrub architecture and photographic evidence indicates

131 that *L. tridentata* expanded over the Five Points area in the past 80 years, particularly in a

132 major shrub-encroachment pulse that took place along a deep and prolonged drought from

133 1951 to 1956 (Gosz 1992, Allen et al. 2008, Turnbull et al. 2008). Livestock grazing was

134 excluded from the area in 1973, following 37 years of rangeland use.

135   The study sites (each 30 × 10 m, Fig. 1c) represent a *L. tridentata*-encroachment

136 gradient (Turnbull et al. 2008, 2010a, 2010b), and encompass a grassland end-member (Site

137 1, 45% and 0% grass and shrub cover, respectively), two transition landscapes (Sites 2 and 3,

138 40%-22% grass and 6%-16% shrub cover) and a shrubland end-member (Site 4, <1% and

139 33% grass and shrub cover, respectively). Vegetation cover is organized in a mosaic of grass

140 and shrub patches (typically <1 m^2 and 0.5-5 m^2 for grass and shrub) interspaced by bare soil

141 interpatches (with average diameter ranging from 0.2 m in sites 1 and 2 to >1 m in sites 3 and

142 4). Soils are alkaline, sandy loams with <10% clay content (Appendix S1: Table S1 for soil

143 characteristics). Surface stoniness and soil organic carbon contents in shrub, grass and bare

144 soil interpatches reflect the typical spatial patterns of erosion/sedimentation and resource-

145 island development in shrub-encroached dry grasslands (Schlesinger et al. 1990, Reynolds et

146 al. 1999, Turnbull et al. 2010a, 2010b).

147

148 *Characterization of vegetation cover and soils*

149   We used the point-intercept method to estimate the distribution of surface types (%

150   cover, relative abundance) in our four sites. Presence of grass patches (Gp), shrub patches

151   (Sp) and bare soil interpatches (Ip) was recorded every 50 cm using a metal rod pointer (2 cm

152   diameter) along five parallel 30-m long linear transects regularly distributed at each site.

153   We collected three composite soil samples per surface type and site (each sample

154   formed by six homogeneously mixed subsamples) from the top 10 cm to characterize soil

155   traits (i.e., soil granulometry and texture, pH, electrical conductivity, inorganic and organic

156   C, total N and plant available P) using standardized methods of soil analysis (Carter and

157   Gregorich, 2008). Soil-water retention at a range of matric potentials ranging from saturation

158   to the permanent wilting point (Ψ= -0.0002, -0.01, -0.03, -0.05, -0.08, -0.4, -0.8 and -1.45

159   MPa) was determined using the pressure-chamber method (Klute, 1986). Parametrization of

160   the soil-water-retention characteristic curves was made according to Brooks and Corey

161   (1964). Details of the parameterized Brooks and Corey curves can be found in Fig. S1 and

162   Table S2 (Appendix S1).

163

164   *Soil seed-bank sampling and analysis*

165   In each site we established four 5 × 5 m plots that were randomly distributed (without

166   overlapping) to measure the soil seed-bank. Gp, Sp and Ip surface cover (%) were visually

167   estimated in nine (0.5 × 0.5 m) quadrats distributed at random within each plot.

168   In November 2013, following seed production and dispersal at the SNWR (Pendleton

169   et al. 2008, Koontz and Simpson 2010) and after the course of an exceptionally wet growing

170   season (June-September rainfall was 243 mm, 75% above the 1988-2015 average), we

171   collected soil samples (36 cm^2 surface area and 3 cm depth) to analyze the composition of the

172   soil seed-bank. In each plot we collected four soil samples per surface type, totaling 156

173   samples. Soil samples were air dried, carefully disaggregated and sieved (to 4 mm) to remove

174   big pebbles and coarse plant fragments. Excluded material was examined manually for seeds

175   and fruits. Some Chihuahuan species need exposure to low temperature to break seed

176   dormancy (Peters 2002a) and therefore the soil samples were stored for four weeks at low

177   temperature (ranging from 0° to 4°C) to break seed dormancy (Coffin and Lauenroth, 1989).

178   The samples were then spread out over a 5 cm vermiculite layer in plastic pots. Availability

179   of viable seeds and floristic composition of the soil seed-bank was determined after

180   germination in a greenhouse with controlled day length and temperature (12 h day length, 20-

181   25 °C). The samples were watered to field capacity at the start of the experiment, in January

182   2014. Then, bottom-up watering was provided as required to keep soil moisture near field

183   capacity. After the first six weeks, we added 40 ppm of fertilizer (5 ml /10 l; NPK

184   3.5:3.5:3.5) mixed with water every month. We counted emerged seedlings at weekly

185   intervals until August 2014.

186   Non-metric multidimensional scaling (NMS) ordination was used to explore

187   differences in floristic composition of the soil seed-bank for the studied sites. The relation

188   between the floristic composition of the soil seed-bank to the distribution of cover types on

189   the plot surface (i.e., Gp, Sp, and Ip abundance) was analyzed by fitting thin plate splines to

190   the NMS ordination, using general additive models. Differences in soil seed densities of

191   viable grass, forb, shrub/subshrub, *B. eriopoda*, *L. tridentata* and total seeds between sites

192   and surface types were tested using Kruskal-Wallis ANOVA and post-hoc Mann-Whitney

193   tests.

194

195   *Seed-germination tests and analysis for B. eriopoda and L. tridentata*

196   Native *B. eriopoda* seeds (unimproved Chihuahuan ecotype 'Nogal'; Leyendecker

197   1975) for the germination tests were obtained from a local seed supplier (USDA Los Lunas

198   Plant Materials Center, Los Lunas, New Mexico). *L. tridentata* seeds for this study were

collected in the field. Chihuahuan *L. tridentata* fruits are generally shed when ripe throughout summer and fall, although *L. tridentata* shrubs can keep small seed crops un-shed for after-ripening periods beyond fall (Barbour 1968, Pendleton et al. 2008). At the time of soil seed-bank sampling for this study (November 2013), we observed significant amounts of ripe, un-shed fruits in SNWR *L. tridentata* shrubs. We therefore collected freshly ripe *L. tridentata* fruits in the Five Points shrublands for our seed-germination tests (approx. 30 fruits per shrub from 50 adult shrubs). Each *L. tridentata* fruit is split into five mericarps, containing one seed per mericarp. The seeds' husks were removed by rubbing the mericarps with sandpaper.

Seedling germination was studied under four temperature levels (15°, 20°, 25° and 30°C) and eight water potentials representing soil-moisture values between saturation and the wilting point (Ψ= 0, -0.2, -0.4, -0.6, -0.85, -1.1, -1.3 and -1.5 MPa). Water-potentials were simulated using polyethylene glycol (PEG) concentrations following the standard equations of Michel et al. (1983). Seven replicates for each water potential and temperature level were prepared. Each replicate consisted of 25 (15) *B. eriopoda* (*L. tridentata*) seeds placed in a 9 cm diameter Petri dish, on a bed composed of a layer of hydrophilic cotton and filter paper. Replicates were moistened with 30 ml of PEG solutions. The experiment took place inside a phytotron under controlled conditions (temperature level ±1°C, 12-h day length, 150 μmol m^{-2} s^{-1} lighting intensity, 75% relative air humidity for four weeks). Germination was monitored every three days, with germination considered successful when a minimum of 4 mm of embryonic root had emerged (at the time of germination, the seedling green cotyledons were, at least, partially visible). Germination rate (Gr, %) was calculated as the total percentage of germinated seeds during the (four-week) germination tests. Time to germination (Gt, days) was determined as the time to reach 90% of total seed germination for each test, which provides a conservative approach to approximate time to seedling emergence in the field using laboratory tests (Moreno-de las Heras et al. 2011).

224   To study the influence of water potential (Ψ, MPa) on seed-germination rate (Gr, %),

225   a sigmoid-shape response function (Moreno-de las Heras et al. 2011) was fitted to the

226   germination results obtained for each species and temperature level:

227
$$G_r = \frac{G_m}{1 + e^{\frac{\psi - a}{b}}},$$
(1)

228   where G_m (%) is the maximum germination rate that is reached at Ψ = 0 MPa, a is the

229   inflection point (MPa), and b is a shape coefficient.

230   Germination rate in eq. 1 rapidly decreases below a particular level of water potential

231   (hereafter defined as the water-potential threshold for seed germination, Ψg, MPa), which can

232   be mathematically determined by equating the fourth derivate of the response function to

233   zero:

234
$$\psi_g = -\ln\left(5 - 2\sqrt{6}\right)b + a \,.$$
(2)

235   We applied a generalized linear model (quasi-binomial distribution and logit-link

236   function) with species (Sp, two levels: *B. eriopoda* and *L. tridentata*) and temperature (T,

237   four levels: 15°, 20°, 25° and 30°C) as factors, water potential (Ψ) as a co-variable, and their

238   interactions to test for their effects on germination rate (Gr). The Sp:Ψ interaction indicates

239   whether Ψg differs for *B. eriopoda* and *L. tridentata*. We also applied factorial ANOVA to

240   test for the effects of Sp, T and Ψ and their interactions on Germination time (Gt).

241

242   *Seedling emergence and early establishment simulations and analysis*

243   Early plant dynamics for *B. eriopoda* and *L. tridentata* are largely controlled by soil

244   moisture dynamics in the top 5 cm of the soil profile for seed germination and field

245   emergence, and in the top 30 cm for early root growth and seedling establishment (Minnick

246   and Coffin 1999, Peters 2000, Woods et al. 2011). Seed germination in arid environments is

247   also influenced by the temporal dynamics of maximum and minimum soil-surface

248   temperature, which constrain the opportunity time for field seedling emergence and further

10

249   plant establishment (Kigel 1995). We model long-term (centennial) soil-moisture contents

250   and soil surface temperature to simulate seedling emergence and early establishment of *B.*

251   *eriopoda* and *L. tridentata*. We use results of these simulations to analyze the influence of

252   shrub-encroachment stage, and growing season rainfall on the early dynamics of both species,

253   with particular detail for the 1950s severe drought, which is recognized as the period with the

254   most important *L. tridentata*-encroachment pulse for the Five Points experimental area (Gosz

255   1992, Allen et al. 2008).

256

257   (i)   Soil moisture and soil surface temperature simulations:

258   We applied an ecohydrological model, DayCent (Parton et al. 1998), parameterized

259   for our field sites, to simulate soil-moisture dynamics at a daily time step (at 0-5 cm and 0-30

260   cm) using daily precipitation records. The model (DayCent version 4.5) was set up to

261   represent soil-moisture records obtained in 2005-06 for our sites (Turnbull et al. 2010a). Soil

262   parameters (i.e., soil particle-size distribution and bulk density) were obtained from intensive

263   soil surveys carried out by Turnbull et al. (2010b). Soil-water content at field capacity and the

264   wilting point were obtained from the Brooks and Corey soil-water retention curves

265   determined in this study (Appendix S1: Table S3). Root distribution by depth was

266   parametrized using typical root-density values of Chihuahuan *B. eriopoda* grasslands and *L.*

267   *tridentata* shrublands (Appendix S1: Table S4). Application of the parameterized model

268   using 2005-06 onsite daily records of precipitation and air temperature (SNWR Five Points

269   station, Fig. 1b) yields a low root-mean-square error (0.02 vol/vol, 9% NRMSE) for soil

270   moisture prediction (Appendix S1: Fig. S2a).

271   Maximum and minimum soil temperatures were estimated using field-based air-soil

272   temperature relations. Our empirical air-soil equations were derived from the application of

273   polynomial regression of the historic 1997-2014 air and soil surface records of the SNWR

11

274 Five Points station (equations and error details in Fig. S2b and c of Appendix S1). Analysis

275 of the field-parameterized air-soil temperature equations yield low levels of root-mean-

276 squared error for the estimation of maximum (3.9°C, 9% NRMSE) and minimum (2.5°C, 7%

277 NMRSE) soil-surface temperature.

278   We simulated soil moisture and soil surface temperature dynamics in our four

279 experimental sites, by applying a centennial (1916-2014) series of daily (maximum and

280 minimum) air temperature and precipitation of a nearby (30 km from the study area)

281 reference weather station (Socorro, US Western Regional Climate Center station code

282 298387, 237 mm mean annual precipitation).

283

284 (ii)   Modeled seedling emergence and early establishment requirements:

285 Occurrence of emergence and early establishment of *B. eriopoda* and *L. tridentata* seedlings

286 was simulated by comparing modelled soil-surface temperature and soil-moisture contents

287 through time with the plant recruitment conditions of the species. The application of

288 conservative criteria derived from seed-germination laboratory tests provides a practical way

289 to simulate seedling emergence in the field (Lauenroth et al. 1994, Kigel 1995, Moreno-de las

290 Heras 2011). Thus, seedling emergence conditions were derived from our seed-germination

291 experiments. A wet period with water potential $\geq \Psi g$ MPa and length Gt (days) at 0-5 cm of

292 the soil profile is required for seedling emergence. Threshold water-potential values for our

293 sites, Ψg, were transformed into soil-moisture content (%, vol/vol) for analysis, by applying

294 the parameterized water-retention curves. We constrained the timing of seedling emergence

295 by imposing a tolerance range of soil-surface temperatures. Species-specific minimum soil-

296 surface temperature (T_{min}) was obtained in our seed germination experiments as the lowest

297 temperature level that showed optimal (i.e., not statistically different from peak) Gr and Gt

298 responses. Maximum soil-surface temperature (T_{max}) for our simulations was established at

12

299   35°C for *L. tridentata* and 40°C for *B. eriopoda*, which constitute the upper tolerance limit

300   for the species (Rivera and Freeman 1979, Minnick and Coffin, 1999).

301   Early seedling establishment for both *B. eriopoda* and *L. tridentata* largely depends

302   on root development, which has been shown to occur at minimum soil-moisture levels similar

303   to those required for seedling emergence (Herbel and Sosebee 1969, Woods et al. 2011). We

304   therefore applied the experimental Ψg water-potential threshold as minimum soil-moisture

305   level to simulate early seedling establishment. *B. eriopoda* seedling establishment depends on

306   the occurrence of suitable soil-moisture conditions for promoting the development of both the

307   seminal and adventitious root systems in the soil (Minnick and Coffin 1999, Peters 2000).

308   Early growth of the seminal roots requires wet conditions (i.e., water potential $\geq \Psi g$ MPa) at

309   0-30 cm during four consecutive days after seedling emergence (Minnick and Coffin 1999,

310   Peters 2000). Growth of the adventitious roots typically is initiated from the near surface 20

311   to 50 days after field emergence, and requires three consecutive days with water potential at

312   or above Ψg at 0-5 cm and a subsequent four-day period with suitable soil moisture at 0-30

313   cm (Peters 2000). The seminal root system has little capacity to support vegetation growth

314   under dry conditions. Hence, seedlings were simulated to die if soil moisture at 0-30 cm

315   drops below Ψg for more than four days between the dates of seedling emergence and

316   adventitious root growth (Minnick and Coffin 1999).

317   Early seedling establishment of *L. tridentata* is also strongly dependent on the

318   occurrence of suitable soil-moisture conditions for the development of the root system in the

319   0-30 cm soil profile. Creosotebush requires four weeks of wet conditions for taproot

320   development in the top 30 cm of the soil (Woods et al. 2011). Death of modeled seedlings

321   was simulated if soil moisture at 0-30 cm drops below Ψg for more than four days within four

322   weeks after the seedling-emergence date, applying a conservative seedling root-desiccation

323   criterion analogous to those described by Minnick and Coffin (1999). *L. tridentata*-root

324 growth requires unusually high oxygen and may also be affected by fungal intrusion due to

325 excessive soil moisture (Valentine and Gerard 1968, Woods et al. 2011). Seedlings were

326 therefore simulated to die if soil moisture at 0-30 cm remains at or above field capacity (-0.03

327 MPa) for more than four days in the (four-week) taproot-development period.

328

329 (iii)   Data analysis of simulated seedling emergence and early establishment:

330   We calculated the seedling emergence/establishment frequency (years^{-1}) and relative

331 frequency by month (%) for the modelled plant-recruitment dynamics in our sites between

332 1916 and 2014. Event-triggering rainfall for modelled seedling emergence (establishment)

333 was determined as the cumulative rainfall amount between the start date of seed germination

334 and seedling emergence (establishment) date. We analyzed the effects of species (two levels:

335 *B. eriopoda* and *L. tridentata*) and site (four levels: sites 1 to 4) on event-triggering rainfall

336 by applying factorial ANOVA. Factorial ANOVA was also applied to test for site effects and

337 differences in monsoonal summer rainfall (i.e., June-September precipitation) between

338 successful and unsuccessful years (i.e., years with and without seedling

339 emergence/establishment, respectively). Finally, we explored in detail the modeled *B.*

340 *eriopoda* and *L. tridentata* seedling-recruitment dynamics for the series 1948-59, which

341 includes the 1950s dry period plus three years immediately before/after that period.

342

343 RESULTS

344 *Soil seed-bank*

345   We identified a total of 21 different plant species in the soil seed-bank, comprising

346 twelve forbs, five grasses and four subshrub/shrub species (Appendix S1: Table S5). The soil

347 seed-bank at all sites is dominated by seeds of annual forbs (particularly *Plantago*

348 *patagonica*). Although species richness is similar in all sites (about 9-14 species), the floristic

349   composition and heterogeneity of the soil seed-bank are significantly affected by shrub

350   encroachment (Fig. 2). NMS ordination of the soil seed-bank data (81% of total data

351   variance) shows important differences in the floristic composition of the sites. Three grass

352   species (*Sporobolus flexuosus*, *Bouteloua eriopoda* and *Muhlenbergia torreyi*) and a

353   subshrub (*Gutierrezia sarothrae*) are indicative of the seed-bank floristic composition in the

354   grass-dominated and transition sites (Site 1 and 2, respectively; Fig. 2b). The soil seed-bank

355   structure in the shrub-transition and dominated sites (Site 3 and 4, respectively) is

356   characterized by the presence of two annual forb species (*Descurainia pinnata* and

357   *Chamaesyce serpyllifolia*), a perennial grass (*Dasyochloa pulchela*), two subshrubs

358   (*Thymophylla acerosa* and *Gutierrezia sphaerocephala*), and a shrub species (*Larrea*

359   *tridentata*, Fig. 2b). Heterogeneity of the soil seed-bank floristic composition (assessed on the

360   basis of mean Euclidean distances for each site in the NMS ordination) increases from the

361   grassland to the shrubland site (0.7, 0.9, 0.9 and 1.2 NMS distance units for Site 1, 2, 3 and 4,

362   respectively). Overall, the NMS representation of the soil seed-bank floristic structure can be

363   explained to a large extent by the abundance of grass and shrub patches in the (5 x 5 m) seed-

364   bank plots (Fig. 2a).

365   Average density of viable seeds in the soil ranges 200-400 seeds m^{-2} (no differences

366   were found between sites at $P<0.05$) and is mainly dominated by annual forbs (Fig. 3a),

367   particularly *Plantago patagonica* and *Phacelia integrifolia* (largely distributed in all sites)

368   and *Descurainia pinnata* and *Chamaesyce serpyllifolia* (broadly distributed in sites 3 and 4).

369   Grass seed abundance in the shrub-dominated Site 4 is negligible (5 seeds m^{-2}, Fig. 3a). *B.*

370   *eriopoda* seed density varies significantly between sites (H=9.7, 3 d.f., $P=0.02$), decreasing

371   from sites 1 and 2 to sites 3 and 4 (from 50-40 to 7-0 seeds m^{-2}, Fig. 3b). *L. tridentata* soil

372   seed abundance differs marginally between sites (H=6.3, 3 d.f., $P=0.08$), with 20 seeds m^{-2} in

373   the shrubland, 6-2 seeds m^{-2} in the transition sites, and no *L. tridentata* seeds in the grassland

374   (Fig. 3c).

375   Availability of viable seeds differs between surface types (H=19.8, 2 d.f., *P*<0.01).

376   Bare soil interpatches show very low densities (typically <150 seeds m^{-2}), while vegetated

377   clumps concentrate most viable seeds, peaking up to maxima of 800 and 1200 seeds m^{-2} for

378   grass and shrub patches, respectively (Fig. 3d). *B. eriopoda* seeds are preferentially

379   distributed in the grass patches (H=13.9, 2 d.f., *P*<0.01, Fig. 3e). We did not find any *L.*

380   *tridentata* seeds outside the shrub patches (Fig. 3f). Seed availability in all sites follows the

381   same surface-type trends, although for Site 1 we did not find in general significant

382   differences between interpatches and vegetated (grass) patches at *P*<0.05 (Appendix S1:

383   Table S6).

384

385   *Seed-germination conditions for B. eriopoda and L. tridentata*

386   Maximum germination rate for both *B. eriopoda* and *L. tridentata* ranges from 75% to

387   80% and is reached at 20-25°C (Fig. 4a and b). We did not find significant differences in seed

388   germination between species at *P*<0.05. Seed germination is affected by temperature

389   ($F_{3, 432}$=52.0, *P*<0.01), and decreases abruptly below 20°C. Seed germination is also affected

390   by water potential ($F_{1, 432}$=2042.2, *P*<0.01), and decreases rapidly below threshold Ψg levels

391   of about -0.45 and -0.55 MPa for *B. eriopoda* and *L. tridentata*, respectively (Fig. 4a and b,

392   supplement Table S7 in Appendix S1). The interaction between species and water potential

393   (Sp:Ψ, $F_{1, 432}$=10.8, *P*<0.01) indicates that threshold Ψg is significantly higher for *B.*

394   *eriopoda*. Soil moisture at Ψg for our sites ranges from 9.0% (for -0.55 MPa in Site 1) to

395   9.7% (for -0.45 MPa in Site 4, Table S3 in Appendix S1).

396   Time to germination is affected by species ($F_{1, 240}$=72.4, *P*<0.01) and water potential

397   ($F_{4, 240}$=14.3, *P*<0.01). *L. tridentata* shows generally longer seed-germination times than *B.*

398 *eriopoda* (Fig. 4c). Time to germination increases for both species significantly by reducing

399 water potential (Fig. 4c). Seed germination time is also affected by temperature ($F_{3, 240}=51.2$,

400 *P*<0.01), and is significantly longer at 15°C (Fig. 4d). The lowest temperature level that

401 shows a quick and strong seed germination response (T_{min}) is 20°C for both species. Time to

402 germination (Gt) at 20-30°C and threshold Ψg water potential (*B. eriopoda*: -0.45 MPa,

403 9.3%-9.7% soil moisture for the study sites; *L. tridentata*: -0.55 MPa, 9.0%-9.3% soil

404 moisture) is about six and ten days for *B. eriopoda* and *L. tridentata*, respectively (Fig. 4d).

405

406 *Simulated seedling emergence and establishment for B. eriopoda and L. tridentata*

407   Simulated conditions for seedling emergence in the study sites take place at a

408 frequency of 0.29-0.37 years^{-1} (recurrence time 2-3 years) for *B. eriopoda* and 0.08-

409 0.15 years^{-1} (recurrence time 6-12 years) for *L. tridentata* (Fig. 5a, supplement Tables S8 and

410 S9 in Appendix S1). Modelled early seedling-recruitment events show a frequency of 0.13-

411 0.22 years^{-1} (recurrence time 5-8 years) for *B. eriopoda*, and 0.04-0.12 years^{-1} (recurrence

412 time 9-25 years) for *L. tridentata* (Fig. 5b, supplement Tables S8 and S9 in Appendix S1).

413 Sites 3 and 4 show in general longer recurrence times for seedling emergence and

414 establishment than sites 1 and 2. Simulated seedling emergence takes place primarily in July

415 and August for both species (Fig. 5a), while early seedling establishment occurs mainly in

416 September for *B. eriopoda* and throughout August and September for *L. tridentata* (Fig. 5b).

417   Triggering rainfall for seedling emergence differs for *L. tridentata* and *B. eriopoda*

418 ($F_{1, 182}=37.9$, *P*<0.01). *B. eriopoda* seedling emergence requires less rainfall than *L. tridentata*

419 (a minimum of 15 mm and 35 mm average rainfall for *B. eriopoda*, compared with 30 mm

420 minimum and 50 mm average for *L. tridentata*, Fig.5c). Triggering rainfall for seedling

421 establishment is also affected by species ($F_{1, 103}=17.3$, *P*<0.01). *B. eriopoda* early seedling

422 establishment takes place in a wide rainfall range (from approx. 50 to 160 mm), while *L.*

17

423 *tridentata* seedling establishment occurs within a narrower window of rainfall amount (from

424 about 50 to 100 mm, Fig. 5c). We did not find any differences between sites at $P<0.05$ on

425 triggering rainfall requirements for both seedling emergence and early establishment.

426   Monsoonal summer rainfall differs for successful and unsuccessful years at both the

427 seedling emergence ($F_{1,\,776}=162.4$, $P<0.01$) and early establishment ($F_{1,\,776}=72.8$, $P<0.01$)

428 stages. In general, successful seedling emergence and establishment for both *B. eriopoda* and

429 *L. tridentata* takes place in years with above-average summer rainfall, while unsuccessful

430 years commonly have lower monsoonal rainfall (Fig. 5d). *L. tridentata* emergence occurs in

431 years with higher monsoonal rainfall than *B. eriopoda* seedling emergence ($F_{1,\,776}=21.3$,

432 $P<0.01$), however we did not find differences between species in summer precipitation for

433 seedling establishment (Fig. 5d). Site does not affect monsoonal requirements for successful

434 seedling emergence/establishment of the species at $P<0.05$.

435   Detailed exploration of seedling emergence and early establishment simulations for

436 1948-59 indicates that the soil-surface temperature and soil-moisture dynamics in (the grass-

437 transition) Site 2 meet episodically the creosostebush recruitment conditions during that

438 period (Fig. 5e). Particularly, modelled dynamics show successful recruitment of *L.*

439 *tridentata* for 1949 and 1957, immediately before and after the 1951-56 drought (1951-56

440 average yearly precipitation is 160 mm, 35% less than the 1916-2014 annual mean). Surface

441 temperature and soil-moisture conditions for *B. eriopoda* seedling recruitment are also

442 episodically met in Site 2 within 1948-59 (years 1949, 1950, 1954 and 1957). Recruitment

443 simulations in sites 1, 3 and 4 show similar patterns (Appendix S1: Fig. S3).

444

445 DISCUSSION

446 *Structure of the soil seed-bank and availability of B. eriopoda and L. tridentata seeds*

447   Shrub encroachment in Chihuahuan landscapes promotes large changes in the

448   structure and spatial pattern of plant communities (Schlesinger et al. 1990, Reynolds et al.

449   1999, Baez and Collins 2008, Peters et al. 2015). Overall, the observed variations in floristic

450   composition of the soil seed-bank over the study grassland-shrubland ecotone (Fig. 2) are

451   consistent with aboveground variations in species composition and also reflect the higher

452   spatiotemporal heterogeneity of subdominant plant communities in areas invaded by *L.*

453   *tridentata* (Baez and Collins 2008).

454   Soil seed-density records in our Chihuahuan study are considerably smaller than

455   typical viable seed amounts in other semi-arid environments, for example the Shortgrass

456   Steppe and Mediterranean grasslands, which have average densities generally above 1000

457   seeds m^{-2} (Coffin and Lauenroth 1989, Espigares and Peco 1993), although they are within

458   the range of viable soil seed densities reported to occur in desert sites (Dwyer and Aguirre

459   1978, Koontz and Simpson 2010). Concentration of the soil seed-bank in densely vegetated

460   microsites is characteristic of many arid and semi-arid landscapes, where vegetation patches

461   generally act as a sink for water, nutrients and sediments, leading to improved soil properties

462   (Bochet 2015). Spatial heterogeneity of soil seed density in these systems is usually explained

463   by the patchy distribution of parental plants (i.e., seed pools generally decrease rapidly away

464   from the parental plants for most gravity dispersed seeds) and patch-to-patch transmission of

465   seeds by wind and/or runoff (Guo et al. 1998, Koontz and Simpson 2010, Thompson et al.

466   2014). Spatial heterogeneity of soil properties (i.e., abundance of fine soil particles, organic

467   matter and nutrients) in Chihuahuan landscapes generally increases with the progression of

468   shrub encroachment, as does the size of bare soil interpatches and intensity of water and wind

469   erosion (Schlesinger et al. 1990, Reynolds et al. 1999, Turnbull et al. 2010a, 2010b). These

470   observations are in agreement with our results, which show that the differences in seed

471   density between vegetated and bare soil patches are more pronounced in the shrub-transition

472   and shrubland sites where bare soil interpatches are in general longer than 1 m (Appendix S1:

473   Table S6).

474   Our observations of low viable seed availability for *B. eriopoda* and *L. tridentata*

475   (<100 seeds m^{-2}; Fig. 3b and c) are in agreement with other studies carried out in Chihuahuan

476   landscapes (Dwyer and Aguirre 1978, Guo et al. 1998, Koontz and Simpson 2010). Previous

477   analysis of *B. eriopoda* seed production and soil seed-bank dynamics in the SNWR indicates

478   that *B. eriopoda* has (short-lived) transient seed-banks, and that viable seed availability in the

479   soil is strongly dependent on the amount of growing season (June-September) precipitation,

480   ranging from 0 seeds m^{-2} for dry years to 200-400 seeds m^{-2} for very wet years (Peters

481   2002a). Our soil seed-bank analysis reflects limited amounts of viable *B. eriopoda* seeds for

482   2013 (average 50 seeds m^{-2} in the grassland and grass-transition sites) despite high growing

483   season precipitation (243 mm, 75% above the historical average), and suggests legacy effects

484   of preceding dry years 2011 and 2012 (80-90 mm summer rainfall, 35% below the historical

485   mean). *B. eriopoda* growth and seed production frequently shows legacy effects on previous

486   year's rainfall conditions (Nelson 1934). *L. tridentata* seeds are also relatively short-lived

487   and, although this shrub species does not show in general persistent soil seed-banks, *L.*

488   *tridentata* growth and seed production are quite stable in time, producing year-round small

489   crops of viable seeds except for growing seasons preceded by extremely low winter

490   temperatures (Valentine and Gerard 1968, Boyd and Brum 1983). *L. tridentata* has a bimodal

491   rooting system (encompassing near-surface and >70 cm depth active roots) that facilitates the

492   use of both (ephemeral) shallow soil water-resources derived from summer rainfall and (more

493   stable) winter-derived deep soil moisture -that is not available to grass species- for plant

494   production, limiting the inter-annual variation in shrub seed production (Reynolds et al. 1999,

495   Ogle and Reynolds 2004, Moreno-de las Heras et al. 2015).

496   The availability of *B. eriopoda* and *L. tridentata* seeds varies at both the plant-patch

497   scale and broader landscape scale over the shrub-encroachment gradient (Fig. 3). This

498   variation may be explained by primary and secondary seed dispersal mechanisms. Primary

499   seed dispersal of *L. tridentata* is typically limited to a few centimeters from the shrubs, thus

500   yielding patch-scale variations in seed abundance, whereas secondary dispersal by runoff

501   during early summer storms may facilitate patch-to-patch transport of significant amounts of

502   *L. tridentata* seeds over longer distances (Chew and Chew 1965, Valentine and Gerard 1968,

503   Boyd and Brum 1983, Thompson et al. 2014). *B. eriopoda* seeds are dispersed more widely

504   by wind (Peters and Yao 2012). However, low flowering and seed-production capacity of *B.*

505   *eriopoda* plants growing in areas co-dominated by *L. tridentata* may strongly limit the

506   availability of viable *B. eriopoda* seeds in transition and shrubland landscapes (Nelson 1934,

507   Peters 2002a).

508

509   *Seed-germination conditions for B. eriopoda and L. tridentata*

510   Soil-water potential and temperature broadly limit seed germination in arid and semi-

511   arid landscapes (Kigel 1995) and control the time and rates of seed germination for *B.*

512   *eriopoda* and *L. tridentata* (Fig. 4). Threshold soil moisture for seed germination obtained in

513   our study for *B. eriopoda* (Ψg: -0.45 MPa) is higher than that applied by Minnick and Coffin

514   (1999) and Peters (2000) to simulate the seedling-emergence dynamics of *B. eriopoda* across

515   the Shortgrass Steppe and Chihuahuan landscapes. They used a threshold water-potential

516   level of -1.0 MPa based on laboratory tests on seeds by Knipe and Herbel (1960), which

517   reported a strong *B. eriopoda* germination response at -1.1 MPa applying a non-conservative

518   criterion (a seed was considered germinated when the radicle had perforated the pericarp,

519   irrespective of radicle elongation). Our seed-germination criterion is more conservative, and

520   requires the elongation of at least 4 mm of radicle. Knipe and Herbel (1960) found very little

521   radicle extension (≤ 2 mm) at water-potential levels below -0.7 MPa, which can hardly

522   support seedling emergence in the field. Laboratory observations of *B. eriopoda* seedling

523   emergence and early growth under controlled conditions indicate that emergence does not

524   occur if daily average soil water potential drops below -0.5 MPa (Herbel and Sosebee, 1969),

525   which is in agreement with our estimate of Ψg for *B. eriopoda*. Our experimental *L.*

526   *tridentata* Ψg threshold level for seed germination (-0.55 MPa) is also within the range of

527   critical osmotic potential (from -0.4 to -0.6 MPa) reported by other studies (Tipton, 1985).

528   Similarly, experimental *B. eriopoda* and *L. tridentata* seed-germination time in our study (6

529   and 10 days, respectively, Fig. 4d) is consistent with field and laboratory observations of seed

530   germination and seedling emergence of these species (Barbour 1968, Rivera and Freeman

531   1979, Minnick and Coffin 1999).

532

533   *Recruitment recurrence times and environmental conditions for field emergence and early*

534   *establishment dynamics of B. eriopoda and L. tridentata*

535   Our results show that environmental conditions for *B. eriopoda* seedling recruitment

536   have recurrence times ranging from 5 to 8 years (Fig. 5b). This result is comparable to that of

537   Neilson (1986), who in a 55-year field study in a northern Chihuahuan site obtained a *B.*

538   *eriopoda* seedling recruitment recurrence time of 8 years. Similarly, Peters (2000) in a

539   simulation study reported *B. eriopoda* recurrence times ranging from 4 to 10 years for

540   Chihuahuan grasslands with 210-330 mm annual precipitation. Our results are also consistent

541   with field observation of bi-decadal (1989-2010) *B. eriopoda* dynamics in grass-dominated

542   landscapes within the SNWR experimental Five Points area, where *B. eriopoda* has increased

543   in cover via both stoloniferous expansion and occasional seed reproduction (Peters and Yao

544   2012, Collins and Xia 2015). The two most important pulses of *B. eriopoda* cover expansion

545   over the Five Points grasslands took place in 1991 and 1999 (Collins and Xia 2015), which

546   coincide with two major pulses of simulated *B. eriopoda* recruitment for our experimental

547   sites, also located within the Five Points area (Appendix S1: Table S8).

548   Our simulations suggest that *B. eriopoda* seedling emergence occurs primarily in July

549   and August (Fig. 5a). Peters (2000) simulated *B. eriopoda* seedling emergence centered in

550   June, by applying less conservative water potential requirements (-1.0 MPa Ψg); however,

551   June is in general a dry month compared to July and August (1919-2014 average precipitation

552   in the area for June is 15 mm and 40-50 mm for July and August). Field observations in north

553   Chihuahuan grasslands and experimental *B. eriopoda* plantings indicate that seedling

554   emergence and plant growth for *B. eriopoda* usually does not start until July (Nelson 1934,

555   Leyendecker 1975, Dwyer and Aguirre 1978). Our simulations indicate that *B. eriopoda*

556   seedling establishment primarily takes place in September (Fig. 5b), which also matches *B.*

557   *eriopoda* early establishment observations in Chihuahuan landscapes during late-summer and

558   early-fall rains (Neilson 1986).

559   Our results suggest that *L. tridentata* recruitment events across the grassland-

560   shrubland ecotone appear to be sporadic, although with considerably longer recurrence times

561   than for *B. eriopoda* (9-25 years, Fig. 5b), which could be expected considering the extremely

562   high life-span (about 400 years) of adult Chihuahuan *L. tridentata* shrubs (Miller and

563   Huenneke 2000). There is little field information on the recurrence times of seedling

564   emergence and establishment for *L. tridentata*. Valentine and Gerard (1968) estimated

565   effective *L. tridentata* seedling emergence pulses to occur with a time-span of 7 years in the

566   Jornada Experimental Range (southern New Mexico), which is in line with our modeled *L.*

567   *tridentata*-seedling emergence frequency of 6-12 years (Fig. 5a). Analysis of *L. tridentata*

568   age and size frequency in a variety of sites across southwestern USA suggests that *L.*

569   *tridentata* seedling recruitment occurs primarily during rare and episodic events, which

570   supports our low frequency early seedling establishment simulations (Chew and Chew 1965,

571   Barbour 1969, Allen et al. 2008). LTER vegetation surveys between 1990-2012 within the

572   SNWR Five Points area indicate that seedling emergence and establishment of *L. tridentata*

573   appears to be low, sporadic and very localized, accounting for a small recruitment pulse that

574   took place at the end of the 1990s (Moore 2011, Peters and Yao 2012). Recent vegetation

575   surveys in the area indicate that other sizeable *L. tridentata* emergence events also took place

576   in 2013 and 2014, although showing variable recruitment success (for the 2014 event all

577   seedlings died within 4-6 months of field emergence; Scott L. Collins, personal

578   communication). Our *L. tridentata* modelling results suggest that the most recent effective

579   recruitment events for the area took place in years 1997, 1999 and 2013 (Appendix S1: Table

580   S9).

581   Field observations of *L. tridentata* seedlings in southwestern USA landscapes indicate

582   that emergence and early plant establishment can take place broadly between early-July and

583   the beginning of October, with timing frequently synchronized with the occurrence of late-

584   summer heavy rainfall (Rivera and Freeman 1979, Boyd and Brum 1983, Bowers et al.

585   2004). Our simulations reproduce field-observed temporal patterns, with *L. tridentata*

586   seedling emergence and early establishment concentrated principally in late summer (August

587   and September, Fig. 5a and b). *L. tridentata* seedling establishment may be facilitated by the

588   activity of Eastern Pacific tropical cyclone remnants, which provide supplemental soil

589   moisture in the arid southwest USA from late-August until October (Ritchie et al. 2011).

590   Whilst our modelling results suggest that seedling recruitment of both *B. eriopoda*

591   and *L. tridentata* is generally associated with the occurrence of strong monsoons (Fig. 5d),

592   triggering rainfall for field emergence and early plant establishment are different for the two

593   species (Fig. 5c). *B. eriopoda* requires moderate amounts of rainfall for seedling emergence

594   (minimum 15 mm, and 35 mm average rainfall), and early plant establishment occurs within

595   a broad range of precipitation during the seedling development period (from 50 to 160 mm).

24

596 These requirements match the rainfall conditions empirically determined by Herbel and

597 Sosebee (1969) for *B. eriopoda* seedling emergence (20-30 mm within six days) and early

598 survival (70-150 mm within a month of seed planting) under typical summer temperatures for

599 northern Chihuahuan landscapes (20-50°C). Conversely, *L. tridentata* seedling emergence

600 requires a minimum of 30 mm and average 50 mm rainfall, which also agrees with field

601 observations and controlled laboratory analysis of *L. tridentata* early plant development

602 (Rivera and Freeman 1979, Boyd and Brum 1983, Bowers et al. 2004, Woods et al. 2011).

603 The smaller range of rainfall conditions for *L. tridentata* early establishment (from 50 to 100

604 mm for 5-6 consecutive weeks) reflects its low seedling tolerance to high soil moisture.

605 Laboratory rhizotron and field experiments indicate that *L. tridentata* has unusually high

606 oxygen requirements for root development, which together with fungal intrusion under high

607 soil moisture regimes can produce high seedling mortality and/or weaken early-established

608 shrubs for longer-term survival (Valentine and Gerard 1968, Woods et al. 2011). Extreme

609 winter temperatures (i.e., cold spells with minimum air temperature below -14°C) may cause

610 further seedling mortality due to freezing-induced xylem cavitation in the SNWR, where *L.*

611 *tridentata* is close to its northern Chihuahuan distribution limit (Martinez-Vilalta and

612 Pockman 2002).

613

614 *Implications for vegetation dynamics at Chihuahuan grassland-shrubland boundaries*

615   Early seedling dynamics can have a great influence on the spatial pattern and rates of

616 vegetation change in drylands (Espigares and Peco 1993, Kigel 1995, Peters 2002b, Bowers

617 et al. 2004, Collins and Xia 2015, Bochet 2015). Our *L. tridentata* seedling simulations along

618 1948-1959 suggest that the 1950s dry period provided an ideal window for the expansion of

619 *L. tridentata* over the SNWR Five Points area (Fig. 5e), with shrub recruitment taking place

620 immediately before and after the 1951-1956 dry period. Soil moisture and surface-soil-

621   temperature dynamics also episodically meet the conditions for *B. eriopoda* recruitment along

622   the 1950s dry decade. However, it is unlikely that grass recruitment pulses would have been

623   taken place, as a result of a lack of persistent *B. eriopoda* soil seed-banks and deficiency of

624   viable seed production in dry years with below-average summer monsoonal precipitation

625   (Nelson 1934, Peters 2002a). In fact, field analysis of *B. eriopoda* basal cover in north

626   Chihuahuan grasslands indicates that, although relatively high summer rainfall in 1949 and

627   1950 resulted in a small increase in *B. eriopoda* cover, the abundance of perennial grass (also

628   affected by cattle grazing in the SNWR at that time) was reduced to very low levels along the

629   1951-1956 dry period (Gibbens and Beck 1988). High drought tolerance of *L. tridentata*

630   plants and synchronization of active shrub recruitment at the temporal boundaries of the dry

631   period may have been contributing factors in facilitating widespread expansion of *L.*

632   *tridentata* over damaged *B. eriopoda* grasslands in the SNWR Five Points area during and

633   shortly after the 1950s severe multiyear drought. Accordingly, analysis of shrub architecture

634   and age-structure indicates that local *L. tridentata* populations are dominated by shrubs that

635   were recruited primarily within or soon after the 1950s (Allen et al. 2008).

636   Observed spatial structure of the *L. tridentata* seed-bank and simulated temporal

637   patterns of shrub recruitment for the study sites suggest that shrub colonization is an episodic

638   and localized phenomenon associated with the occurrence of late-summer strong

639   precipitation. Viable soil seed concentration near adult shrubs due to limited *L. tridentata*

640   large-range seed dispersal (Fig. 3f) can constrain the spatial extension of active shrub

641   recruitment events. Similarly, the narrow range of rainfall conditions for early seedling

642   establishment (Fig. 5c) strongly limit effective *L. tridentata* recruitment pulses to episodic

643   decadal events, especially in Chihuahuan landscapes where local (diploid) *L. tridentata*

644   populations appear to establish and regenerate exclusively by seed reproduction (Miller and

645   Huenneke 2000, Pendleton et al. 2008, Peters and Yao 2012). In support of this result, field

646 observations at the SNWR area indicate that *L. tridentata* infill across the Five Points area

647 happens slowly, and is characterized by the occurrence of rare and sporadic events that are

648 localized in space (Allen et al. 2008, Moore 2011, Peters and Yao 2012). Erratic spacing of

649 summer storms may also be partially responsible of the localized nature for the observed

650 recruitment events (Valentine and Gerard 1968). Overall, the grassland-shrubland boundary

651 at the Five Points area appears to be stable at present (Moreno-de las Heras et al. 2015). In

652 the absence of cattle overgrazing, no significant shrub encroachment is expected for the area

653 unless extensive grass suppression by an extreme event similar to the severe and prolonged

654 1950s drought occurs (Peters 2002b). However, an increment of the activity of tropical

655 cyclones that provide supplemental late-summer and fall precipitation for the area may

656 facilitate *L. tridentata* infill and long-term destabilization of *B. eriopopda-L. tridentata*

657 transition landscapes in the region, particularly under the future context of increased aridity in

658 southwestern USA due to higher summer temperatures and increased drought frequency

659 (Garfin et al. 2013).

660   Environmental conditions for seedling recruitment and soil seed-bank structure also

661 have a critical role for the re-establishment of *B. eriopoda* in landscapes now dominated by *L.*

662 *tridentata*, and particularly for those areas lacking *B. eriopoda* remnants that impedes

663 vegetative plant recovery via stolons. Our simulations indicate that shrub encroachment has a

664 net impact on *B. eriopoda* seedling dynamics, reducing the frequency of soil-moisture

665 conditions for early establishment from 0.22 years^{-1} in the grassland sites down to 0.13 years^{-}

666 ^{1} in the shrub-transition and shrubland landscapes (Fig. 5b). The longer seedling recurrence

667 times in areas where shrubs are dominant can be explained by site variations in soil properties

668 and soil-surface hydrology that are induced by the shrub-encroachment process (Schlesinger

669 1990, Reynolds 1999, Peters 2002b, Stewart et al. 2014). Previous analysis of soil-surface

670 hydrology of our study sites indicates that increased (bare soil) interpatch size and surface

671 stoniness for the shrub-transition and dominated sites 3 and 4 intensify runoff production and

672 soil erosion, which feedbacks into plant dynamics by reducing plant-available soil moisture

673 (Turnbull et al. 2010a, 2010b). Furthermore, the lack of viable *B. eriopoda* seeds in those

674 landscapes (Fig. 3b) makes *B. eriopoda* seedling recruitment highly unfeasible in areas now

675 dominated by the shrubs. In fact, recent bi-decadal field observations of *B. eriopoda*

676 dynamics across the Five Points area indicate that although *B. eriopoda* cover has broadly

677 increased since 1989 in grassland sites via both stoloniferous expansion and episodic seed

678 reproduction, no *B. eriopoda* recovery has been detected in shrubland and shrub-transition

679 sites, even 16 years after the application of experimental shrub removal (Peters and Yao

680 2012, Collins and Xia 2015). Similarly, long-term (1930-present) experiments of (spatially

681 extensive) *L. tridentata* removal in other Chihuahuan shrublands have shown little impact on

682 grass recovery (Rango et al. 2005). Peters (2002b), using a dynamic vegetation model,

683 proposed that increases in summer precipitation could facilitate the re-establishment of *B.*

684 *eriopoda* in areas now dominated by *L. tridentata*. However, lack of viable *B. eriopoda* seeds

685 and tighter environmental conditions for grass establishment in shrubland sites may greatly

686 limit the rates of *B. eriopoda* recovery even under favorable climate conditions, which thus

687 has significant implications for the restoration of native grasslands. Re-seeding in targeted

688 areas (e.g., shallow gullies, gently sloping arroyos and small artificial dikes) strategically

689 applied with punctual supplemental irrigation may help re-establish *B. eriopoda* patches in

690 areas invaded by *L. tridentata* (Herrick et al. 1997). Our study provides biophysical criteria to

691 regulate supplemental irrigation efforts for satisfying soil moisture conditions along the *B.*

692 *eriopoda* emergence and early seedling establishment stages.

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707

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868

SUPPORTING INFORMATION

870 APPENDIX S1. Supplementary results: characterization of soils and soil-water retention

871 curves, parameterization and error assessment details of soil moisture/temperature

872 simulations, supplementary seed-bank and seed germination results, and detailed records of

873 seedling emergence/establishment simulations.

874

875 FIGURE CAPTIONS

876 FIG. 1. Study area: (a) location of the Sevilleta National Wildlife Refuge (SNWR) and

877 distribution of New Mexico biomes, (b) location of the study sites (Five Points area,

878 McKenzie Flats), and (c) study sites (10 x 30 m each). Biome distribution in panel (a) follows

879 the Sevilleta LTER classification (http://sev.lternet.edu/about). Spatial distribution of surface

880 types (%) was determined in the field by applying the point intercept method (details in

881 methods). Graphical representation of surface types (panel c) for sites 1, 3 and 4 was taken

882 from Turnbull et al. (2010a). For Site 2, surface types were digitalized from a recent (2014)

883 high-resolution (20 cm pixel^{-1}) aerial image available through GoogleEarth™. Source for

884 background image in panels (a) and (b): 2014 National Aerial Imagery Program (USDA

885 Farm Service Agency).

886

887 FIG. 2. NMS (non-metric multidimensional scaling) ordination of the soil seed-bank data: (a)

888 projection of cases (5 x 5 m soil seed-bank plots), and (b) projection of plant species (for

889 simplification, only plant species that significantly correlate with the ordination axes at

890 $P<0.05$ are shown; tested using Spearman's R). Grass and shrub patch abundances are

891 represented in panel (a) by the fitted thin plate spline surfaces (R^2 and significance of the

892 fitted surfaces is shown). Species symbols in panel (b) follow the USDA Plant Database

893 codes (http://plants.usda.gov/java/): MUPO2, *Muhlenbergia torreyi*, GUSA2, *Gutierrezia*

894 *sarothrae*, SPFL2, *Sporobolus flexuosus*, BOER2, *Bouteloua eriopoda*, DEPI, *Descurainia*

895 *pinnata*, GUSP, *G. sphaerocephala*, DAPU7, *Dasyochloa pulchela*, CHES6, *Chamaesyce*

896 *serpyllifolia*, LATR2, *Larrea tridentata*, THAC, *Thymophylla acerosa*. Sig. codes: **,

897 *P*<0.01.

898

899 F<small>IG</small>. 3. Differences in soil seed density (viable seeds m^{-2}) for (a-c) sites (area-weighed values)

900 and (d-f) surface types. Total seeds (a and d), *B. eriopoda* seeds (b and e), and *L. tridentata*

901 seeds (c and f). Shrub/subshrub, forb and grass seed densities are detailed in different patterns

902 in panels (a) and (b). Different letters indicate differences at *P*<0.05. Tested using Kruskal-

903 Wallis ANOVA and post-hoc Mann-Whitney tests.

904

905 F<small>IG</small>. 4. Seed germination environmental conditions: germination vs. water potential curves for

906 (a) *B. eriopoda* and (b) *L. tridentata* (parameter estimates of the fitted curves are detailed in

907 the supplement Table S7 of Appendix S1), (c) impact of water potential level on seed

908 germination time (pooled data for the studied range of temperatures), and (d) impact of

909 temperature on seed germination time (pooled data for water potential at -0.4 and -0.6 MPa).

910 Different temperatures are indicated by different markers and line patterns in panels (a) and

911 (b). Water potential threshold for seed germination (Ψg) is indicated in each curve by the

912 vertical arrows (panels a and b). Different letters indicate differences at *P*<0.05 (plots c and

913 d). Tested using factorial ANOVA and post-hoc Tukey HSD tests. Sig. Codes for panels (a)

914 and (b): '**', *P*<0.01.

915

916 F<small>IG</small>. 5. Simulated seedling emergence and early establishment for *B. eriopoda* and *L.*

917 *tridentata*: frequency (year^{-1}) and relative frequency by month (%) of seedling emergence (a)

918 and establishment (b) for the studied sites, (c) differences in event (seedling

919   emergence/establishment) triggering rainfall between species, (d) differences in monsoonal

920   summer rainfall (from June to September) between years that showed successful seedling

921   emergence/establishment (successful years) and years without occurrence of seedling

922   emergence/establishment (unsuccessful years), (e) simulated 1948-1959 seedling recruitment

923   dynamics for Site 2 (modeled soil surface temperature and soil moisture at 0-5 cm and 0-30

924   cm are shown along with seasonal precipitation, daily rainfall, and simulated seedling

925   establishment events). Seedling recruitment simulation results for Site 1, 3 and 4 can be

926   found in the supplement Figure S3 of Appendix S1. Different letters in panels (c) and (d)

927   indicate differences within event type (seedling emergence or establishment) at $P<0.05$.

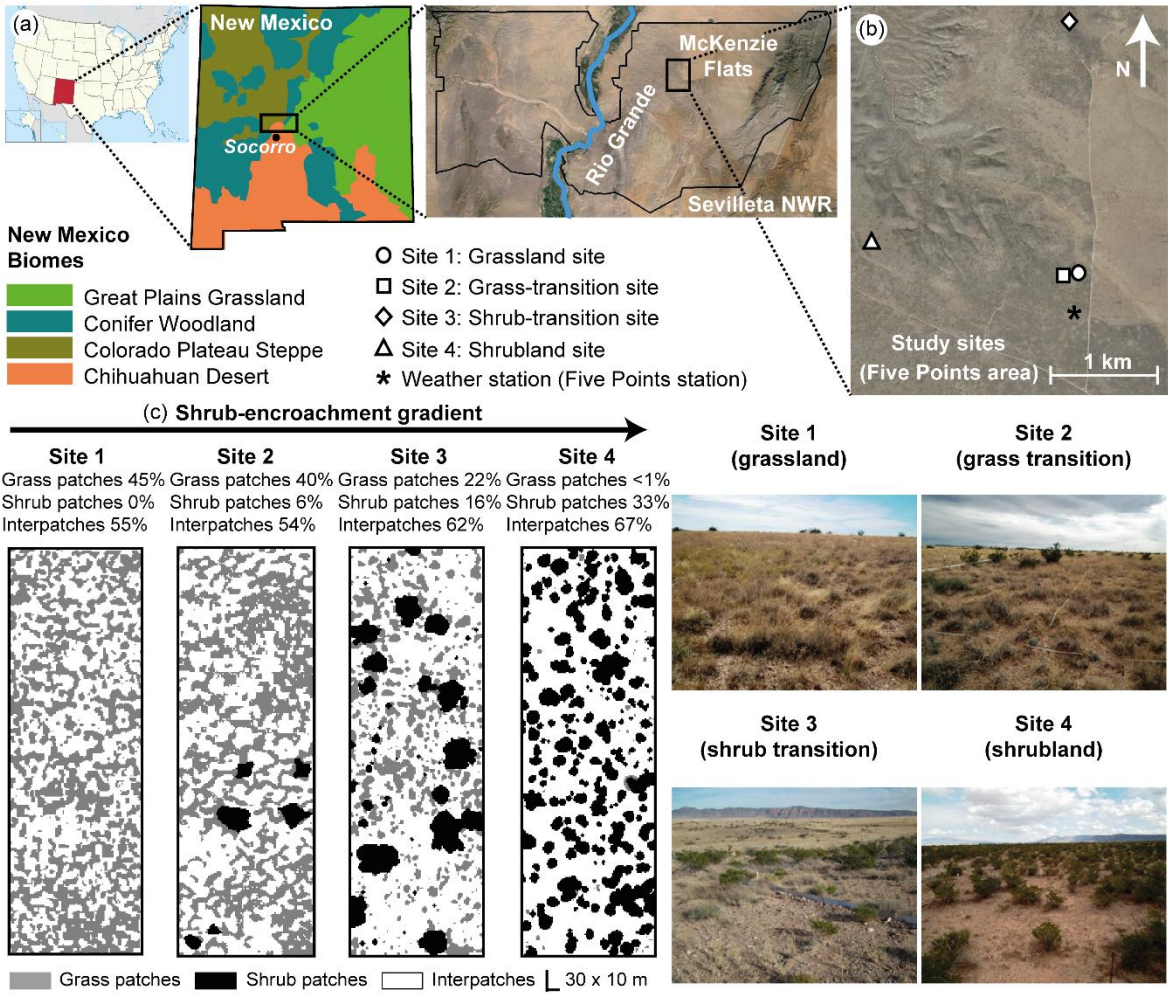928   Tested using factorial ANOVA and post-hoc Tukey HSD tests.

929

(a)

New Mexico Biomes

- ■ Great Plains Grassland
- ■ Conifer Woodland
- ■ Colorado Plateau Steppe
- ■ Chihuahuan Desert

○ Site 1: Grassland site
□ Site 2: Grass-transition site
◇ Site 3: Shrub-transition site
△ Site 4: Shrubland site
✱ Weather station (Five Points station)

(b) Study sites (Five Points area)   1 km   N

(c) **Shrub-encroachment gradient**

Site 1	Site 2	Site 3	Site 4
Grass patches 45%	Grass patches 40%	Grass patches 22%	Grass patches <1%
Shrub patches 0%	Shrub patches 6%	Shrub patches 16%	Shrub patches 33%
Interpatches 55%	Interpatches 54%	Interpatches 62%	Interpatches 67%

■ Grass patches   ■ Shrub patches   □ Interpatches   └ 30 x 10 m

Site 1 (grassland)   Site 2 (grass transition)
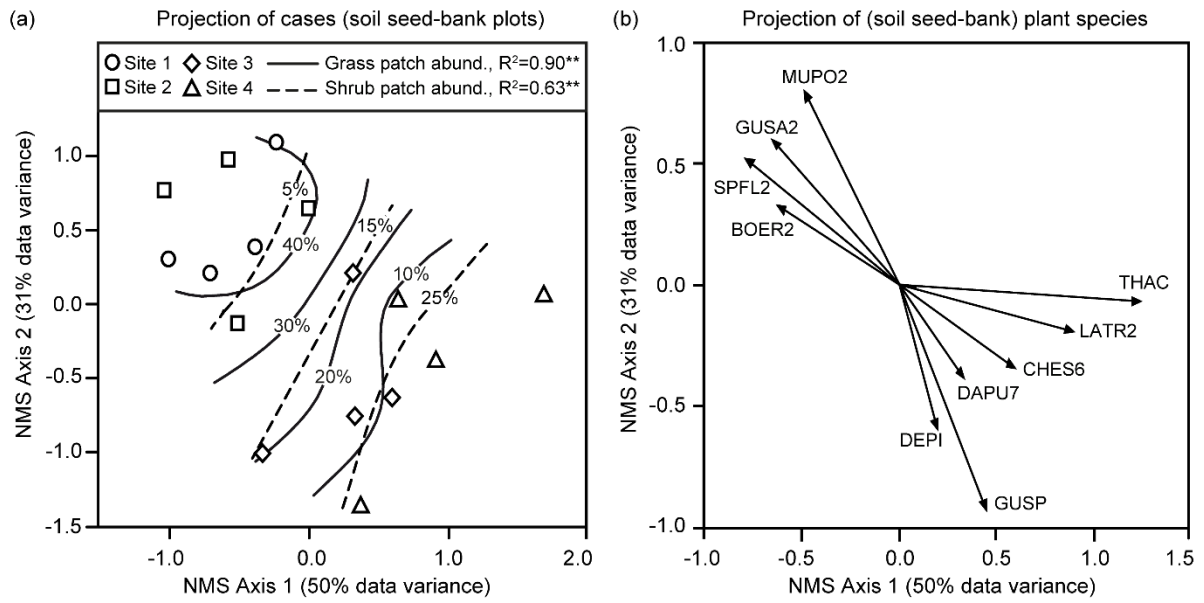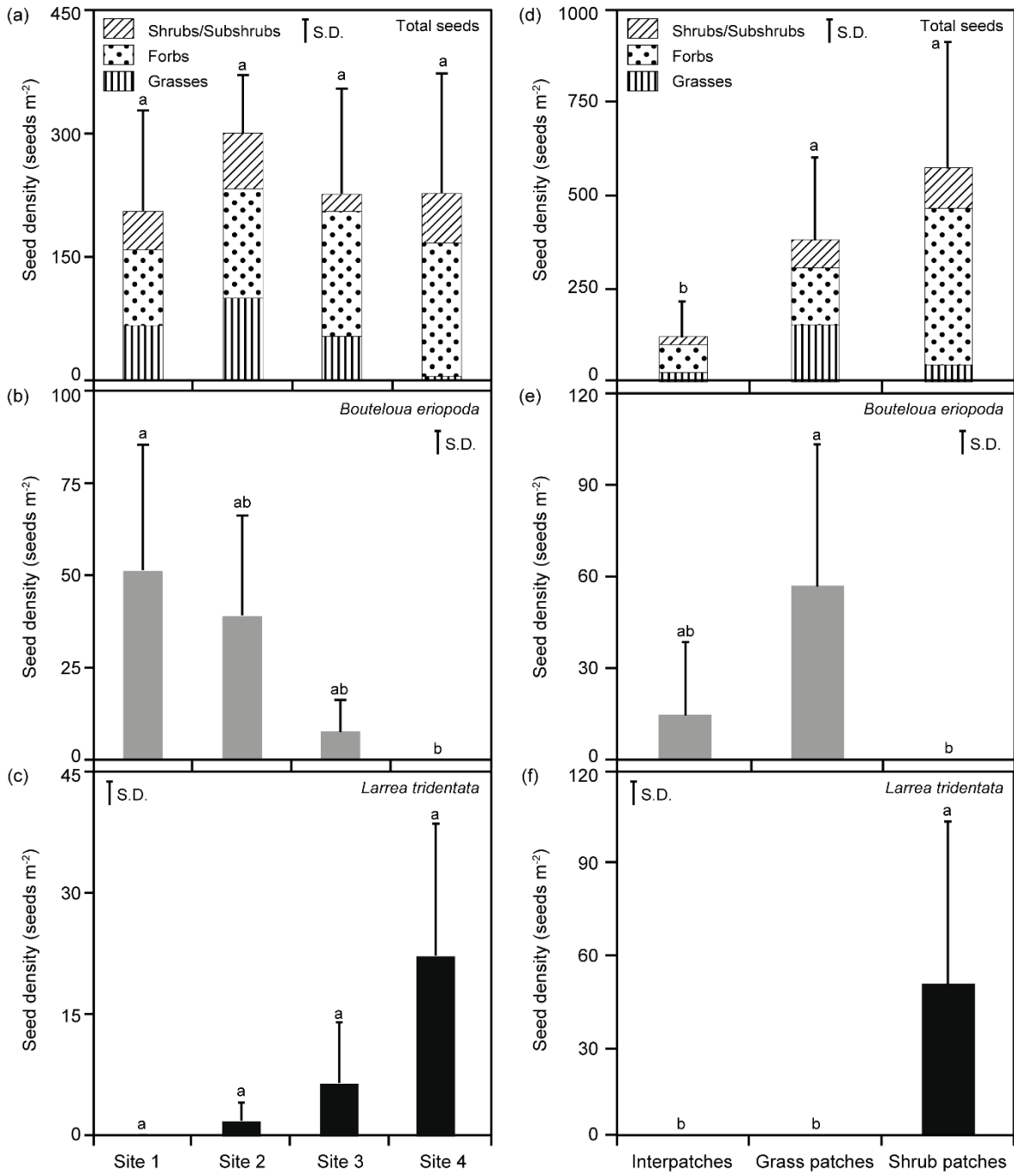
Site 3 (shrub transition)   Site 4 (shrubland)

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.