1 **Effect of grazing and drought on seed bank in semi-arid patchy rangelands of**

2 **northern Patagonia, Argentina**

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Abstract

The soil seed bank (SSB) plays a critical role in vegetation regeneration after stress and/or disturbance events. Here we analyzed the SSB of shrub patches and interpatches in a rangeland from northern Patagonia under different grazing intensity, at the end of a multi-year drought and two years after the end of the drought. Soil samples were collected immediately before seed dispersion of the dominant species in order to study the persistent seed bank. Seeds were separated from the mineral soil by elutriation, and grouped into four life-forms: annual grasses, perennial grasses, forbs and shrubs. In all sampling conditions the SSB was markedly dominated by forbs. With a few exceptions, total seed density and seed density of every life-form were higher in shrub patches than in interpatches, although not influenced by sampling time and grazing intensity in both type of microsites. The results suggest that in patchy rangelands of northern Patagonia the shrub patches represent microsites of SSB enhanced preservation during multi-year droughts, particularly under high grazing intensity, and that recovery of the SSB after drought is a slow process both in the shrub patches and interpatches.

Introduction

The soil seed bank (SSB) plays a crucial role in plant communities' regeneration after stress and/or disturbance events (Harper, 1977), therefore having the potential of driving vegetation change (Fenner, 2000). The SSB is commonly characterized by seed density and species composition, richness, and diversity (Fenner and Thompson, 2005). Based on seed longevity, the SSB can be classified as transient, short- and long-term persistent (Thompson et al., 1997). Seeds of transient seed banks persist in the soil for no more than a year, whereas seeds of short- and long-term persistent seed banks can survive in the soil for one to five years or for more than five years, respectively. Recruitment from persistent SSB plays an essential role in restoration after stress and/or disturbance events (López-Mariño et al., 2000; Thompson and Grime, 1979).

Grazing by large herbivores can induce changes in SSB density, composition, richness and diversity (Bertiller, 1992; Milberg, 1995; Peco et al., 1998). Livestock grazing can impact the SSB directly through depressing seed production of preferred species (O'Connor and Pickett, 1992; Pehrsson, 1988) or indirectly by inducing modifications in species composition (Loucougaray et al., 2004; Moreno García et al., 2014; Pol et al., 2014). The intensity and direction of grazing-induced modifications in the SSB may vary according to the evolutionary history of grazing, herbivory level and resources availability of the site (Milchunas et al., 1988). For instance, in unproductive drylands with short evolutionary history of herbivory the model predicts changes in composition and reduction in density, richness and diversity of the SSB at increasing grazing intensities. Modifications in botanical composition (Kinukan and Smeins, 1992; O'Connor and Pickett, 1992) and reductions in richness and diversity (Sternberg et al.,

63   2003) of the SSB have been documented in heavy grazed unproductive rangelands. The

64   impact of grazing on the SSB is normally exacerbated during drought conditions.

65   Drought can differentially affect seed production of the species in a community, leading

66   to modifications in composition, density, richness and diversity of the SSB (del Cacho

67   and Lloret, 2012; Fenner and Thompson, 2005; Pakeman, 2011). For example, in

68   rangelands seed production of perennial grasses is highly sensitive to drought stress

69   (Schwinning and Sala, 2004). Consequently, after a prolonged drought period seeds of

70   perennial grasses are commonly poorly represented in the SSB compared to annual

71   grasses and forbs (Gutiérrez et al., 2000; O'Connor, 1991).

72   In grazed steppes of northern Patagonia, Argentina, vegetation is organized in shrub-

73   dominated patches alternating with sparsely vegetated interpatch areas (Bisigato and

74   Bertiller, 1997; Funk et al., 2017; Kröpfl et al., 2013). Shrub patches act as a trap for

75   seeds transported by wind and water (Bertiller, 1998; Pazos and Bertiller, 2008).

76   Moreover, shrub patches provides a better environment for understory plant growth

77   compared with interpatch areas (Pazos and Bertiller, 2008). The canopy of woody plants

78   mitigates abiotic stress and can give protection from grazing (Soliveres et al., 2012).

79   Both seed entrapment and improved growth environment determine a marked seed

80   concentration in shrub patches (Bertiller, 1998). However, there is still scarcity of

81   information on the polled effect of multi-year drought and sustained heavy grazing on

82   the SSB.

83   We hypothesize that in the shrub steppes of northern Patagonia (1) shrub patches

84   represent sites of SSB preservation during multi-year droughts, particularly in

85   combination with sustained heavy grazing; and (2) SSB recovery after multi-year

86   droughts is faster in the shrubs patches than in interpatches, especially under high

87   grazing intensity. To test these hypotheses we analyzed the SSB in shrub patches and

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88   interpatches under different grazing intensities at the end of a multi-year drought and

89   two years after the end of the drought event.

90

91   **Methods**

92

93   *Study area*

94   The study was conducted in north-eastern Patagonia, Argentina (40º 41'- 40° 46' S, 64º

95   06'- 64° 30' W). Overall characteristics of climate, soil and vegetation has been

96   described by Godagnone and Bran (2009). Dominant soils are classified as Haplargids

97   and Haplocalcids. The climate of the regions is temperate and semi-arid. Mean monthly

98   air temperatures range from 7 ºC in July to 24 ºC in January, with an annual mean of 14

99   ºC. Mean annual rainfall is 270 mm (C.V. 40%), with peaks in autumn and spring, and

100   the mean annual potential evapotranspiration is 800 mm. In the study period 2007-2011

101   the annual precipitation summed 139, 175, 117, 225 and 253 mm, respectively. The first

102   three years encompassed multi-year drought conditions in the study region.

103   The study area belongs to the Monte Phytogeographical Province (Cabrera, 1971). The

104   vegetation physiognomy resembles a shrub-grass steppe. Dominant shrubs are the

105   evergreen *Chuquiraga erinacea* and *Condalia microphylla*. Perennial grasses are

106   mainly represented by *Nasella tenuis* (Syn. *Stipa tenuis*), and to a lesser extent by *Poa*

107   *ligularis*, *Piptochaetium napostaense*, *Jarava plumosa* (Syn. *Stipa papposa*),

108   *Pappostipa speciosa* (Syn. *Stipa speciosa*) and *Nasella longiglumis* (Syn. *Stipa*

109   *clarazii*). The most abundant annual grasses are *Bromus secalinus* (Syn. *Bromus mollis*)

110   and *Schismus barbatus*, and the most abundant forb is *Erodium cicutarium*. All

111   dominant herbaceous plants are cool-season species. There is also a cryptogamic layer

112   composed of cyanobacteria, bacteria, algae, bryophytes, and lichens. Scientific names

113   are according to the Flora Argentina (Zuloaga and Morrone, 2007).

114   Presently, after almost 100 years of livestock grazing, vegetation is spatially distributed

115   in shrub patches alternating with interpatches of either bare soil or low herbaceous

116   cover (Fig. 1). For the purpose of this study, we defined a shrub patch as a discrete unit

117   of the spatial pattern of vegetation with an upper shrub layer and a lower layer

118   composed of perennial grasses, annual species and/or cryptogams, associated with soil

119   mounding. Interpatches are characterized by scarce vegetation and soil degradation.

120   The study area remained unexploited by domestic livestock until the beginning of last

121   century. At that time the physiognomy of the vegetation resembled grassland with

122   scattered shrubs, the main large herbivore was the guanaco (*Lama guanicoe*), and

123   summer fires occurred frequently (d'Orbigny and Cepeda, 1945; Villarino and de

124   Angelis, 1972). Since then, the vegetation has been heavily grazed by sheep and cattle,

125   under commercial ranching operations.

126

127   *Sampling design*

128   We analyzed the SSB in grazed and ungrazed areas in September 2009 (last year of the

129   multi-year drought period) and September 2011 (second year with near long-term

130   average precipitation after the multi-year drought). Soil samples were taken

131   immediately before seed dispersion of the dominant species in order to study the

132   persistent seed bank (Thompson and Grime, 1979). They were taken in three sampling

133   sites, each in a different commercial ranch. Sites were separated from one another by no

134   more than 30 km. They had in common a similar grazing history (ca. 0.36 dry sheep

135   equivalent per hectare, year round grazing), soil type and plant community type. Each

136   sampling site comprised a square paddock of 625 ha, with a water source located at one

137 corner. Within each paddock we sampled at two distances from the water source: 600 m

138 and 2,500 m, within an area of ca. 1 ha. We assume theses distances to represent

139 different grazing intensities, based on the creation of piosphere effects which determine

140 a decreasing intensity of grazing radiating out from the water source (Jeltsch et al.,

141 1997). We also sampled grazing exclosures adjacent to each paddock, which were 1 ha

142 in size and had not been grazed for 10 or more years. In such a way we arbitrarily

143 defined three grazing intensities: heavy grazing at 600 m from the water source (HG),

144 moderate grazing at 2,500 m from the water source (MG), and ungrazed at exclosures

145 (UG).

146 At all sampling sites and level of grazing intensity we collected soil samples for seed

147 bank analysis in two different microsites: shrub patches and interpatches. In each

148 sampling date we took 90 samples, 10 samples per grazing intensity and per site. Of

149 these 10 samples, five corresponded to shrub patches and five to in interpatches. Each

150 soil sample consisted of a soil core (10 cm in diameter and 5 cm deep) and the litter

151 above it. Samples were stored in sealed bags and taken to the lab, where they were air-

152 dried for 20 days before processing. Dried samples were elutriated with tap water as

153 many times as necessary for them to pass through a set of graduated stacked sieves (15

154 cm diameter) with mesh sizes of 1 mm, 0.71 mm, 0.5 mm and 0.25 mm. After

155 elutriation, samples were allowed to air-dry for 72 h. All material retained in each of

156 these sieves was observed under a dissecting microscope in order to extract and count

157 all viable seeds, which were determined to the genus or species level (for details see

158 Loydi et al., 2012). Seeds were considered to be viable when they resisted light hand

159 pressure with a tweezer (Busso and Bonvissuto, 2009). Seeds were grouped into the

160 dominant plant life-form in the area: annual grasses, perennial grasses, forbs and shrubs.

161 Only viable seeds were considered for analysis.

162

164 Data were analyzed according a randomized complete block split-plot ANOVA design,

165 where the year (2009 vs. 2011) was considered the main factor and grazing intensity as

166 the split factor. Analyses were performed for each microsite (shrub patches vs.

167 interpatches) separately since this factor probed to have a significant interaction

168 ($p<0.05$) with grazing intensity and year. Analyses were done for total seed density and

169 for seed density of each life-form. Prior to analyses, data were transformed to their

170 $\sqrt{(x+1)}$ to meet ANOVA assumptions. In the Results section we presented data before

171 transformation. Tukey HSD was used as a posthoc test ($p=0.05$) for mean comparisons.

172 Seed density between shrub patches and interpatches was compared using a paired

173 Wilcoxon test, since data were not normally distributed and homoscedasticity was not

174 possible to achieve. We also calculated species richness under the different

175 experimental conditions, and made comparisons using corrected confidence intervals

176 obtained by *bootstrap* method (500 iterations) (Pla and Matteucci, 2011). Non

177 overlapping confidence intervals were considered as significant differences between

178 means. All analyses were made using software InfoStat (Di Rienzo et al., 2009).

179

180 **Results**

181 For total seed density and for seed density of each plant life-form (annual grasses,

182 perennial grasses, forbs, shrubs), the interaction term between grazing intensity (heavy-

183 HG, moderate-MG, ungrazed-UG) and year (2009 –drought conditions, and 2011 –near

184 normal precipitation conditions) was not significant ($p> 0.05$), at the level of both shrub

185 patches and interpatches (Fig. 2).

186   Total seed density increased (p<0.05) or tended to increase (p=0.08) after the drought in

187   the interpatches and the shrub patches, respectively. Seed density of forbs in 2011

188   almost doubled that in 2009 in both microsites, although the difference was significant

189   for interpatches only. For the rest of the plant life-form seed density was similar

190   between sampled years in both interpatches and shrub patches.

191   In the shrub patches, total seed density was significantly highest at HG, and similar

192   between MG and UG. This response was mainly due to enhanced (p<0.05) seed density

193   of forbs at increasing grazing intensity, since for the other plant life-forms no significant

194   differences was observed among grazing intensities. On the other hand, total seed

195   density and seed density of every plant life-form were similar at all grazing intensities

196   in the interpacthes.

197   Total seed density was higher ($p<0.01$) in the shrub patches than in the interpatches at

198   all grazing intensities during both sampled years. At the end of the multi-year drought

199   (2009), total seed density in the shrub patches was three, five or nine times higher than

200   in the interpatches for the UG, MG and HG, respectively. Two years after the multi-year

201   drought (2011), total seed density was three times (UG and MG) or eleven times (HG)

202   higher in the shrubs patches than in the interpatches. In 2009 seed density of all plant

203   life-form was higher (p<0.05) in the shrub patches than in the interpatches at all levels

204   of the grazing intensity, except for perennial grasses in the UG condition. In 2011 the

205   same response was observed in the HG condition, while there were no differences

206   between microsites for either annual or perennial grasses in MG or forbs in UG.

207   In both shrub patches and interpatches the SSB was markedly dominated by forbs,

208   equally in 2009 and 2011 (Fig. 3). In the shrub patches species richness increased or

209   decreased at increasing grazing intensity in 2009 and 2011, respectively (Fig. 4). On the

210 other hand, in the interpatches species richness was similar among the grazing

211 intensities in 2009, whereas it was higher in UG and MG compared to HG in 2011.

212

213 **Discussion**

214 Our results supported the hypothesis of the shrub patches as the major reservoir of seeds

215 in the soil bank during a multi-year drought, particularly under heavy sustained grazing.

216 On the other hand, we did not find support for the hypothesis of a faster recovery of the

217 SSB in the shrub patches two years after a multi-year drought event at all grazing

218 intensities.

219 The enhanced seed density in the soil bank of the shrub patches, accentuated under

220 heavy sustained grazing, can be attributed to reduce physical stress (Bertness and

221 Callaway, 1994)and protection against herbivory (Callaway et al., 2005; Milchunas and

222 Noy-Meir, 2002) on the understory vegetation. The combined effects of mitigating

223 stress and disturbance forces benefit understory plant growth and seed production,

224 potentially increasing SSB density. Moreover, wind- and water- transported seeds

225 accumulate in the shrub patches (Aerts et al., 2006; Aguiar and Sala, 1997; Bertiller,

226 1998; Kinloch and Friedel, 2005). According the stress-gradient hypothesis (Bertness

227 and Callaway, 1994), the beneficial effects of shrubs on the understory herbaceous

228 vegetation is expected to be magnified in multi-year drought events, since as stress

229 increases the improved environmental condition in the shrub patches depart further from

230 those in the interpatches (although see below).

231 Contrary to expectation, we did not observe a faster recovery of the SSB from the

232 different plant life-form in the shrub patches compared to in the interpatches two years

233 after the multi-year drought event. Moreover, the improvement in the SSB of forbs was

234 higher in the interpatches than in the shrub patches. A possible explanation is that,

10

235 beyond a certain level of water stress, negative interactions offset positive interactions

236 between shrubs and the understory herbaceous vegetation (Pugnaire et al., 2011).

237 Dominant shrubs in the study system have not only deep roots but also shallow roots

238 (Rodríguez et al., 2007), which may allow them to compete strongly for water in the

239 superficial soil layers under severe drought conditions. For instance, survival of

240 perennial grasses was similar or higher in the interpatches than in the shrub patches in

241 the study area at the end of the muti-year drought (Funk et al., 2017). Structural

242 attributes of vegetation such as seeds can be strongly reduced after multi-year droughts

243 and may constrain the ability of ecosystems to respond to a subsequent increase in

244 precipitation (Oesterheld et al., 2001). This argument is consistent with the fact that

245 SSB density responded in the same way to the different grazing intensity at the end of

246 the multi-year drought (2009) and after two years with precipitations close to the long-

247 term average precipitation (2011). Also, better environmental conditions in the shrub

248 patches than in the interpatches after the drought event may have resulted in a higher

249 transference to the seedling bank in the former than in the latter microsite (Marone et

250 al., 2000).

251 Forbs seeds were the most abundant in the SSB at all levels of the studied factors (i.e.

252 grazing intensity, year and microsite), which has also been observed in rangelands

253 communities in different parts of the world (e.g. Bertiller, 1992; Gutiérrez and Meserve,

254 2003; Solomon et al., 2006). Frequently forbs have small isodiametric seeds with hard

255 cover that facilitate seed penetration into and persistence in the soil (Burmeier et al.,

256 2010; Fenner and Thompson, 2005; Sternberg et al., 2003), which may explain their

257 widespread dominance. We also observed that grazing can markedly increase the

258 abundance of forbs in the SSB, which agrees with results from previous studies (e.g.

259 Kinukan and Smeins, 1992; Loydi et al., 2012; O'Connor and Pickett, 1992; Russi et al.,

260   1992). On the other hand, shrub seeds were the second more abundant in the SSB. Even

261   though shrub seeds were much more plentiful in the shrub patches than in the

262   interpatches, the latter microsites would gather the best environmental conditions for

263   shrub seedling establishment under grazed conditions (Bisigato and Bertiller, 1999).

264   This would boost shrub encroachment, a common phenomena over much of the arid and

265   semiarid regions of the world (Eldridge et al., 2011).

266   Perennial grasses were comparatively less represented in the SSB than the previous life-

267   forms (forbs and shrubs). Normally, the persistence of perennial grasses depend to a

268   great extent on vegetative reproduction and a relatively long lifespan of established

269   individuals, which make them less dependent on the SSB compared to annual species

270   (Caballero et al., 2008; Milberg, 1995). Also, seeds of perennial grasses have relatively

271   limited longevity, leading to the development of short-term persistent seed banks (<5

272   years, Bertiller and Aloia, 1997; Peco et al., 1998; Sternberg et al., 2003). Moreover,

273   seeds of perennial grasses can suffer high depredation by insect (particularly ants),

274   rodents and birds (Mayor et al., 2003; Pazos and Bertiller, 2008). Nonetheless, perennial

275   grasses were still fairly represented in the SSB -to a greater extent in the shrub patches

276   than in the interpatches under heavy and moderate grazing conditions- at the end of the

277   drought event. Since multi-year droughts can induce the death of established individuals

278   of perennial grasses in the study region (Funk et al., 2017), the SSB is important for the

279   post-drought recovery of their populations. On the other hand, overall seed density of

280   annual grasses was rather similar to the seed density of perennial grasses, departing

281   from the expected superiority of the former over the latter plant life-form (Grime,

282   2001). It is possible that the methodology employed here for quantifying SSB have led

283   to an underestimation of annual grasses since its main contributing species, *Schismus*

284   *barbatus*, has very small seeds (seed mass ca. 0.075 mg, Seed information database,

285   Kew botanical garden). Gross (1990) argued that smaller seeds may be underestimated

286   when using the elutriaton method to recover seeds from soil samples.

287   Overall, species richness was increased by precipitation and depressed by heavy

288   sustained grazing. A more stressful competitive environment in the shrub patches of the

289   exclosures (ungrazed condition) may explain the increased species richness in this type

290   of microsites under moderate and heavy grazing at the end of the drought event.

291   Variations in species richness were mainly due to changes in forbs and shrubs species,

292   which is consistent with finding in other arid and semi-arid ecosystems (Kinukan and

293   Smeins, 1992; Pugnaire and Lázaro, 2000; Tessema et al., 2011).

294   In conclusion, in shrubby steppes of northern Patagonia the shrub patches represented

295   microsites of SSB enhanced preservation for the different plant life-forms at the end of a

296   multi-year drought, particularly at high grazing intensity. Although at relatively low

297   density, perennial grasses were still fairly represented in the SSB at the end of the multi-

298   year drought. With only one exception (forbs in the interpatches), two year after the end

299   of the drought there was not a significant recovery of the SSB for all plant life-forms.

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306

307   **Appendix A. Supplementary data**

308   Supplementary data related to this article can be found at http://

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458

459 **Figure Captions**

460

461 **Fig. 1.** View of the vegetation in a heavy grazed shrubland of the study area at the end

462 of a multi-year drought.

463

464 **Fig. 2.** Mean seed density (n= 3) in shrub patches and interpacthes measured at the end

465 of the multi-year drought (2009) and at the second year with near long-term average

466 precipitation after the multi-year drought (2011), in areas with different grazing

467 intensity: ungrazed (white bars), moderate grazed (grey bars), and heavy grazed (black

468 bars). T: total seed density, AG: annual grasses, PG: perennial grasses, F: forbs, S:

469 shrubs. Bars represent the SE of the means.

470

471 **Fig. 3.** Proportional contribution of each plant life-form to the soil seed bank in the

472 shrub patches (a 2009, c 2011) and in the interpatches (b 2009, d 2011). PG: perennial

473 grasses, AG: annual grasses, F: forbs, S: shrubs.

474

475 **Fig. 4.** Mean species richness (n= 3) in shrub patches (SP) and interpatches (IP)

476 measured at the end of the multi-year drought (2009) and at the second year with near

477 long-term average precipitation after the multi-year drought (2011), in areas with

478 different grazing intensity: ungrazed (white bars), moderate grazed (grey bars) and

479 heavy grazed (black bars) grazing intensity. Bars represent ± 1 confidence interval.

480 Overlapping bars indicate non significant differences between means.