1   **Legume germination is delayed in dry soils and in sterile soils devoid of microbial**

2   **mutualists: implications for upward range expansions**

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Abstract

21 Climate change is affecting species and their mutualists and can lead to interaction weakening

22 and loss. Through independent shifts in partner phenology and distribution, climatic stress can

23 separate mutualists, leading to alterations in partner functional traits and fitness. Here, we

24 explored the effects of drying soils and the loss of microbial mutualists via soil sterilization on

25 legume germination success and phenology, focusing on how a loss of mutualisms with soil

26 microbial species can alter legume early life traits. In particular, we assessed the effects of

27 mutualism loss via soil sterilization, increased drought, or introduction to novel soils found

28 beyond the current distributions of two focal legume species in subalpine environments. Through

29 common garden experiments in controlled environments, we found evidence that soil

30 sterilization (and consequent microbial absence) and dry soils caused phenological delays of 2-5

31 weeks in germination date, likely as a result of interaction loss between legumes and

32 germination-promoting soil microbes, such as mutualistic rhizobia. Delays in germination caused

33 by a mismatch between legumes and beneficial microbes could negatively affect legume fitness

34 through increased plant-plant competition later in the season. Additionally, we found evidence of

35 the presence of beneficial microbes beyond the current elevational range of our focal legumes

36 which may allow for expansion of the leading edge, though harsh abiotic factors in the alpine

37 may hinder this. Alterations in the strength of soil microbe-legume mutualisms may lead to

38 reduced fitness and altered demography for both soil microbes and legumes.

39 Keywords: *climate change; distribution; germination; legume; microbes; mutualism.*

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Introduction

Mutualisms are essential species interactions which profoundly influence the structure, productivity, and stability of communities (Wardle *et al.*, 2004; Leff *et al.*, 2018). Mutualistic interactions provide ecosystem services such as nutrient cycling (Wall and Moore, 1999) and increase partner stress tolerance (Lau and Lennon, 2012; de Zelicourt, Al-Yousif and Hirt, 2013; David *et al.*, 2020). Mutualisms between soil microbes and plants are especially ecologically important for plant growth and fitness, as well as community composition and dynamics (Klironomos, 2002; Reynolds *et al.*, 2003; Van Der Heijden and Bardgett, 2008; Mangan *et al.*, 2010). These mutualisms may become increasingly important as the environment becomes more stressful as a result of anthropogenically-induced global climate change (Allan and Soden, 2008; Dai, 2012; Lau and Lennon, 2012; Gehring *et al.*, 2017; Porter *et al.*, 2020). Environmental context can determine the level of investment made by mutualists, such that the net benefits of mutualisms are often greater in more stressful environments (Pringle *et al.*, 2013; Remke *et al.*, 2021). However, some soil microbes enter a state of dormancy in stressful environments, meaning they do not interact with the plant host under increased stress, which can affect host plant distributions and functional traits (Stanton-Geddes and Anderson, 2011; Simonsen *et al.*, 2017; Werner *et al.*, 2018). Indeed, declines in active partner abundances can destabilize mutualisms (Tylianakis *et al.*, 2008; Kiers *et al.*, 2010), altering plant functional traits and overall fitness (Worchel, Giauque and Kivlin, 2013; Fitzpatrick, Mustafa and Viliunas, 2019).

Mutualistic interactions may become decoupled if one partner is dormant or non-receptive for part of the year while the other is not (Rafferty, CaraDonna and Bronstein, 2015). Bacteria in particular, including the naturally and agriculturally important nitrogen (N)-fixing rhizobial bacteria (Harris, Pacovsky and Paul, 1985) and plant growth-promoting rhizobacteria

65 (PGPR), are susceptible to desiccation and death in dry conditions (Schimel, Balser and

66 Wallenstein, 2007; de Vries and Shade, 2013; Ngumbi and Kloepper, 2016; de Vries *et al.*, 2018;

67 Xu and Coleman-Derr, 2019). To avoid losing water to their environment, many bacterial cells

68 can enter a state of dormancy (Lennon and Jones, 2011; Schimel, 2018). In N-fixing rhizobia,

69 dry soils have been shown to induce dormancy in free-living cells and inhibit N-fixation in

70 symbiotic bacteroid cells, leading to denodulation (Zahran, 1999; Hungria and Vargas, 2000;

71 Vriezen, de Bruijn and Nüsslein, 2006; Aldasoro, Larrainzar and Arrese-Igor, 2019) and short-

72 term mutualism loss. Similarly, active, free-living bacterial cells in dry soils may have difficulty

73 forming interactions with plant roots because low soil moisture negatively affects the signaling

74 abilities of soil bacteria and plants (Schimel, 2018; Williams and de Vries, 2020). As a result, the

75 mutualism between legumes and soil microbial species, such as rhizobia and PGPR, can weaken

76 in the short term due to climate change-induced soil drying.

77   When active, soil microbial mutualists often ameliorate environmental stress and

78 overcome limitations for their plant hosts, allowing plants to persist in conditions otherwise

79 intolerable (Defossez *et al.*, 2011; Redman *et al.*, 2011; Lau and Lennon, 2012; Rodríguez-

80 Echeverría, Lozano and Bardgett, 2016; Petipas *et al.*, 2017; David, Thapa-Magar and Afkhami,

81 2018; Bennett and Meek, 2020; David *et al.*, 2020). For this reason, soil microbe-plant

82 mutualistic partners are often able to inhabit a broad range of habitats (Stachowicz, 2001; Bruno,

83 Stachowicz and Bertness, 2003; Rodriguez-Cabal, Noelia Barrios-Garcia and Nunez, 2012;

84 Afkhami, McIntyre and Strauss, 2014; Harrison *et al.*, 2018). For example, by increasing plant

85 access to N, phosphorus (P), and water, N-fixing bacteria and arbuscular mycorrhizal fungi

86 (AMF) allow plants to expand their ranges into otherwise unsuitable, nutrient-poor habitats

87 (Halvorson, Smith and Franz, 1991; Hayward *et al.*, 2015; Harrison *et al.*, 2018). Additionally,

88   agricultural studies have demonstrated that some soil microbial species are able to promote host

89   seed germination by excreting phytohormones, thereby increasing germination success in newly

90   colonized habitats (Atzorn *et al.*, 1988; Noel *et al.*, 1996; Bastian *et al.*, 1998; Tsavkelova *et al.*,

91   2007; Miransari and Smith, 2009; Kumar, Dubey and Maheshwari, 2011; Namvar and Sharifi,

92   2011; Meena *et al.*, 2012; Ngumbi and Kloepper, 2016; Wu *et al.*, 2016). Conversely, the

93   absence of mutualists can negatively affect population persistence and limit species distributions

94   (Pellmyr, 2003; Nuñez, Horton and Simberloff, 2009; Mueller *et al.*, 2011; Harrower and

95   Gilbert, 2018; Benning and Moeller, 2021b). Indeed, this has been documented in some soil

96   microbe-plant mutualisms (Stanton-Geddes and Anderson, 2011; Simonsen *et al.*, 2017). Thus,

97   the ability of a plant to successfully establish in a new habitat depends on not only dispersal and

98   the physical conditions in the novel range but also biotic factors, including the presence of

99   mutualists (HilleRisLambers *et al.*, 2012; Brown and Vellend, 2014).

100   The role of soil microbe-plant mutualisms in shaping the geographic distributions of plant

101   species is little-understood (Classen *et al.*, 2015; Bueno de Mesquita *et al.*, 2016; Benning and

102   Moeller, 2021a). As plants, including legumes, continue to expand their leading range edges up

103   altitudinally and poleward latitudinally in response to climate change (Chen *et al.*, 2011;

104   Harrison *et al.*, 2018), they may encounter harsh environments, made potentially more stressful

105   by the absence of mutualistic partners. Legumes often require exposure to soils that have been

106   pre-inoculated with compatible soil microbes to establish and persist in dry N- and P-poor soils

107   such as those found in the alpine and subalpine (Parker, 2001; Simonsen *et al.*, 2017; Bueno de

108   Mesquita *et al.*, 2020). The absence or reduced abundance of microbial mutualists beyond the

109   current range of a population could impair plant fitness and hinder leading range expansion

110   (Miransari, 2010; Peay, Garbelotto and Bruns, 2010; Lankau and Keymer, 2016; Wu and Ying-

111   Ning, 2017; Hu *et al.*, 2022). Non-co-dispersed, horizontally transmitted symbionts, including

112   legumes, rhizobia, and some PGPR, may be at high risk of becoming spatially mismatched as

113   they may track climate differently (Keeler, Rose-Person and Rafferty, 2021). Legumes that

114   interact with specialized mutualists may be less likely to find a compatible partner in novel

115   habitats and thus may fail to establish (Simonsen *et al.*, 2017), though legumes expanding into

116   novel ranges without a historical partner may have relaxed partner choice mechanisms (Harrison

117   *et al.*, 2017; Younginger and Friesen, 2019). Compared to the historical interactions, new

118   interactions in a novel habitat may not confer equivalent benefits to the host plant (Bueno de

119   Mesquita *et al.*, 2018; Werner *et al.*, 2018).

120   A spatial or dormancy-induced loss of a mutualism, even for part of a season, could lead

121   to reduced germination stimulation by soil microbes which could decrease plant germination

122   success (David *et al.*, 2020; Eldridge *et al.*, 2021), delay host plant germination phenology, and

123   alter downstream phenophases, such as flowering onset (Namvar and Sharifi, 2011), which will

124   alter phenological overlap and interaction strengths among host plants and pollinators (Rafferty

125   and Ives, 2012; Rafferty, Bertelsen and Bronstein, 2016). A shift in flowering phenology without

126   a corresponding shift in pollinator phenology could decrease the fitness of both mutualists

127   (Rafferty and Ives, 2011; Schenk, Krauss and Holzschuh, 2018; Kudo and Cooper, 2019).

128   Germination phenology is especially important, as germinants are particularly vulnerable to

129   stress relative to other stages of the plant life cycle. Seedlings that emerge in stressful

130   environments, including in environments lacking certain facultative mutualists, have lower

131   survival (Donohue *et al.*, 2010). Germination timing also shapes competitive outcomes and

132   reproductive success (Fowler, 1984; Leverett, 2017). Studies evaluating the interactions between

133   seeds and soil microbes during the germination process in natural systems are limited, though the

134   importance of these interactions could become greater in a changing climate; seed germination

135   stimulation by microbes may strengthen (David *et al.*, 2020), or weaken via environmental

136   stress-induced microbial dormancy (Schimel, 2018).

137   Here, we explore how early life traits of two legumes are affected by the absence of their

138   mutualisms with soil microbial species. Environmental stress, namely drought, and the possible

139   absence of suitable soil microbes in the expected future ranges of our focal legumes may affect

140   legume germination success and timing. Because germination success can be stimulated by

141   microbes, we hypothesize that legumes in sterilized soils devoid of microbes will have lower

142   germination success and delayed germination phenology. Conversely, if partners co-occur, we

143   predict that interaction strength will increase with increasing stress (David *et al.*, 2020), leading

144   germination success and germination phenology to be similar in potentially high-stress (novel

145   range or drought) and ambient conditions. To investigate the potential consequences of short-

146   term loss of the interactions among legumes and soil microbes, we ask if the success and timing

147   of germination are affected by i) soil sterilization, ii) foreign soils collected from elevations

148   higher than the current distributions of these legumes, or iii) limited soil moisture.

149   **Methods**

150   *Study system*

151   This study was conducted using seeds and soils collected from The Rocky Mountain Biological

152   Laboratory (RMBL; N 38° 52.2928', W 106° 58.671') located in the Maroon Bells-Snowmass

153   Wilderness area near Gothic, Colorado, USA. The RMBL area is characterized by vast, open

154   subalpine meadows dominated by perennial wildflowers and patches of aspen-fir forests.

155   Subalpine plant communities, like those at RMBL, are especially sensitive to changes in climate

156 due to short growing seasons and upward range limitations (Parmesan, 2006; Hülber, Winkler

157 and Grabherr, 2010). These subalpine plant communities therefore offer an excellent system to

158 answer questions about mutualisms and anthropogenic climate change. In the last several

159 decades at RMBL, snowpack has decreased, the date of spring snowmelt has shifted earlier (3.5

160 days earlier per decade from 1974-2012), and year-to-year variation in snowmelt date increased

161 by 20% from 1974-2008 (Lambert, Miller-Rushing and Inouye, 2010; CaraDonna, Iler and

162 Inouye, 2014). Additionally, June precipitation has decreased significantly since the 1980s and

163 July monsoon rains are delayed or non-existent (data accessible at https://www.gothicwx.org/).

164 Temperatures at RMBL are expected to continue to increase over the next century and total

165 precipitation is expected to decrease (Overpeck and Udall, 2010). Decreased snowpack, earlier

166 snowmelt, and decreased June precipitation are predicted to result in earlier, longer dry seasons

167 prior to July monsoon rains (Clow, 2010; Kittel *et al.*, 2015), which is likely to affect the species

168 and their interactions in this system.

169 *Selection of plant species*

170 We studied the two native, nectar-producing legume plant species present in the Maroon Bells-

171 Snowmass Wilderness area, *Lathyrus lanszwertii* var. *leucanthus* and *Vicia americana*

172 (Fabaceae). Both are perennial vines that are common in the RMBL area and produce nutrient-

173 rich rewards that attract native pollinators (unpublished data, A. M. Keeler). We verified that

174 both of these species form root nodules and host N-fixing bacteria in the field and in controlled

175 common garden settings (Image 1), and that they host arbuscular mycorrhizal fungi (AMF) and

176 dark septate endophytes (DSE) in the field. These species are known to host AMF across their

177 range and facultative DSE at higher elevations near RMBL (unpublished data, RMBL).

178 However, after staining and microscopy (Schmidt *et al.*, 2008), neither fungal group was found

179   on the roots of these legumes in our controlled common garden setting, likely because fungal

180   spores can quickly decay in cool, wet soils if stored there for a month or more (Gottlieb, 1950;

181   Varga *et al.*, 2015), as our soils were. We note that, using the same staining methods, we have

182   verified the presence of AMF in roots of other species grown from seeds collected at some of the

183   same sites near RMBL, increasing our confidence that AMF were absent in our soils for this

184   study. Along with AMF, DSE, and rhizobia, it is likely that various phylotypes of Acidobacteria,

185   nitrifying taxa (e.g., *Nitrospira* spp. and *Thaumarchaeota* spp.), *Thelephora* (Agaricomycetes),

186   *Hebeloma* (Agaricomycetes), *Archaeorhizomyces* (Archaeorhizomycetes), and *Tetracladium*

187   (Leotiomycetes) were in our soils, as these taxa are common in the soils around RMBL after

188   snowmelt (Sorensen *et al.*, 2020).

189   *Vicia americana* is widely distributed across North America while *L. leucanthus* is

190   located solely in mountainous regions of western North America. The ranges of *L. leucanthus*

191   and *V. americana* in the RMBL region extend from ~2700 to 3500 m in elevation. Observational

192   data suggest that the elevational ranges of both species (and their bumblebee pollinators) have

193   expanded upward in the last 40 years, and leading edges are expected to continue to expand

194   (Pyke, 1982; Pyke, Inouye and Thomson, 2012; Pyke *et al.*, 2016). These observations come

195   from systematic surveys conducted in the 1970s (Pyke, 1982), wherein the presence/absence of

196   *L. leucanthus* and *V. americana* was noted along transects that span elevational gradients in the

197   RMBL area, and the systematic resurveying in 2015-2018 of some of those same transects and

198   others that span similar elevations (described herein). For example, whereas neither species was

199   recorded as present at the highest-elevation survey point (3394-3442 m) along the Washington

200   Gulch transect in the 1970s (Pyke, 1982), both species were found there in 2015-2018.

201   *Collection of soils and seeds*

202   We collected soils and seeds from multiple populations across gradients that span the

203   current and expected future elevational ranges of our focal plant species at RMBL in summer

204   2017 and summer 2018 to understand how the loss of microbial partners, elevational range

205   expansions, and drying soils may affect subalpine legume early life traits. Soils and seeds were

206   collected within 10 m of transects that traverse the Washington Gulch (403), Gothic Mountain,

207   and Baldy Mountain trails (3200-3500 m in elevation; Image 2). Within the current range of our

208   focal legume species, soils were collected from within a 10 cm radius of the nearest legume to a

209   depth of 15 cm, just past the rooting depth of *L. leucanthus* and *V. americana*, and where

210   beneficial soil microbial species are likely to be at higher densities in the soil (Komatsu and

211   Simms, 2020). To collect soils from elevations beyond the upper range limits of *L. leucanthus*

212   and *V. americana* populations (>3500 m, just beyond treeline), we sampled at least 3 sites per

213   transect after verifying that neither species occurred at those elevations, which ranged from

214   3500-3800 m. All soils were put on ice and transported back to the RMBL field station where

215   they were stored at 4° C. Soils were kept on ice for a day during transportation to UC Riverside,

216   then stored at 4° C. Soils from each elevational zone were homogenized each year to standardize

217   nutritional content and soil texture. Seeds were haphazardly collected from mature fruits (i.e.,

218   pods) within 10 m of these same transects; seeds were collected from plants if pods were

219   beginning to dehisce. One to six pods were collected per maternal plant and each pod contained

220   one to three seeds. Only unparasitized seeds were used in experiments. In total, 347 *L.*

221   *leucanthus* and 1059 *V. americana* seeds were collected and used for these experiments. Seeds

222   were cold stratified at 4° C for 45 days, the recommended time for these species (personal

223   communication, Mike Bone, Denver Botanic Gardens). All seeds were weighed to the nearest

224   0.1 mg before sowing. The average seed mass was 15.7 ± 7.4 mg (mean ± SD) for *L. leucanthus*

225   and 10.9 ± 8.7 mg for *V. americana*.

226   *Experimental design and setup*

227   We grew our focal legume species in sterile background soils inoculated with field-

228   collected soils (Collins, 2019; David *et al.*, 2020). To control for abiotic differences across soil

229   collection points, background soils were sterilized (double autoclaved within 12 hours at 121˚ C

230   for 90 minutes) and then were added to sterile pots (66 mL with drainage holes; Ray Leach

231   Cone-tainer, Stuewe & Sons, Tangent, Oregon, USA); background soils consisted of 57% sand,

232   43% peat moss, and various minerals. We then added field-collected soil inoculum to these

233   sterile background soils; 85% of the total soil volume was made up of sterile background soil,

234   while the other 15% of total soil volume was made up of field-collected soil inoculum. In

235   addition to twice sterilizing pots and background soils, half of all field-collected soils were twice

236   autoclaved prior to use; in sterile soils there can be no interactions between legumes and soil

237   microbes, mimicking a complete loss of the possible mutualisms. Wild-collected *L. leucanthus*

238   and *V. americana* seeds were surface sterilized for 20 minutes in a 10% bleach solution, briefly

239   soaked in four subsequent sterile water baths, then allowed to dry (Oyebanji *et al.*, 2009; Collins,

240   2019); seeds were sterilized to isolate the effects of the soil type and moisture treatments. Sterile,

241   dry seeds were weighed, sown directly into separate soil-filled pots at the same depth, then

242   covered with ~10 g of treatment soil. Each treatment soil was housed on a separate tray to reduce

243   movement of microbes from pot to pot via watering or air circulation (Image 3). Legumes were

244   placed in a growth chamber (Conviron MTR30) at a temperature and photoperiod regime

245   reflecting that of the early growing season (germination period of the focal species) at RMBL

246   (day: 21° C, 50% relative humidity; night: 4.4° C, 20% relative humidity; 12:12 h light:dark

247   cycle).

248   *Legume early life traits in sterilized soils and soils from beyond the current elevational range*

249   We grew individuals of both legume species in separate factorial experiments that

250   manipulated the presence of soil microbes (unsterilized vs. sterilized soil conditions) and

251   elevational origin of the soil (within the current ranges vs. beyond the current ranges of the focal

252   legumes) in soils collected from RMBL. Soil treatments comprised: unsterilized, current range

253   soils (unmanipulated, microbially active, current range of the focal legumes; *L. leucanthus*: n =

254   95 seeds; *V. americana*: n = 76 seeds); unsterilized, beyond the current range soils

255   (unmanipulated, microbially active, beyond the current range of the focal legumes; *L.*

256   *leucanthus*: n = 78 seeds; *V. americana*: n = 59 seeds); sterilized, current range soils (double

257   autoclaved, microbially sterile, current range of the focal legumes; *L. leucanthus*: n = 96 seeds;

258   *V. americana*: n = 71 seeds); and sterilized, beyond the current range soils (double autoclaved,

259   microbially sterile, beyond the current range of the focal legumes; *L. leucanthus*: n = 78 seeds; *V.*

260   *americana*: n = 53 seeds).

261   *Legume early life traits in dry soil conditions*

262   Separately, we grew *V. americana* in factorial experiments that manipulated the presence

263   of microbes (unsterilized vs. sterilized conditions) and the soil moisture level (dry vs. well-

264   watered), where all soils were from within the current range and began at the same soil moisture

265   VWC%. Half of all pots were placed into the well-watered treatment while the other half were

266   placed in the dry treatment. The well-watered treatment was watered with sterile, twice

267   autoclaved water every other day for 10 weeks while the dry treatment was watered every other

268 day for 2 weeks then once every week for 8 weeks; approximately 3 mL of water was added to

269 each pot at every watering. Soil treatments comprised: unsterilized, well-watered soils (n = 200

270 seeds); sterilized, well-watered soils (n = 200 seeds); unsterilized, dry soils (n = 200 seeds); and

271 sterilized, dry soils (n = 200 seeds).

272 *Data collection*

273 Seedling germination phenology, or the date of germinant emergence from the soil, was

274 monitored every other day for 10 weeks. In total, we documented the timing of germination for

275 225 seeds (16% of the 1406 seeds sown germinated). Many alpine species, including our focal

276 legumes, are highly clonal and are therefore expected to have a low rate of germination

277 (Angevine, 1983; Eriksson, 1989; Callaghan *et al.*, 1992). Indeed, germination success in both

278 species tends to be low (4-11%) in natural conditions around RMBL and in laboratory settings,

279 even when methods to crack the seed coats (e.g., sulfuric acid treatment) are used (personal

280 communication, Mike Bone, Denver Botanic Gardens; unpublished data, N. E. Rafferty). Seeds

281 that did not successfully germinate within 10 weeks were removed from pots and replanted in

282 unsterilized soils with adequate water and monitored for 10 additional weeks; none of these

283 seeds germinated after replanting.

284 *Data analysis*

285 To examine variation in germination success in soils that differed in microbe presence

286 and elevational origin, we constructed generalized linear models (GLM) with binomial error. To

287 investigate variation in germination latency in soils that differed in microbe presence and

288 elevational origins, we used linear models (LM). To these models (with either germination

289 success or latency as the response), we introduced species, seed mass, and soil type as predictors.

290   The importance of seed mass for germination success or latency may depend on soil type, such

291   that seed mass may be positively related to germination in sterilized soils but less important for

292   seeds in unsterilized soils; we therefore also included the interaction between seed mass and soil

293   type as a predictor. Because species was a significant predictor in all models, we fitted separate

294   models for *L. leucanthus* and *V. americana*. We used the same approach to examine variation in

295   germination success and latency in response to soil moisture, except we omitted species as a

296   predictor because only *V. americana* was used in that experiment. We used likelihood ratio tests

297   to compare model fit, and report the best fitting models. Post-hoc Tukey tests were used to test

298   for significant pairwise differences between categorical soil treatments. All analyses were

299   conducted in R version 4.0.2 (R Core Team, 2019).

300   **Results**

301   *Legume early life traits in sterilized soils*

302   Germination success of *L. leucanthus* did not differ in sterilized vs. unsterilized soils

303   from the current range (Tukey test: $p < 0.17$; Table 1 and Figure 1). Regardless of soil type,

304   heavier seeds were more likely to germinate than lighter seeds (GLM: 0.02 ± 0.01, $z_{347} = 2.33$, p

305   < 0.02; Figure 2). Similarly, for *V. americana*, germination success did not differ in sterilized vs.

306   unsterilized current range soils (Tukey test: $p < 0.26$; Table 1 and Figure 1). For this species,

307   seed mass did not significantly affect germination success (GLM: 0.02 ± 0.02, $z_{259} = 1.04$, $p <$

308   0.29).

309   For *L. leucanthus* in current range soils, the onset of germination in sterilized soils was

310   delayed by about two weeks compared to germination in unsterilized soils (Tukey test: $p < 0.03$;

311   Table 1 and Figure 1). In unsterilized, current range soils, *L. leucanthus* seeds germinated $28.0 \pm$

312   14.8 (mean ± SE) days after sowing, while in sterilized soils, seeds germinated in 69.0 ± 26.0

313   days. Seed mass was a significant predictor of germination timing in *L. leucanthus,* where

314   heavier seeds germinated faster than lighter seeds (LM: -2.18 ± 0.48, t_{15} = -4.48, $p < 0.0004$;

315   Figure 2). For *V. americana*, the delay in germination in sterilized vs. unsterilized current range

316   soils approached significance (Tukey test: $p < 0.08$; Table 1 and Figure 1); seeds in unsterilized

317   current range soils germinated in 30.2 ± 5.3 days vs. 40.0 ± 6.9 days in sterilized soils. Seed

318   mass was similarly important for germination timing in *V. americana* (LM: -1.91 ± 0.33, t_{35} = -

319   5.71, $p < 0.0001$; Figure 2). Nodules were found on some of the plant roots of both species in the

320   unsterilized but not in the sterilized treatments.

321   *Legume early life traits in soils from beyond the current elevational range*

322   Germination success of *L. leucanthus* did not differ in unsterilized soils from within vs.

323   beyond the current elevational range (Tukey test: $p < 0.80$; Table 1 and Figure 1). For *V.*

324   *americana*, germination success was significantly lower in unsterilized soils from beyond the

325   current elevational range compared to that within the range (Tukey test: $p < 0.01$; Table 1 and

326   Figure 1).

327   Seeds of *L. leucanthus* and *V. americana* sown in unsterilized soils from within vs.

328   beyond their current elevational range showed no significant difference in germination timing (*L.*

329   *leucanthus* Tukey test: $p < 0.73$; *V. americana* Tukey test: $p < 0.91$; Table 1 and Figure 1).

330   Heavier *L. leucanthus* seeds germinated more quickly than lighter seeds did, and this trend was

331   especially pronounced in sterilized soil from beyond the current elevational range (Figure 2).

332   *Legume early life traits in dry soil conditions*

333   *Vicia americana* seeds in unsterilized, dry soils had lower germination success than those

334   in unsterilized, well-watered soils (Tukey test: $p < 0.03$; Table 1 and Figure 3). Germination

335   success was $26 \pm 2\%$ in unsterilized, well-watered soils, higher than the $15 \pm 2\%$ seen in

336   unsterilized, dry soils. Seeds in unsterilized, dry conditions also experienced delayed germination

337   compared to those in unsterilized, well-watered soils (Tukey test: $p < 0.0001$; Table 1 and Figure

338   3). In unsterilized soils under dry conditions, seeds germinated in 50.2 ± 1.8 days, while in

339   unsterilized soils under well-watered conditions, seeds germinated in 35.2 ± 1.9 days. Lastly, *V.*

340   *americana* seeds planted in unsterilized, well-watered soils germinated more quickly and readily

341   than those in sterile, dry conditions (germination latency Tukey test: $p < 0.01$; germination

342   success Tukey test: $p < 0.001$; Table 1 and Figure 3).

343   **Discussion**

344   Short term weakening or loss of beneficial partnerships is becoming increasingly

345   common as our climate rapidly changes and repeated instances of loss can lead to a mutualism

346   breakdown (Kiers *et al.*, 2010; Werner *et al.*, 2018). The loss of a mutualism can alter the

347   distribution, functional traits, and survival of both partners. In this study, we found that a forced

348   loss of interaction between legumes and root mutualists can have consequences for plant traits,

349   where legume germination timing, but not success, was negatively affected by sterilized,

350   microbe-free, soils. Specifically, our results demonstrate that the onset of germination of *L.*

351   *leucanthus* in sterilized soils depleted of microbes was delayed by about two weeks (Figure 1),

352   suggesting that some legume seeds and seedlings may benefit from interacting with plant

353   growth-promoting soil microbes shortly after germinating and possibly even before germination

354   via germination stimulation. Germination timing is a particularly important trait, as it affects a

355   plant's competitive interactions and hardiness to frost and drought, and individuals that survive

356   this fragile life stage are more likely to reach reproductive stages. The phenomenon observed

357   here of germination stimulation, or the manipulation of the timing of germination, by soil

358   microbial mutualists is well known in systems such as the orchid-fungal mutualism (Arditti,

359   1967; Dressler, 1981), in certain legume-rhizobia mutualisms (Miransari and Smith, 2009), and

360   with *Pseudomonas fluorescens* (Moeinzadeh *et al.*, 2010) but this is the first known evidence of

361   this phenomenon occurring in the legumes of this subalpine system. This work contributes to the

362   growing number of findings that the community of soil microorganisms around a seed influences

363   germination timing, which may affect plant fitness (Mordecai, 2012; Lamichhane *et al.*, 2018;

364   Das *et al.*, 2019; Eldridge *et al.*, 2021).

365   As climatic patterns shape the natural distributions of species, changing climate

366   conditions strongly influence species' ranges (Chen *et al.*, 2011; Becker-Scarpitta, Vissault and

367   Vellend, 2019), where shifts typically involve a range expansion towards higher latitudes and

368   elevations and a range contraction away from lower latitudes and elevations (Davis and Shaw,

369   2001; Parmesan, 2006; Lenoir and Svenning, 2015). Because the presence of mutualists can

370   serve to expand the range of a partner by ameliorating abiotic stressors in novel environments

371   (Stachowicz, 2001; Afkhami, McIntyre and Strauss, 2014; Harrison *et al.*, 2018) and the absence

372   of a mutualist can constrict the range of a partner (Nuñez, Horton and Simberloff, 2009;

373   Simonsen *et al.*, 2017; Harrower and Gilbert, 2018), these mutualist-hosting legumes may not be

374   able to expand their elevational ranges upward if compatible soil microbes are not present

375   beyond the current range. This study demonstrates that the leading range edges of *L. leucanthus*

376   may not be restricted to 3500 m as active, compatible microbial species may be present at higher

377   elevations; germination timing and success of this species in higher-elevation soils mirrors that

378   in current range soils. Contrary to this, *V. americana* germination success was over five times

379   lower in novel, beyond the range soils, indicating that beneficial microbe strains specific to *V.*

380   *americana* plants may be absent or at low abundances at higher elevations, leading to reduced

381   germination success. Although sequencing and quantification of both within- and beyond the

382   range soils are needed to confirm the presence and abundance, or absence of active microbes, the

383   comparable germination timing and success in *L. leucanthus* in novel vs. current elevational

384   range soils suggests that compatible *L. leucanthus*-specific soil microbes may facilitate the

385   leading range expansion of this legume.

386   Germination-triggering soil wetting events are becoming less frequent and less intense in

387   many areas due to climate change (Saatkamp *et al.*, 2019). One of the predictions of the stress

388   gradient hypothesis is that mutualistic interactions increase in strength with increasing stress

389   (Callaway *et al.*, 2002; David *et al.*, 2020). Legumes grown in stressful conditions may not

390   exhibit reduced germination success and early life survival relative to those in less stressful

391   conditions as long as microbial mutualists can buffer the abiotic stress by stimulating

392   germination, provisioning N, P, and water, reducing root parasitism, thereby increasing plant

393   performance (Figueiredo *et al.*, 2008; Pawar *et al.*, 2014; Jemo *et al.*, 2017; Marinković *et al.*,

394   2019; Tankari *et al.*, 2019). Conversely, stressful, dry soils promote microbial dormancy,

395   meaning beneficial microbes are not able to stimulate germination or interact with the seed or

396   plant (de Vries *et al.*, 2018). In this controlled common garden study, legume seeds subjected to

397   dry conditions were less likely to germinate and had significantly delayed germination timing

398   compared to those in well-watered soils, likely due to stressful abiotic conditions for both the

399   seed and the microbes. This reduced germination stimulation may reflect a drought-induced loss

400   of the mutualism. If a plant germinates later than the optimal time, plant-plant competition will

401   be greater and the ability to acquire limited resources such as water, P, and N will be reduced

402 (Lloret, Casanovas and Peñuelas, 1999; Leverett, 2017). Downstream phenological patterns,

403 such as flowering time, could also be delayed, affecting pollinator visitation rates and

404 reproductive output (Rafferty and Ives, 2011). If germination occurs too early, the plant may

405 desiccate or be subject to frost damage (Inouye, 2008; CaraDonna and Bain, 2016; Pardee *et al.*,

406 2019).

407 Though microbes compatible with *L. leucanthus* may be present at high elevations,

408 abiotic conditions at these elevations are relatively harsh; low soil moisture, high UV exposure,

409 and high winds, among other factors common above the treeline, could limit legume

410 establishment beyond 3500 m (Normand *et al.*, 2009). In addition to this, mammalian seed and

411 seedling herbivores may limit seedling establishment in novel areas (Lynn, Miller and Rudgers,

412 2021). In this study, stressful, dry conditions led to decreased percent germination and delayed

413 germination; drier alpine conditions may not allow this mutualism to establish or persist and may

414 hinder a continued upward range expansion. Interestingly, *Lupinus argenteus*, a co-occurring

415 rhizobia- and AMF-hosting legume, occupies higher elevations than *L. leucanthus* or *V.*

416 *americana.* The PGPR which interact with *L. argenteus* may stimulate the germination of other

417 legume species (Hirsch, Lum and Allan Downie, 2001), though sequencing of both within- and

418 beyond the range soils is needed to confirm the observational evidence of soil microbes

419 occurrence in both ranges. Another avenue to be explored is that of the seed microbiome

420 (Nelson, 2018), specifically the epiphytic microbial community for horizontally transmitted

421 mutualists like rhizobia and other PGPR bacteria. The seed coats of both focal legume species

422 are porous; if seeds fall to the ground and accumulate mutualists before dispersal, an upward

423 range expansion may be more likely to occur because the partners would co-occur spatially,

424 though joint dispersal of legumes and rhizobia and other microbes has seldom been studied

425   (Porter, Faber-Hammond and Friesen, 2018; Wendlandt *et al.*, 2021), and joint dispersal does not

426   always imply interaction (Wornik and Grube, 2010). Sequencing of soils and dispersed seeds

427   would be useful to test this possibility.

428   In addition to soil microbe-mediated germination stimulation, seed traits, such as seed

429   mass, are important components of germination success and timing (Lord, Westoby and

430   Leishman, 1995; Venable *et al.*, 1998; Thompson, 2008). It is thought that heavier seeds are an

431   adaptation for overcoming stressful conditions, like drought, during seedling establishment

432   (Wullf, 1986), as larger seeds increase seedling persistence via greater internal resource

433   provisioning (Leishman and Westoby, 1994; Lebrija-Trejos *et al.*, 2016; Harrison and LaForgia,

434   2019). The transition from seed to seedling can be a defining period in the life history of a plant

435   (Muscarella *et al.*, 2013; Larson *et al.*, 2015), and here, we found that heavier seeds germinated

436   more quickly than did lighter seeds, and that heavier seeds had greater germination success. This

437   trend was especially pronounced when seeds were planted in the most stressful conditions,

438   sterile soils collected from beyond the current range of *L. leucanthus* (Figure 2). In the absence

439   of epiphytic microbes, other factors such as seed mass and abiotic components of the seed

440   environment (e.g., soil moisture, available nutrients) become more important (Lamichhane *et al.*,

441   2018). In these scenarios, heavier seeds are predicted to be more vigorous, and thus germinate at

442   a higher and faster rate.

443   A drawback to using a sterile soil treatment is that sterilization removes not only plant

444   growth-promoting soil mutualists but also all other potential soil microbes. Based on field

445   observations near the study area, *L. leucanthus* and *V. americana* interact not only with rhizobia

446   but also with AMF and DSE (unpublished data, RMBL). Through staining and microscopy,

447   neither of these fungal symbionts were found on or in any of the plant roots in this study, but

448   nodules were found on some of the plants in natural, current elevation soils. As simultaneous

449   infection by multiple belowground mutualists can additively benefit the plant (Afkhami and

450   Stinchcombe, 2016), future work that assesses the effects of native rhizobial, AMF, DSE, and

451   other PGPR bacterial infection on legume functional traits would be valuable.

452   Symbiotic rhizobia in extreme environments have lost the segment of their genome

453   responsible for the initiation and maintenance of their mutualism with legumes due to natural

454   selection; the maintenance of this non-essential portion of the genome is costly to the bacteria in

455   harsh environments (Sullivan *et al.*, 1996; Sullivan and Ronson, 1998; Denison and Kiers, 2004;

456   Sachs, Skophammer and Regus, 2011). The loss of this segment of DNA ultimately causes a

457   complete breakdown of the mutualism, only likely after numerous short-term losses of the

458   mutualism. A climate change-induced breakdown in the mutualism between legumes and

459   rhizobia will have significant effects on legume germination, phenology, and N-acquisition,

460   which could affect higher-order mutualists, such as pollinators (Keeler, Rose-Person and

461   Rafferty, 2021), and plant community structure (Suttle, Thomsen and Power, 2007). Just as floral

462   traits such as nectar quality can be directly related to soil nutrient availability (Mevi-Schutz and

463   Erhardt, 2005; Burkle and Irwin, 2009), short- or long-term loss of the interaction between plants

464   and soil microbial species due to mutualism loss or breakdown will indirectly affect floral traits

465   by altering host plant nutrient acquisition (Gwata *et al.*, 2003; Megueni *et al.*, 2006; Namvar and

466   Sharifi, 2011; Ballhorn, Kautz and Schädler, 2013), which could cascade to affect pollinator

467   behavior and legume fitness (Keeler, Rose-Person and Rafferty, 2021). The long-term fitness

468   consequences of this particular mutualism loss or potential breakdown are generally unknown

469   (Berg *et al.*, 2010; Kiers *et al.*, 2010), though slower growth and lower quality floral rewards in

470   these pollinator-dependent, pollen-limited plants (Xingwen, 2021) may further decrease

471   reproductive success and thus recruitment in a warming, drying climate.

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482   **Literature Cited**

483   Afkhami, M.E., McIntyre, P.J. and Strauss, S.Y. (2014) 'Mutualist-mediated effects on species'
484   range limits across large geographic scales', *Ecology Letters*, 17(10), pp. 1265–1273.

485   Afkhami, M.E. and Stinchcombe, J.R. (2016) 'Multiple mutualist effects on genomewide
486   expression in the tripartite association between *Medicago truncatula*, nitrogen-fixing bacteria
487   and mycorrhizal fungi', *Molecular Ecology*, 25(19), pp. 4946–4962.

488   Aldasoro, J., Larrainzar, E. and Arrese-Igor, C. (2019) 'Application of anti-transpirants
489   temporarily alleviates the inhibition of symbiotic nitrogen fixation in drought-stressed pea
490   plants', *Agricultural Water Management*, 213, pp. 193–199.

491   Allan, R.P. and Soden, B.J. (2008) 'Atmospheric warming and the amplification of precipitation
492   extremes', *Science*, 321(5895), pp. 1481–1484.

493   Angevine, M.W. (1983) 'Variations in the Demography of Natural Populations of the Wild

494   Strawberries *Fragaria vesca* and *F. virginiana*', *The Journal of Ecology*, 71(3), pp. 959–974.

495   Arditti, J. (1967) 'Factors affecting the germination of orchid seeds', *The Botanical Review,*
496   33(1), pp. 1–97.

497   Atzorn, R. *et al.* (1988) 'Production of gibberellins and indole-3-acetic acid by *Rhizobium*
498   *phaseoli* in relation to nodulation of *Phaseolus vulgaris* roots', *Planta*, 175(4), pp. 532–538.

499   Ballhorn, D.J., Kautz, S. and Schädler, M. (2013) 'Induced plant defense via volatile production
500   is dependent on rhizobial symbiosis', *Oecologia*, 172(3), pp. 833–846.

501   Bastian, F. *et al.* (1998) 'Production of indole-3-acetic acid and gibberellins A by *Acetobacter*
502   *diazotrophicus* and *Herbaspirillum seropedicae* in chemically-defined culture media', *Plant*
503   *Growth Regulation*, 24, pp. 7–11.

504   Becker-Scarpitta, A., Vissault, S. and Vellend, M. (2019) 'Four decades of plant community
505   change along a continental gradient of warming', *Global Change Biology*, 25(5), pp. 1629–1641.

506   Bennett, A.E. and Meek, H.C. (2020) 'The Influence of Arbuscular Mycorrhizal Fungi on Plant
507   Reproduction', *Journal of Chemical Ecology*, 46(8), pp. 707–721.

508   Benning, J.W. and Moeller, D.A. (2021a) 'Microbes, mutualism, and range margins: testing the
509   fitness consequences of soil microbial communities across and beyond a native plant's range',
510   *The New phytologist*, 229(5), pp. 2886–2900.

511   Benning, J.W. and Moeller, D.A. (2021b) 'Plant-soil interactions limit lifetime fitness outside a
512   native plant's geographic range margin', *Ecology*, 102(3), p. e03254.

513   Berg, G. *et al.* (2010) 'Symbiotic Plant–Microbe Interactions: Stress Protection, Plant Growth
514   Promotion, and Biocontrol by Stenotrophomonas', in Seckbach, J. and Grube, M. (eds)
515   *Symbioses and Stress: Joint Ventures in Biology*. Dordrecht: Springer Netherlands, pp. 445–460.

516   Brown, C.D. and Vellend, M. (2014) 'Non-climatic constraints on upper elevational plant range
517   expansion under climate change', *Proceedings. Biological sciences / The Royal Society*,
518   281(1794), p. 20141779.

519   Bruno, J.F., Stachowicz, J.J. and Bertness, M.D. (2003) 'Inclusion of Facilitation into Ecological
520   Theory', *Trends in Ecology & Evolution*, 18(3), pp. 119–125.

521   Bueno de Mesquita, C.P. *et al.* (2016) 'Incorporating biotic factors in species distribution
522   modeling: are interactions with soil microbes important?', *Ecography*, 39(10), pp. 970–980.

523   Bueno de Mesquita, C.P. *et al.* (2018) 'Patterns of root colonization by arbuscular mycorrhizal
524   fungi and dark septate endophytes across a mostly-unvegetated, high-elevation landscape',
525   *Fungal Ecology*, 36, pp. 63–74.

526   Bueno de Mesquita, C.P. *et al.* (2020) 'Growing-season length and soil microbes influence the
527   performance of a generalist bunchgrass beyond its current range', *Ecology*, 101(9), p. e03095.

528 Burkle, L.A. and Irwin, R.E. (2009) 'The Effects of Nutrient Addition on Floral Characters and
529 Pollination in Two Subalpine Plants, *Ipomopsis aggregata* and *Linum lewisii*', *Plant Ecology*,
530 203(1), pp. 83–98.

531 Callaghan, T.V. *et al.* (1992) 'Clonal Plants and Environmental Change: Introduction to the
532 Proceedings and Summary', *Oikos* , 63(3), pp. 341–347.

533 Callaway, R.M. *et al.* (2002) 'Positive interactions among alpine plants increase with stress',
534 *Nature*, 417(6891), pp. 844–848.

535 CaraDonna, P.J. and Bain, J.A. (2016) 'Frost sensitivity of leaves and flowers of subalpine plants
536 is related to tissue type and phenology', *The Journal of Ecology*, 104(1), pp. 55–64.

537 CaraDonna, P.J., Iler, A.M. and Inouye, D.W. (2014) 'Shifts in flowering phenology reshape a
538 subalpine plant community', *PNAS*, 111(13), pp. 4916–4921.

539 Chen, I.-C. *et al.* (2011) 'Rapid Range Shifts of Species Associated with High Levels of Climate
540 Warming', *Science*, pp. 1024–1026.

541 Classen, A.T. *et al.* (2015) 'Direct and indirect effects of climate change on soil microbial and
542 soil microbial-plant interactions: What lies ahead?', *Ecosphere*, 6(8), pp. 1–21.

543 Clow, D.W. (2010) 'Changes in the Timing of Snowmelt and Streamflow in Colorado: A
544 Response to Recent Warming', *Journal of Climate*, 23(9), pp. 2293–2306.

545 Collins, C.G. (2019) 'Above- and below-ground consequences of woody plant range expansion
546 in alpine ecosystems', Ph.D. Dissertation, *University of California, Riverside*. University of
547 California.

548 Dai, A. (2012) 'Increasing drought under global warming in observations and models', *Nature
549 Climate Change*, 3(1), pp. 52–58.

550 Das, S. *et al.* (2019) 'Isolation and characterization of indole acetic acid producing bacteria from
551 rhizosphere soil and their effect on seed germination', *International Journal of Current
552 Microbiology Applications in Science*, 8(3), pp. 1237–1245.

553 David, A.S. *et al.* (2020) 'Do plant–microbe interactions support the Stress Gradient
554 Hypothesis?', *Ecology,* 101, pp. 8.

555 David, A.S., Thapa-Magar, K.B. and Afkhami, M.E. (2018) 'Microbial mitigation-exacerbation
556 continuum: a novel framework for microbiome effects on hosts in the face of stress', *Ecology*,
557 99(3), pp. 517–523.

558 Davis, M.B. and Shaw, R.G. (2001) 'Range shifts and adaptive responses to Quaternary climate
559 change', *Science*, 292(5517), pp. 673–679.

560 Defossez, E. *et al.* (2011) 'Do interactions between plant and soil biota change with elevation? A
561 study on Fagus sylvatica', *Biology Letters*, 7(5), pp. 699–701.

562 Denison, R.F. and Kiers, E.T. (2004) 'Lifestyle alternatives for rhizobia: mutualism, parasitism,
563 and forgoing symbiosis', *FEMS microbiology letters*, 237(2), pp. 187–193.

564 Donohue, K. *et al.* (2010) 'Germination, Postgermination Adaptation, and Species Ecological
565 Ranges', *Annual Review of Ecology, Evolution, and Systematics,* 41, pp. 293-319.

566 Dressler, R.L. (1981) *The orchids: natural history and classification*. Harvard University Press.

567 Eldridge, D.J. *et al.* (2021) 'Experimental evidence of strong relationships between soil
568 microbial communities and plant germination', *The Journal of Ecology*.

569 Eriksson, O. (1989) 'Seedling dynamics and life histories in clonal plants', *Oikos* , 55(2), p. 231.

570 Figueiredo, M.V.B. *et al.* (2008) 'Alleviation of drought stress in the common bean (*Phaseolus*
571 *vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*', *Applied Soil*
572 *Ecology*, 40(1), pp. 182–188.

573 Fitzpatrick, C.R., Mustafa, Z. and Viliunas, J. (2019) 'Soil microbes alter plant fitness under
574 competition and drought', *Journal of Evolutionary Biology*, 32(5), pp. 438–450.

575 Fowler, N.L. (1984) 'The Role of Germination Date, Spatial Arrangement, and Neighbourhood
576 Effects in Competitive Interactions in Linum', *The Journal of Ecology*, 72(1), pp. 307–318.

577 Gehring, C.A. *et al.* (2017) 'Tree genetics defines fungal partner communities that may confer
578 drought tolerance', *PNAS*, 114(42), pp. 11169–11174.

579 Gottlieb, D. (1950) 'The physiology of spore germination in fungi', *The Botanical Review;*
580 *Interpreting Botanical Progress*, 16(5), pp. 229–257.

581 Gwata, E.T. *et al.* (2003) 'Pollen morphology and in vitro germination characteristics of
582 nodulating and nonnodulating soybean (*Glycine max* L.) genotypes', *Theoretical and Applied*
583 *Genetics*, 106(5), pp. 837–839.

584 Halvorson, J.J., Smith, J.L. and Franz, E.H. (1991) 'Lupine influence on soil carbon, nitrogen
585 and microbial activity in developing ecosystems at Mount St. Helens', *Oecologia*, 87(2), pp.
586 162–170.

587 Harris, D., Pacovsky, R.S. and Paul, E.A. (1985) 'Carbon economy of soybean-rhizobuim-
588 glomus associations', *The New Phytologist*, 101, pp. 427–440.

589 Harrison, S. and LaForgia, M. (2019) 'Seedling traits predict drought-induced mortality linked to
590 diversity loss', *PNAS*, 116(12), pp. 5576–5581.

591 Harrison, T.L. *et al.* (2017) 'No evidence for adaptation to local rhizobial mutualists in the
592 legume *Medicago lupulina*', *Ecology and Evolution*, 7(12), pp. 4367–4376.

593 Harrison, T.L. *et al.* (2018) 'More partners, more ranges: generalist legumes spread more easily
594 around the globe', *Biology Letters*, 14(11), p. 20180616.

595 Harrower, J. and Gilbert, G.S. (2018) 'Context-dependent mutualisms in the Joshua tree-yucca
596 moth system shift along a climate gradient', *Ecosphere* , 9(9), p. e02439.

597 Hayward, J. *et al.* (2015) 'A single ectomycorrhizal fungal species can enable a Pinus invasion',
598 *Ecology*, 96(5), pp. 1438–1444.

599 HilleRisLambers, J. *et al.* (2012) 'Rethinking Community Assembly through the Lens of
600 Coexistence Theory', *Annual Review of Ecology, Evolution, and Systematics*, 43, pp.227-248.

601 Hirsch, A.M., Lum, M.R. and Allan Downie, J. (2001) 'What Makes the Rhizobia-Legume
602 Symbiosis So Special?', *Plant Physiology*, pp. 1484–1492.

603 Hülber, K., Winkler, M. and Grabherr, G. (2010) 'Intraseasonal climate and habitat-specific
604 variability controls the flowering phenology of high alpine plant species', *Functional Ecology*,
605 24(2), pp. 245–252.

606 Hungria, M. and Vargas, M.A.T. (2000) 'Environmental factors affecting N2 fixation in grain
607 legumes in the tropics, with an emphasis on Brazil', *Field Crops Research*, 65(2), pp. 151–164.

608 Hu, Y. *et al.* (2022) 'The Role of Arbuscular Mycorrhiza Fungi in Drought Tolerance in Legume
609 Crops: A Review', *Legume Research-An International Journal*, 1, p. 9.

610 Inouye, D.W. (2008) 'Effects of climate change on phenology, frost damage, and floral
611 abundance of montane wildflowers', *Ecology*, 89(2), pp. 353–362.

612 Jemo, M. *et al.* (2017) 'Comparative Analysis of the Combined Effects of Different Water and
613 Phosphate Levels on Growth and Biological Nitrogen Fixation of Nine Cowpea Varieties',
614 *Frontiers in Plant Science*, 8, p. 2111.

615 Keeler, A.M., Rose-Person, A. and Rafferty, N.E. (2021) 'From the ground up: Building
616 predictions for how climate change will affect belowground mutualisms, floral traits, and bee
617 behavior', *Climate Change Ecology*, 1, p. 100013.

618 Kiers, T. *et al.* (2010) 'Mutualisms in a changing world: an evolutionary perspective', *Ecology*
619 *Letters*, 13(12), pp. 1459–1474.

620 Kittel, T.G.F. *et al.* (2015) 'Contrasting long-term alpine and subalpine precipitation trends in a
621 mid-latitude North American mountain system, Colorado Front Range, USA', *Plant Ecology &*
622 *Diversity*, 8(5-6), pp. 607–624.

623 Klironomos, J.N. (2002) 'Feedback with soil biota contributes to plant rarity and invasiveness in
624 communities', *Nature*, 417(6884), pp. 67–70.

625 Komatsu, K.J. and Simms, E.L. (2020) 'Invasive legume management strategies differentially
626 impact mutualist abundance and benefit to native and invasive hosts', *Restoration Ecology*, pp.
627 378–386.

628 Kudo, G. and Cooper, E.J. (2019) 'When spring ephemerals fail to meet pollinators: mechanism

629 of phenological mismatch and its impact on plant reproduction', *Proceedings. Biological*
630 *sciences / The Royal Society*, 286(1904), p. 20190573.

631 Kumar, H., Dubey, R.C. and Maheshwari, D.K. (2011) 'Effect of plant growth promoting
632 rhizobia on seed germination, growth promotion and suppression of Fusarium wilt of fenugreek
633 (*Trigonella foenum-graecum* L.)', *Crop Protection* , 30, p. 1396e1403.

634 Lambert, A.M., Miller-Rushing, A.J. and Inouye, D.W. (2010) 'Changes in snowmelt date and
635 summer precipitation affect the flowering phenology of *Erythronium grandiflorum* (glacier lily;
636 Liliaceae)', *American Journal of Botany*, 97(9), pp. 1431–1437.

637 Lamichhane, J.R. *et al.* (2018) 'Abiotic and biotic factors affecting crop seed germination and
638 seedling emergence: a conceptual framework', *Plant and Soil*, 432(1), pp. 1–28.

639 Lankau, R.A. and Keymer, D.P. (2016) 'Ectomycorrhizal fungal richness declines towards the
640 host species' range edge', *Molecular Ecology*, 25(13), pp. 3224–3241.

641 Larson, J.E. *et al.* (2015) 'Seed and seedling traits affecting critical life stage transitions and
642 recruitment outcomes in dryland grasses', *Journal of Applied Ecology*, pp. 199–209.

643 Lau, J.A. and Lennon, J.T. (2012) 'Rapid responses of soil microorganisms improve plant fitness
644 in novel environments', *PNAS*, 109(35), pp. 14058–14062.

645 Lebrija-Trejos, E. *et al.* (2016) 'Species with greater seed mass are more tolerant of conspecific
646 neighbours: a key driver of early survival and future abundances in a tropical forest', *Ecology*
647 *Letters*, 19(9), pp. 1071–1080.

648 Leff, J.W. *et al.* (2018) 'Predicting the structure of soil communities from plant community
649 taxonomy, phylogeny, and traits', *The ISME Journal*, 12(7), pp. 1794–1805.

650 Leishman, M.R. and Westoby, M. (1994) 'The Role of Seed Size in Seedling Establishment in
651 Dry Soil Conditions -- Experimental Evidence from Semi-Arid Species', *The Journal of*
652 *Ecology*, 82(2), pp. 249–258.

653 Lennon, J.T. and Jones, S.E. (2011) 'Microbial seed banks: the ecological and evolutionary
654 implications of dormancy', *Nature reviews. Microbiology*, 9(2), pp. 119–130.

655 Lenoir, J. and Svenning, J.-C. (2015) 'Climate-related range shifts - a global multidimensional
656 synthesis and new research directions', *Ecography*, pp. 15–28.

657 Leverett, L.D. (2017) 'Germination phenology determines the propensity for facilitation and
658 competition', *Ecology*, pp. 2437–2446.

659 Lloret, F., Casanovas, C. and Peñuelas, J. (1999) 'Seedling survival of Mediterranean shrubland
660 species in relation to root:shoot ratio, seed size and water and nitrogen use', *Functional Ecology*,
661 13(2), pp. 210–216.

662 Lord, J., Westoby, M. and Leishman, M. (1995) 'Seed Size and Phylogeny in Six Temperate

663 Floras: Constraints, Niche Conservatism, and Adaptation', *The American Naturalist*, 146(3), pp.
664 349–364.

665 Lynn, J.S., Miller, T.E.X. and Rudgers, J.A. (2021) 'Mammalian herbivores restrict the
666 altitudinal range limits of alpine plants', *Ecology Letters*, 24(9), pp. 1930–1942.

667 Mangan, S.A. *et al.* (2010) 'Negative plant–soil feedback predicts tree-species relative
668 abundance in a tropical forest', *Nature*, 466(7307), pp. 752–755.

669 Marinković, J. *et al.* (2019) 'Performance of different Bradyrhizobium strains in root nodule
670 symbiosis under drought stress', *Polish Academy of Sciences, Committee of Plant Physiology*
671 *Genetics and Breeding*, 41(3), p. 37.

672 Meena, K.K. *et al.* (2012) 'Epiphytic pink-pigmented methylotrophic bacteria enhance
673 germination and seedling growth of wheat (*Triticum aestivum*) by producing phytohormone',
674 *Antonie van Leeuwenhoek*, 101(4), pp. 777–786.

675 Megueni, C. *et al.* (2006) 'Responses of soybean (*Glycine max* L.) to soil solarization and
676 rhizobial field inoculation at dang ngaoundere, Cameroon', *Asian Journal of Plant Science*, 5(5),
677 pp. 832–837.

678 Mevi-Schutz, J. and Erhardt, A. (2005) 'Amino acids in nectar enhance butterfly fecundity: a
679 long-awaited link', *The American Naturalist*, 165(4), pp. 411–419.

680 Miransari, M. (2010) 'Contribution of arbuscular mycorrhizal symbiosis to plant growth under
681 different types of soil stress', *Plant Biology* , 12(4), pp. 563–569.

682 Miransari, M. and Smith, D. (2009) 'Rhizobial Lipo-Chitooligosaccharides and Gibberellins
683 Enhance Barley (*Hordeum vulgare* L.) Seed Germination', *Biotechnology*, pp. 270–275.

684 Moeinzadeh, A. *et al.* (2010) 'Biopriming of Sunflower ('*Helianthus annuus*' L.) Seed with
685 '*Pseudomonas fluorescens*' for Improvement of Seed Invigoration and Seedling Growth',
686 *Australian Journal of Crop Science*, 4(7), pp. 564–570.

687 Mordecai, E.A. (2012) 'Soil moisture and fungi affect seed survival in California grassland
688 annual plants', *PloS One*, 7(6), p. e39083.

689 Mueller, U.G. *et al.* (2011) 'Frontier mutualism: coevolutionary patterns at the northern range
690 limit of the leaf-cutter ant-fungus symbiosis', *Proceedings. Biological sciences / The Royal*
691 *Society*, 278(1721), pp. 3050–3059.

692 Muscarella, R. *et al.* (2013) 'Life-history trade-offs during the seed-to-seedling transition in a
693 subtropical wet forest community', *The Journal of Ecology*, 101(1), pp. 171–182.

694 Namvar, A. and Sharifi, R.S. (2011) 'Phenological and morphological response of chickpea
695 (*Cicer arietinum* L.) to symbiotic and mineral nitrogen fertilization', *Zemdirbysté*, 2, p. 121.

696 Nelson, E.B. (2018) 'The seed microbiome: Origins, interactions, and impacts', *Plant and Soil*,

697   422(1), pp. 7–34.

698   Ngumbi, E. and Kloepper, J. (2016) 'Bacterial-mediated drought tolerance: Current and future
699   prospects', *Applied Soil Ecology*, 105, pp. 109–125.

700   Noel, T.C. *et al.* (1996) '*Rhizobium leguminosarum* as a plant I growth-promoting
701   rhizobacterium: direct growth promotion of canola and lettuce', *Canadian Journal of*
702   *Microbiology*, 42, pp. 279–283.

703   Normand, S. *et al.* (2009) 'Importance of abiotic stress as a range-limit determinant for European
704   plants: insights from species responses to climatic gradients', *Global Ecology and Biogeography*,
705   18(4), pp. 437–449.

706   Nuñez, M.A., Horton, T.R. and Simberloff, D. (2009) 'Lack of belowground mutualisms hinders
707   Pinaceae invasions', *Ecology*, 90(9), pp. 2352–2359.

708   Overpeck, J. and Udall, B. (2010) 'Climate change. Dry times ahead', *Science*, 328(5986), pp.
709   1642–1643.

710   Oyebanji, O.B. *et al.* (2009) 'Simple, effective and economical explant-surface sterilization
711   protocol for cowpea, rice and sorghum seeds', *African Journal of Biotechnology*, 8(20).

712   Pardee, G.L. *et al.* (2019) 'The individual and combined effects of snowmelt timing and frost
713   exposure on the reproductive success of montane forbs', *The Journal of Ecology*, 107(4), pp.
714   1970–1981.

715   Parker, M.A. (2001) 'Mutualism as a constraint on invasion success for legumes and rhizobia',
716   *Diversity & Distributions*, 7(3), pp. 125–136.

717   Parmesan, C. (2006) 'Ecological and Evolutionary Responses to Recent Climate Change',
718   *Annual Review of Ecology, Evolution, and Systematics*, 37(1), pp. 637–669.

719   Pawar, V.A. *et al.* (2014) 'Effect of Rhizobium on seed germination and growth of plants',
720   *Journal of Academia and Industrial Research*, 3(2), pp. 84–88.

721   Peay, K.G., Garbelotto, M. and Bruns, T.D. (2010) 'Evidence of dispersal limitation in soil
722   microorganisms: isolation reduces species richness on mycorrhizal tree islands', *Ecology*,
723   91(12), pp. 3631–3640.

724   Pellmyr, O. (2003) 'Yuccas, Yucca Moths, and Coevolution: A Review', *Annals of the Missouri*
725   *Botanical Garden*, p. 35.

726   Petipas, R.H. *et al.* (2017) 'Habitat-specific AMF symbioses enhance drought tolerance of a
727   native Kenyan grass', *Acta Oecologica*, 78, pp. 71–78.

728   Porter, S.S. *et al.* (2020) 'Beneficial microbes ameliorate abiotic and biotic sources of stress on
729   plants', *Functional Ecology*, 34(10), pp. 2075–2086.

Porter, S.S., Faber-Hammond, J.J. and Friesen, M.L. (2018) 'Co-invading symbiotic mutualists of Medicago polymorpha retain high ancestral diversity and contain diverse accessory genomes', *FEMS Microbiology Ecology*, 94(1).

Pringle, E.G. *et al.* (2013) 'Water stress strengthens mutualism among ants, trees, and scale insects', *PLoS Biology*, 11(11), p. e1001705.

Pyke, G.H. (1982) 'Local geographic distributions of bumblebees near Crested Butte, Colorado: Competition and community structure', *Ecology*, 63(2), pp. 555–573.

Pyke, G.H. *et al.* (2016) 'Effects of climate change on phenologies and distributions of bumble bees and the plants they visit', *Ecosphere*, 7(3), p. e01267.

Pyke, G.H., Inouye, D.W. and Thomson, J.D. (2012) 'Local geographic distributions of bumble bees near Crested Butte, Colorado: competition and community structure revisited', *Environmental Entomology*, 41(6), pp. 1332–1349.

Rafferty, N.E., Bertelsen, C.D. and Bronstein, J.L. (2016) 'Later flowering is associated with a compressed flowering season and reduced reproductive output in an early season floral resource', *Oikos,* 125(6), pp. 821–828.

Rafferty, N.E., CaraDonna, P.J. and Bronstein, J.L. (2015) 'Phenological shifts and the fate of mutualisms', *Oikos,* 124(1), pp. 14–21.

Rafferty, N.E. and Ives, A.R. (2011) 'Effects of experimental shifts in flowering phenology on plant-pollinator interactions', *Ecology Letters*, 14(1), pp. 69–74.

Rafferty, N.E. and Ives, A.R. (2012) 'Pollinator effectiveness varies with experimental shifts in flowering time', *Ecology*, 93(4), pp. 803–814.

R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria (2019). Available at: https://www.R-project.org/.

Redman, R.S. *et al.* (2011) 'Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change', *PloS One*, 6(7), p. e14823.

Remke, M.J. *et al.* (2021) 'Sympatric pairings of dryland grass populations, mycorrhizal fungi and associated soil biota enhance mutualism and ameliorate drought stress', *The Journal of Ecology*, 109(3), pp. 1210–1223.

Reynolds, H.L. *et al.* (2003) 'Grassroots ecology: Plant–microbe–soil interactions as drivers of plant community structure and dynamics', *Ecology*, 84(9), pp. 2281–2291.

Rodriguez-Cabal, M.A., Noelia Barrios-Garcia, M. and Nunez, M.A. (2012) 'Positive interactions in ecology: filling the fundamental niche', *Ideas in Ecology and Evolution,* 5.

Rodríguez-Echeverría, S., Lozano, Y.M. and Bardgett, R.D. (2016) 'Influence of soil microbiota in nurse plant systems', *Functional Ecology*, 30(1), pp. 30–40.

Saatkamp, A. *et al.* (2019) 'A research agenda for seed-trait functional ecology', *The New Phytologist*, 221(4), pp. 1764–1775.

Sachs, J.L., Skophammer, R.G. and Regus, J.U. (2011) 'Evolutionary transitions in bacterial symbiosis', *PNAS*, 108 Suppl 2, pp. 10800–10807.

Schenk, M., Krauss, J. and Holzschuh, A. (2018) 'Desynchronizations in bee-plant interactions cause severe fitness losses in solitary bees', *The Journal of Animal Ecology*, 87(1), pp. 139–149.

Schimel, J., Balser, T.C. and Wallenstein, M. (2007) 'Microbial stress-response physiology and its implications for ecosystem function', *Ecology*, 88(6), pp. 1386–1394.

Schimel, J.P. (2018) 'Life in Dry Soils: Effects of Drought on Soil Microbial Communities and Processes', *Annual Review of Ecology, Evolution, and Systematics*.

Schmidt, S.K. *et al.* (2008) 'Mycorrhizal and Dark-Septate Fungi in Plant Roots Above 4270 Meters Elevation in the Andes and Rocky Mountains', *Arctic, Antarctic, and Alpine Research*, 40(3), pp. 576–583.

Simonsen, A.K. *et al.* (2017) 'Symbiosis limits establishment of legumes outside their native range at a global scale', *Nature Communications*, 8, p. 14790.

Sorensen, P.O. *et al.* (2020) 'The Snowmelt Niche Differentiates Three Microbial Life Strategies That Influence Soil Nitrogen Availability During and After Winter', *Frontiers in Microbiology*, 11, p. 871.

Stachowicz, J.J. (2001) 'Mutualism, Facilitation, and the Structure of Ecological Communities: Positive interactions play a critical, but underappreciated, role in ecological communities by reducing physical or biotic stresses in existing habitats and by creating new habitats on which many species depend', *Bioscience*, 51(3), pp. 235–246.

Stanton-Geddes, J. and Anderson, C.G. (2011) 'Does a facultative mutualism limit species range expansion?', *Oecologia*, 167(1), pp. 149–155.

Sullivan, J.T. *et al.* (1996) 'Four unnamed species of nonsymbiotic rhizobia isolated from the rhizosphere of *Lotus corniculatus*', *Applied and Environmental Microbiology*, 62(8), pp. 2818–2825.

Sullivan, J.T. and Ronson, C.W. (1998) 'Evolution of rhizobia by acquisition of a 500-kb symbiosis island that integrates into a phe-tRNA gene', *PNAS*, 95(9), pp. 5145–5149.

Suttle, K.B., Thomsen, M.A. and Power, M.E. (2007) 'Species interactions reverse grassland responses to changing climate', *Science*, 315(5812), pp. 640–642.

Tankari, M. *et al.* (2019) 'Leaf Gas Exchange, Plant Water Relations and Water Use Efficiency of *Vigna Unguiculata* L. Walp. Inoculated with Rhizobia under Different Soil Water Regimes', *Water*, 11(3), p. 498.

798   Thompson, K. (2008) 'Seeds and seed banks', *The New Phytologist*, 106, pp. 23–34.

799   Tsavkelova, E.A. *et al.* (2007) 'Bacteria associated with orchid roots and microbial production of
800   auxin', *Microbiological Research*, 162(1), pp. 69–76.

801   Tylianakis, J.M. *et al.* (2008) 'Global change and species interactions in terrestrial ecosystems',
802   *Ecology Letters*, 11(12), pp. 1351–1363.

803   Van Der Heijden, M.G.A. and Bardgett, R.D. (2008) 'The unseen majority: soil microbes as
804   drivers of plant diversity and productivity in terrestrial ecosystems', *Ecology,* 11, pp. 296-310.

805   Varga, S. *et al.* (2015) 'Arctic arbuscular mycorrhizal spore community and viability after
806   storage in cold conditions', *Mycorrhiza*, 25(5), pp. 335–343.

807   Venable, D.L. *et al.* (1998) 'Seed morphometrics and adaptive geographic differentiation,
808   *Evolution*, 52(2), pp. 344–354.

809   de Vries, F.T. *et al.* (2018) 'Soil bacterial networks are less stable under drought than fungal
810   networks', *Nature Communications*, 9(1), p. 3033.

811   de Vries, F.T. and Shade, A. (2013) 'Controls on soil microbial community stability under
812   climate change', *Frontiers in Microbiology*, 4, p. 265.

813   Vriezen, J.A.C., de Bruijn, F.J. and Nüsslein, K. (2006) 'Desiccation responses and survival of
814   *Sinorhizobium meliloti* USDA 1021 in relation to growth phase, temperature, chloride and sulfate
815   availability', *Letters in Applied Microbiology*, 42(2), pp. 172–178.

816   Wall, D.H. and Moore, J.C. (1999) 'Interactions Underground: Soil biodiversity, mutualism, and
817   ecosystem processes', *Bioscience*, 49(2), pp. 109–117.

818   Wardle, D.A. *et al.* (2004) 'Ecological linkages between aboveground and belowground biota',
819   *Science*, 304(5677), pp. 1629–1633.

820   Wendlandt, C.E. *et al.* (2021) 'Decreased coevolutionary potential and increased symbiont
821   fecundity during the biological invasion of a legume-rhizobium mutualism', *Evolution,* 75(3),
822   pp. 731–747.

823   Werner, G.D.A. *et al.* (2018) 'Symbiont switching and alternative resource acquisition strategies
824   drive mutualism breakdown', *PNAS*, 115(20), pp. 5229–5234.

825   Williams, A. and de Vries, F.T. (2020) 'Plant root exudation under drought: implications for
826   ecosystem functioning', *The New Phytologist*, 225(5), pp. 1899–1905.

827   Worchel, E.R., Giauque, H.E. and Kivlin, S.N. (2013) 'Fungal symbionts alter plant drought
828   response', *Microbial Ecology*, 65(3), pp. 671–678.

829   Wornik, S. and Grube, M. (2010) 'Joint dispersal does not imply maintenance of partnerships in
830   lichen symbioses', *Microbial Ecology*, 59(1), pp. 150–157.

831   Wullf, R.D. (1986) 'Seed size variation in *Desmodium paniculatum* I. factors affecting seed
832   size', *The Journal of Ecology*, 74, pp. 87–97.

833   Wu, Q.-S. and Ying-Ning, Z. (2017) 'Arbuscular Mycorrhizal Fungi and Tolerance of Drought
834   Stress in Plants', *Springer Nature Singapore*, pp. 25–41.

835   Wu, Y.-N. *et al.* (2016) 'Beneficial soil microbe promotes seed germination, plant growth and
836   photosynthesis in herbal crop *Codonopsis pilosula*', *Crop & Pasture Science*, 67(1), pp. 91–98.

837   Xingwen, L. (2021) *The effects of anthropogenic change on pollination in plant-pollinator
838   communities*. Edited by B. Brosi. Ph.D. Emory University.

839   Xu, L. and Coleman-Derr, D. (2019) 'Causes and consequences of a conserved bacterial root
840   microbiome response to drought stress', *Current opinion in microbiology*, 49, pp. 1–6.

841   Younginger, B.S. and Friesen, M.L. (2019) 'Connecting signals and benefits through partner
842   choice in plant–microbe interactions', *FEMS microbiology letters*, 366(18).

843   Zahran, H.H. (1999) 'Rhizobium-legume symbiosis and nitrogen fixation under severe
844   conditions and in an arid climate', *Microbiology and Molecular Biology Reviews*, 63(4), pp.
845   968–89.

846   de Zelicourt, A., Al-Yousif, M. and Hirt, H. (2013) 'Rhizosphere microbes as essential partners
847   for plant stress tolerance', *Molecular Plant*, 6(2), pp. 242–245.

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856　**Tables & Figures**

857　**Table 1.** Results of post-hoc pairwise Tukey tests for soil treatments following species-specific

858　(generalized) linear models, with either germination success or germination latency as the

859　response variable. Predictors included seed mass and soil treatment. Significant p-values are

860　given in bold; marginally significant p-values are given in italics.

Species	Soil treatment comparison	Germination success (p-value)	Germination latency (p-value)
L. leucanthus	unsterilized, current - sterilized, current	0.17	**0.03**
	unsterilized, current - unsterilized, beyond	0.80	0.73
V. americana	unsterilized, current - sterilized, current	0.26	*0.08*
	unsterilized, current - unsterilized, beyond	**0.01**	0.91
V. americana	unsterilized, well-watered - unsterilized, drought	**0.03**	**0.0001**
	unsterilized, well-watered - sterilized, drought	**0.001**	**0.01**

861

862　**Figure 1.** Microbial absence (sterile soil) did not significantly alter *L. leucanthus* or *V.*

863　*americana* germination success (A), but significantly delayed *L. leucanthus* germination within

864   the current range (B; compare unsterilized, within the current range vs. sterilized, within the

865   current range); this trend approached significance for *V. americana*. *Vicia americana* seeds

866   planted in soils from beyond their elevational range had lower germination success than seeds

867   planted in soils from within their current range (A; compare unsterilized, beyond the current

868   range vs. unsterilized, within the current range). Each point represents the mean germination

869   value ± SE.

870   **Figure 2.** Predicted days to *L. leucanthus* (A) and *V. americana* (B) germination by seed mass.

871   For *L. leucanthus*, heavier seed mass was especially important for germination timing in

872   sterilized, beyond the current range soils. Shaded areas represent 95% confidence intervals for

873   each soil type.

874   **Figure 3.** *Vicia americana* germination success was lower in unsterilized, dry soils than in

875   unsterilized, well-watered soils (A). In dry soils, unsterilized or sterilized, seeds germinated later

876   than those in unsterilized, well-watered soils (B). Germination success and timing did not differ

877   significantly between seeds planted in dry conditions (unsterilized, drought vs. sterilized,

878   drought) or well-watered conditions (unsterilized, well-watered vs. sterilized, well-watered).

879   Each point is the mean germination value ± SE.

880

881   **Image Legends**

882   Image 1. Root nodules found on plants grown in unsterilized, current range soils.

883

884   Image 2. Soil collection sites at RMBL in Gunnison County, CO. Yellow dots are approximate

885   areas where soil was collected beyond the current range of the legumes on Mt. Baldy (upper left

886   peak; 38.9926°N, 107.0462°W) and Mt. Gothic (lower right peak; 38.9564°N, 107.0106°W).

887   Orange dots are approximate locations where soil was collected near *L. leucanthus* and *V.*

888   *americana* patches.

889

890   Image 3. Common garden experimental design. Each tray contained a single treatment type to

891   reduce movement of water and/or microbes between pots. There were 96 pots and 96 seeds per

892   tray. We show 4 example trays, but there were 15 total trays.

893

894   Data Accessibility: Complete germination success and phenology data will be uploaded to Dryad

895   or similar at the time of manuscript acceptance.