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Evidence of plant-soil feedback in South Texas grasslands associated with invasive Guinea grass

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2 **grass.**

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4 Running title: Evidence of plant-soil feedback in Guinea grass invaded South Texas grasslands

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16

17 **Abstract**

18 Plant-soil feedback (PSF) processes play an integral role in structuring plant communities. In
19 native grasslands, PSF has a largely negative or stabilizing effect on plant growth contributing to
20 species coexistence and succession, but perturbations to a system can alter PSF leading to long-
21 term changes. Through additions of novel root exudates and litter which alter soil microbial
22 communities and nutrient cycling, invasion by non-native plants has a strong impact on
23 belowground processes with broad shifts in historical PSFs. Guinea grass, *Megathyrsus*
24 *maximus*, an emerging invasive in South Texas, can efficiently exclude native plants possibly
25 due to its fast growth rate and high biomass accumulation, but its impacts on belowground
26 processes are unknown. Here, we provide a first look at PSF processes in South Texas savannas
27 currently undergoing invasion by Guinea grass. We addressed the question of how the presence
28 of the invasive *M. maximus* may alter PSF compared to non-invaded grasslands. Under
29 greenhouse conditions, we assessed germination and growth of Guinea grass and the seed bank
30 in soil collected from native grasslands and grasslands invaded by Guinea grass. We found that
31 Guinea grass grown in soil from invaded grasslands grew taller and accumulated higher biomass
32 than in soil from non-invaded grasslands. Plants grown from the seed bank were more species
33 rich and abundant in soil from non-invaded grasslands but had higher biomass in soil from
34 invaded grasslands. In South Texas savannas, we found evidence to support shifts in the
35 direction of PSF processes in the presence of Guinea grass with positive feedback processes
36 appearing to reinforce invasion and negative feedback processes possibly contributing to species
37 coexistence in non-invaded, native grasslands. Future work is needed to determine the
38 mechanisms behind the observed shifts in PSF and further explore the role PSF has in Guinea
39 grass invasion.

40

41 Keywords: Guinea grass, *Megathyrsus maximus*, *Panicum maximum*, invasive species, South

42 Texas, grassland, plant-soil feedback, whole-soil inoculum

43 **Introduction**

44 Invasive species are an increasingly widespread concern due to their negative impacts on
45 ecosystems and difficulty in controlling their spread (Assessment 2005, Pyšek and Richardson
46 2010). Invasion by non-natives reduces plant diversity with extreme cases resulting in
47 monodominant plant stands and subsequent declines of wider biodiversity (Assessment 2005,
48 Dogra et al. 2010). At the ecosystem level, invasion disrupts nutrient cycling, disturbance
49 regimes, and microbial communities above- and belowground with some changes persisting for
50 decades (Hawkes et al. 2005, D'Antonio and Flory 2017). A difficulty we face in predicting and
51 preventing invasions is that the outcome of an introduction is largely context dependent varying
52 with initial plant density, life history, and dispersal traits of the invasive plant (Suding et al.
53 2013). To address this variability, more examples of invasion need to be studied to discern
54 overarching patterns and to inform management opportunities for distinct invasive species and
55 geographical locations.

56

57 In a process called plant-soil feedback (PSF), plants modify their soil environment via root
58 exudates and litter which can impact nutrient cycling and soil microbial communities (Bever
59 1994, Bennett and Klironomos 2019). In native grasslands, PSF has a largely negative or
60 stabilizing effect on plant growth which contributes to species coexistence and succession
61 through negative-density dependent processes (e.g. competition, pathogens, herbivory)
62 (Kulmatiski et al. 2008, Hawkes et al. 2013, Lekberg et al. 2018). Non-natives, if sufficiently
63 distinct from established plant species, can alter root microbial communities and decomposition
64 rates (Reinhart and Callaway 2006, Hawkes et al. 2013, Zhang et al. 2019, Fehmi et al. 2021).
65 These changes can impact subsequent plant growth reducing native plant establishment and

66 disrupt historical PSF processes in native communities (see Batten et al., 2006; Belnap et al.,
67 2005; Hawkes et al., 2005; Levine et al., 2006; Wolfe & Klironomos, 2005). PSF studies are
68 increasing in frequency, but only about 46% (32 of 69) of studies have looked at non-native
69 species; of these, 65% (21 of 32) focused on grasses with only 23 genera and 34 species within
70 Poaceae represented (Crawford et al. 2019). Although some species of Poaceae become effective
71 and widespread invaders, others fail to establish or establish locally, but are unsuccessful at
72 expanding their range. Non-native grasses that have been studied weaken negative PSFs that
73 dominate native grasslands indicating that this could be a contributing factor in invasion success
74 (Crawford et al. 2019), but studies on a wider range of non-native species, including both
75 noxious invaders and naturalized species, need to be conducted to understand this pattern and
76 what drives this shift.

77

78 In South Texas, Guinea grass, *Megathyrsus maximus* (Jacq.) B.L. Simon and Jacobs, is emerging
79 as a problematic invasive (CABI 2021). A perennial bunchgrass native to Africa, Guinea grass
80 has been introduced in tropical areas globally as a pasture grass due to its fast growth, high
81 biomass accumulation, and stress tolerance, but these same traits also make it a successful
82 invader (Rhodes et al. 2021a). For instance, the fast growth rates and high biomass accumulation
83 of Guinea grass results in displacement of many native species through direct competition for
84 space and resources (Ho et al. 2016). After senescence, native seedling germination is restricted
85 directly by a thick layer of Guinea grass litter (Rhodes et al. 2021a), but native plant regeneration
86 is also reduced in areas without a litter layer or where Guinea grass has been removed previously
87 (pers. obs). This could indicate factors other than direct shading are inhibiting native plant
88 germination and growth. A study conducted in Hawaii comparing establishment of natives from

89 seeds versus out-plantings after removal of Guinea grass found that field germination from seeds
90 was extremely low ranging from 0.5% to 2.3% (Ammond et al. 2013). Natives that were
91 transplanted into sites performed better, although there was still a high level of variation with
92 38% to 67% surviving (Ammond et al. 2013). Research into methods to control Guinea grass
93 invasion have focused on removal of Guinea grass with herbicides, burn treatments, and grazing
94 followed by reintroduction of natives from seeds or out-plantings, but results have been mixed
95 (Ramirez-Yanez et al. 2007, Ammond and Litton 2012, Ammond et al. 2013, Ellsworth et al.
96 2015). If Guinea grass does alter PSF processes toward a positive feedback for conspecifics, the
97 reestablishment of native plants could be hindered even when Guinea grass is removed prior to
98 plantings (Reinhart and Callaway 2006). To date, the effect of PSF on germination and growth of
99 plants from established seed banks is relatively, although soil microbes are known to impact seed
100 germination and survival which could slow recovery of native communities (Zalamea et al. 2015,
101 Sarmiento et al. 2017). Overall, aboveground contributions to the high competitive ability of
102 Guinea grass are well documented (Ammond and Litton 2012, Ho et al. 2016, D'Antonio and
103 Flory 2017, Rhodes et al. 2021b, 2021a), but little is known about how Guinea grass impacts
104 belowground processes (but see Chou & Young, 1975) and what role this may have in
105 facilitating invasion.

106

107 Our goals for this study were to assess the study system in South Texas for evidence of PSF in
108 native grasslands and grasslands invaded by Guinea grass specifically addressing the question:
109 how does the presence of invasive Guinea grass alter PSF compared to non-invaded grasslands?
110 We hypothesized that germination and growth of Guinea grass would be higher in soil from
111 invaded grasslands than soil from non-invaded grasslands due to an overall shift toward a

112 positive PSF in the presence of Guinea grass. In contrast, plants from the seedbank will not
113 experience a similar increase in germination and growth in soil from invaded grasslands possibly
114 due to inhibition by Guinea grass (Chou and Young 1975). In non-invaded, native grassland
115 soils, growth and germination of both native plants and Guinea grass will be lower than in soils
116 from invaded areas, but species richness of plants from the seedbank may be higher than in soils
117 from invaded sites due to the presence of negative PSF processes in native grasslands
118 (Kulmatiski et al. 2008; Hawkes et al. 2013; Lekberg et al. 2018). This is the first study to assess
119 PSF processes in South Texas savannas and to address whether Guinea grass may impact
120 historical PSF patterns. Our research provides a baseline for understanding the role of PSF in
121 Guinea grass invasion into native ecosystems.

122

123 **Methods**

124 To test our hypotheses, we conducted a greenhouse experiment that used soil collected in August
125 2020, from grasslands invaded by Guinea grass, *Megathyrus maximus*, and non-invaded
126 grasslands in Kleberg County, Texas (latitude: 27.433, longitude: -97.67). Here in its
127 unmanipulated state, grasses form the matrix of a savanna punctuated by clumps or mottes of
128 diverse shrubs and low trees dominated by mesquite (*Prosopis glandulosa*). The area receives
129 on average 73.6 cm of rain per year (U.S. Climate Data). Sampled grasslands were located
130 between 4 to 8 km apart spanning an area of approximately 5.5 km². Soil from the three sites
131 sampled in this study was composed predominantly of sand (mean 92% ± 1.8%) with minor
132 amounts of silt and clay (mean 5.7% ± 0.8% and 2.3% ± 1.5%, respectively). Two of the sample
133 sites were in grasslands that had remained intact at least since the 1980's, while the third site had

134 been mechanically treated in 2000 to partially remove encroaching mesquite (Supplementary
135 Fig. S1). These grasslands are grazed annually with occasional prescribed burns.

136

137 *Sampling and experimental design*

138 Within each of three sites, we sampled soil from plots invaded by Guinea grass and non-invaded
139 plots (i.e., predominantly native with no Guinea grass present) that were located within 10 m of
140 each other to minimize the confounding effects of distance on soil microbial communities or soil
141 traits (Supplementary Fig. S1). We collected two sets of soil from invaded and non-invaded sites:
142 a) bulk soil for use as the growth medium and b) soil for use as additional inoculum. For both
143 sets of soil, we removed the litter layer and excavated the soil using a hand trowel to a depth of
144 15 cm. Bulk soil was collected from two locations in each plot. For the additional inoculum, we
145 collected five soil cores from each plot with individual cores located approximately 1 m apart.
146 Additional inoculum soil was collected individually in plastic bags and stored in a 4°C fridge.
147 Bulk soil (hereafter referred to as whole-soil inoculum) was stored at room temperature in a
148 climate-controlled building (~20-22°C). Within one week of collection, we sieved all the soil
149 (i.e., whole soil inoculum and additional inoculum) using a 2 mm soil sieve to remove leaf litter
150 and plant roots. Between each use, the sieve was sterilized with 0.5% NaOCl for five minutes,
151 washed with tap water, and allowed to air dry.

152

153 For our experiment, we chose to use whole-soil inoculum due to concerns that autoclaving
154 impacts soil nutrient availability and composition/abundance of microbial communities. To
155 confirm the effect autoclaving has on soil nutrient availability, we conducted a small assessment
156 on soil nutrients in the whole-soil inoculum pre-autoclaving and after two autoclave times (30

157 minutes and 60 minutes). We found that autoclaving increased levels of phosphorus, sulfur,
158 sodium, and electrical conductivity with autoclave time (ANOVA results in Supplementary
159 Table S1, also see Skipper and Westermann 1973; Tuominen et al. 1994). Studies on the effect
160 of autoclaving on microbial communities demonstrate that sterilization is incomplete with a
161 subset of the fungal and bacterial communities persisting (Skipper and Westermann 1973,
162 Tuominen et al. 1994, Bárcenas-Moreno et al. 2011). Therefore, we chose not to autoclave the
163 soil to limit the influence of these confounding factors (see **Discussion**).

164

165 Since we were unable to refrigerate the whole-soil inoculum due to its large quantity, we added
166 inoculum that was kept at 4°C to counter any changes in the microbial community in the whole-
167 soil inoculum. For this, we created two sets of additional inoculums: a pooled inoculum referred
168 to hereafter as a mixed soil sample (MSS) and an unpooled inoculum referred to as individual
169 soil sample (ISS). To create the MSS inoculum, we pooled inoculum based on soil origin
170 (invaded or uninvaded grasslands) for each of the three sites to create a common inoculum that
171 was applied to replicates (n = 6 inoculum pools used for MSS treatments). For ISS inoculum, we
172 used distinct (i.e., unpooled) soil cores for each replicate.

173

174 For the experiment, treatments included soil origin (invaded grassland, non-invaded grassland)
175 and soil handling method (ISS, MSS). Each cross was replicated five times with soil from three
176 separate sites (20 samples per site, 60 samples total). We filled black plastic pots (2.5 quarts)
177 with the same amount of unautoclaved whole-soil inoculum (2640 g) and then added the
178 additional soil inoculum (3% mass : mass) to each pot (79.2 g) (Van Der Putten et al. 2007b).
179 Pots were randomized in the greenhouse to account for variation in temperature and lighting. We

180 matched the whole-soil inoculum and the additional inoculum by soil origin (site and invasion
181 status), i.e. MSS and ISS inoculum treatments from invaded sites were added to bulk soil also
182 from the same invaded site. Soil samples from each of the treatments were submitted for nutrient
183 analysis at the Texas A&M AgriLife Extension Service Soil, Water, and Forage Testing
184 Laboratory. Soils were analyzed for pH, nitrate, phosphorus, potassium, electrical conductivity,
185 calcium, magnesium, sodium, and sulfur (Schofield and Taylor 1955, Mehlich 1984, Rhoades
186 1984).

187

188 In each pot, we sowed approximately 0.015 g of Guinea grass seed (approximately 15 seeds)
189 collected from the same area and time in South Texas. Although we were unable to quantify the
190 seed bank, we standardized the amount of soil that went into each pot to normalize the seed
191 bank. During the sieving process, we homogenized the whole-soil inoculum based on site and
192 soil origin as described above, then placed the same amount of whole-soil inoculum and
193 additional inoculum as stated above into each pot. We visually assessed the sieved litter for seeds
194 to assess whether larger seeds were removed during soil sieving (i.e. size sorting of seeds), but
195 noted only plant leaves and roots in the material removed during sieving.

196

197 *Germination and growth of Guinea grass*

198 After three weeks, we counted the total number of Guinea grass seedlings and thinned them to a
199 single seedling per pot. We did not normalize Guinea grass seedling number as the number of
200 seeds put into each pot was normalized by weight (see *Sampling and experimental design*). We
201 monitored growth of these seedlings over the course of the experiment (14 weeks), after which
202 plants were carefully removed from pots to keep as much of the root intact as possible. We

203 measured the plant height at the end of the experiment, then separated the aboveground tissue
204 from roots at the root collar and placed both in a drying oven at 65°C for 3-5 days in labeled
205 paper bags. We measured the dry weight of both above- and below-ground tissue.

206

207 *Germination and growth of seed bank*

208 Plants germinating from the seed bank were monitored in the same pots as Guinea grass. We
209 monitored the total number of plant seedlings sprouting from the seed bank weekly. At the end
210 of the experiment, we counted the number of plants present within each pot noting how many
211 were monocots and dicots. We were unable to identify seedlings to species as the plants were
212 juveniles and did not have flowering structures. Therefore, to quantify species richness, we used
213 phenotypic differences to distinguish morphospecies within each pot (hereafter, referred to as
214 species richness). To measure dry weight (total biomass) of the seedbank community, we placed
215 above- and below-ground tissue in drying ovens at 65°C for five days before weighing.

216

217 *Statistical analyses*

218 All statistical analyses were conducted in R and code is available for reproducibility (see **Code**
219 **availability**). To assess the effect of soil origin (invaded or uninvaded grasslands) and soil
220 handling method on Guinea grass growth and germination, we used a mixed effect model to
221 analyze germination, height, root length, and dry biomass. We treated soil origin and soil
222 handling method as fixed variables and site as a random variable. We considered Guinea grass
223 germination as the total number of seedlings and did not normalize this number as we used the
224 same mass of seeds (0.015 g) per pot. We evaluated all data for normality and homogeneity of

225 variance prior to analysis. Germination, height, and biomass data were log-transformed prior to
226 analysis. Three pots had no Guinea grass growth and were removed from analyses.

227

228 The effect of soil origin and soil handling method on germination and growth of the seedbank

229 plant community was also assessed using mixed-effects models as above. Here we also treated

230 germination as the total number of seedlings that germinated as the amount of whole-soil

231 inoculum and additional inoculum used was the same across all treatments and replicates. As

232 above, all data were assessed to see if they met the assumptions for parametric analysis.

233 Germination counts and plant abundance were log-transformed prior to analysis, whereas species

234 richness and biomass were transformed using the formula $\log(x + 1)$.

235

236 To assess for differences in soil characteristics as a function of invasion, we used a t-test and

237 included only data from unautoclaved soil (n = 6 samples; 3 from invaded sites and 3 non-

238 invaded sites). Electrical conductivity, phosphorus, and sulfur were log transformed prior to

239 analysis.

240

241 **Results**

242 *Effect of soil origin (invaded and non-invaded grasslands)*

243 We found a significant difference in Guinea grass growth between invaded and non-invaded sites

244 (Fig. 1; Table 1). Height, root length, and biomass of Guinea grass were higher when grown in

245 soil from invaded sites (height: 46.6 cm \pm 17.4; root length: 14.1 cm \pm 4.2; biomass: 0.8 g \pm 0.7)

246 versus non-invaded sites (height: 22.1 cm \pm 8.3; root length: 9.8 cm \pm 4.2; biomass: 0.01 g \pm

247 0.1). Germination of Guinea grass did not differ in invaded or non-invaded soil (Table 1), but

248 germination within the first week was higher in soil from invaded sites ($F_{1,54} = 6.86, p = 0.0114$;
 249 Supplementary Fig. S2). Within the first week, average germination of Guinea grass in invaded
 250 soil was 4.1 ± 4.4 seedlings compared to 1.9 ± 2.3 in soil from non-invaded sites.

251

252 Table 1: Results of ANOVA mixed effect model to assess the effect of soil origin (invasion
 253 status) and soil handling method on Guinea grass germination and growth. Seedling count here is
 254 the total seedling number of seedlings in the first three weeks.

	Soil origin		Soil handling method		Interaction	
	$F_{1,51}$	p	$F_{1,51}$	p	$F_{1,51}$	p
Seedling count	0.71	0.4057	7.20	0.0098	2.44	0.1245
Height	38.60	< 0.0001	0.98	0.3278	0.00	0.9789
Root length	14.55	0.0004	0.20	0.6601	0.39	0.5350
Biomass	31.22	< 0.0001	0.08	0.7852	0.03	0.8740

255

256 Plant abundance and species richness of plants from the seed bank were significantly higher in
 257 soil from non-invaded sites than invaded sites (Fig. 2; Table 2). Average plant abundance was 54
 258 ± 21 in non-invaded soil and 41 ± 17 in invaded soil, and average species richness was 8 ± 2
 259 species versus 6 ± 2 , respectively. Seedling germination overall was higher in non-invaded sites
 260 (Fig. 2; Table 2), but when we looked at seedling germination within the first week, we found
 261 that seedling germination was initially higher in soil from invaded sites ($F_{1,54} = 32.74, p <$
 262 0.0001 ; Supplementary Fig. S3). The seedbank community had higher total biomass (mean 1.3 g
 263 ± 0.4) in soil from invaded sites than non-invaded sites (mean $0.6 \text{ g} \pm 0.3$) (Fig. 2; Table 2).

264 When we broadly separating plants from the seedbank into dicots and monocots, we found that
 265 monocots had significantly higher species richness and abundance in soil from non-invaded sites

266 than invaded sites (species richness: Kruskal-Wallis $X_1^2 = 13.4, p = 0.0002$; plant abundance:
 267 Kruskal-Wallis $X_1^2 = 18.1, p < 0.0001$; Fig. 3). Dicots showed no difference.

268

269 Table 2: Results of ANOVA mixed effect model to assess the effect of soil origin (invasion
 270 status) and soil handling method on germination and growth of plants from the seedbank.

271 Seedling count here is the total seedling number of seedlings in the first three weeks.

	Soil origin		Soil handling method		Interaction	
	F _{1,54}	p	F _{1,54}	p	F _{1,54}	p
Seedling count	49.31	< 0.0001	0.14	0.7116	4.52	0.0382
Plant abundance	9.05	0.004	5.57	0.0219	3.09	0.0843
Total biomass	51.65	< 0.0001	4.26	0.0439	0.10	0.7539
Species richness	4.52	0.0382	0.02	0.8877	0.08	0.7850

272

273 *Effect of soil handling method*

274 Guinea grass germination, seedbank plant abundance, and total biomass of the seedbank plant
 275 community showed significant differences between the two soil handling methods we tested.
 276 Soil handling method significantly influenced Guinea grass germination with MSS treatments
 277 having higher germination (mean 21 ± 15) than ISS treatments (mean 12 ± 11) (Supplementary
 278 Fig. S4; Table 1). Within the seedbank plant community, plant abundance and total biomass
 279 were higher in ISS treatments (plant abundance: mean 53 ± 21 ; total biomass: mean 1.04 ± 0.5)
 280 than MSS (plant abundance: mean 43 ± 17 ; total biomass 0.85 ± 0.5) (Fig. 2; Table 2).

281

282 *Soil nutrients*

283 We found that no significant difference between soil nutrients in invaded and non-invaded sites,
284 although some nutrients trended higher in invaded sites (Fig. 4; Supplementary Table S2).

285

286 **Discussion**

287 We conducted an observational study to compare the effect of soil from Guinea grass invaded
288 and non-invaded, native grasslands on the germination and growth of Guinea grass, as well as
289 plants emerging from the seed bank. Our experiment presents novel data on PSF processes in the
290 mesquite savannas in South Texas, the impact of Guinea grass invasion on PSF in native
291 grasslands, and the response of seedbanks to shifts in PSF. We found that, consistent with our
292 hypothesis, soil from grasslands already invaded by Guinea grass had a positive effect on
293 conspecific growth with plants growing taller and accumulating more biomass than Guinea grass
294 grown in soil from non-invaded grasslands (Fig. 1, Table 1). In contrast, plants germinating from
295 the seed bank had higher species richness (delimited based on plant morphology) and abundance
296 in soil from non-invaded grasslands. The observed decrease in species richness and higher
297 biomass accumulation of plants from the seedbank in soil from invaded grasslands could indicate
298 a release from negative PSF processes in non-invaded grasslands (Fig. 2, Table 2). Interestingly,
299 we found evidence of a broad phylogenetic signal in the response of monocots and dicots to
300 invaded and non-invaded soil (Fig. 3) indicating that Guinea grass may have a stronger negative
301 impact on more closely related plant species. These results suggest the presence of distinct
302 patterns of PSF in invaded and non-invaded grasslands in South Texas with evidence of positive
303 PSF on Guinea grass in invaded grasslands and an overall negative PSF in non-invaded, native
304 grasslands. Although we did not condition soil under controlled conditions making it difficult to
305 assign the difference in the direction of PSF to the presence or absence of Guinea grass, the low

306 spatial distance between the invaded and non-invaded grasslands we sampled suggests a minor
307 role of environmental factors, such as precipitation and temperature, in driving these differences.

308

309 For a non-native to be a successful invader, it needs to be able to colonize, establish, and
310 disseminate to new environments (Theoharides and Dukes 2007). During colonization, seed
311 germination requires both an appropriate climate and soil conditions, such as texture, nutrients,
312 and microbial community (Theoharides and Dukes 2007, Sarmiento et al. 2017). Despite no
313 difference in climate or soil texture, and no statistical difference in soil nutrients, we observed
314 faster initial germination of Guinea grass (Supplementary Fig. S2) and the seed bank
315 (Supplementary Fig. S3) in invaded soil possibly indicating an effect of the soil microbial
316 community on germination. For instance, a low abundance of seed pathogens in the soil can
317 release seeds from negative density dependence processes found in native grasslands (Gilbert
318 and Parker 2006, Halbritter et al. 2012). Ultimately, germination from the seedbank was higher
319 in native grasslands which could indicate higher propagule pressure in these sites. It would be
320 expected that with increasing invasion time, there would be a decrease in native seeds in the
321 seedbank (Robertson and Hickman 2012), but we expect this difference to be small as our
322 invaded and non-invaded plots bordered each other (within 10 m) indicating a relatively short
323 time since invasion and allowing for the introduction of seeds from nearby non-invaded areas.

324

325 Successful establishment of non-native plants is reliant on their fast growth rate, competitive
326 ability with native plants, and efficient resource usage (Theoharides and Dukes 2007). The fast
327 growth rate of Guinea grass has been noted (Rhodes et al. 2021a), but here we show that the
328 presence of Guinea grass in already invaded areas further increases its growth and biomass

329 accumulation. This result in combination with the observed higher biomass of plants from the
330 seedbank in soil from invaded grasslands suggests that either the microbial community or soil
331 nutrients play a role in re-enforcing invasion. Although soil nutrients were marginally higher in
332 invaded soil than in non-invaded soil (Fig. 4, Supplementary Table S2), these differences were
333 not statistically significant. As even small differences could still be biologically significant, the
334 effect of soil nutrients as a possible contributor warrants deeper exploration. Invasion is
335 generally found to be associated with shifts in nutrient availability and cycling (reviewed in
336 Ehrenfeld 2003; Sardans et al. 2017). Non-native species can alter nutrient cycling by releasing
337 nitrogen from their litter faster than natives and thereby increasing soil nitrogen availability for
338 themselves and co-occurring natives (Allison and Vitousek 2004).

339

340 Invasion has been shown to impact soil microbial communities through multiple pathways (e.g.
341 phytochemicals, litter inputs) altering community processes (reviewed in Wolfe and Klironomos
342 2005, Reinhart and Callaway 2006, Van Der Putten et al. 2007a). Shifts in soil communities by
343 invasive species can indirectly cause alterations in nutrient cycling by supporting decomposers
344 and rhizosphere mutualists (Zhang et al. 2019). Plant-associated microbes have also been found
345 to impact invasion success. For instance, microbial mutualists can directly influence the ability
346 of non-natives to invade native ecosystems (Rudgers et al. 2005). Additionally, the enemy
347 release hypothesis posits that movement of plants to novel environments causes a decrease in
348 negative pressures from pathogen, herbivores, and parasites found in their home range (Keane
349 and Crawley 2002). The observed effect of our soil handling methods supports a possible
350 difference in the microbial communities in invaded and non-invaded grasslands. Guinea grass
351 germination was higher in MSS treatments than ISS, but abundance and biomass of plants from

352 the seedbank was higher in ISS than MSS (Supplementary Fig. S4). These results could indicate
353 that the native plant community is influenced strongly by localized soil microbial communities
354 that are overwhelmed by the wider community upon pooling (e.g. due to rareness or competitive
355 ability) (Batten et al. 2006, Mummey and Rillig 2006, Rúa et al. 2016). Although we did not
356 assess microbial community composition in this study, differences between the soil handling
357 methods could be influenced by shifts in the relative abundance of particular community
358 members after pooling inocula or specificity of PSF effects on native versus non-native plant
359 species (Pernilla Brinkman et al. 2010, van de Voorde et al. 2012). As part of ongoing research,
360 we are evaluating soil microbial communities, litter decomposition rates, and levels of soil
361 nutrients across invasion and disturbance types to assess changes in the soil environment more
362 fully.

363

364 Invasion of non-native plants may cause cascading effects on conspecific native species through
365 allelopathy as examples of the novel weapons hypothesis (Callaway and Ridenour 2004).
366 Allelochemicals can directly impact fitness of native species or indirectly through conditioning
367 of the soil microbial community, such as has been shown with *Centaurea diffusa* (Callaway et al.
368 2004, Wolfe and Klironomos 2005). Allelopathic effects have been found to decrease with
369 increasing phylogenetic distance (Zhang et al. 2021), such that species of monocots should be
370 more negatively impacted than dicots in invaded grasslands. When we assessed differences in the
371 effect of PSF on monocots and dicots, monocot species richness and plant abundance were
372 higher in soil from non-invaded sites than invaded sites, whereas dicots showed no difference
373 (Fig. 3). Although overall PSF in soil from invaded grasslands was positive, these results
374 indicate that in invaded grasslands monocot species that are more closely related to Guinea grass

375 phylogenetically experience negative feedback. These results raise questions that we will test in
376 the future, such as whether negative PSF is driving species coexistence in non-invaded
377 communities, whether the switch to an overall positive feedback mechanism in invaded
378 grasslands is due to nutrient availability, and whether allelopathy or pathogen accumulation is
379 suppressing other monocot species post-invasion. In ongoing research, we aim to parse out the
380 effect of Guinea grass invasion on soil nutrients, allelopathy, and soil microbial communities to
381 better understand how Guinea grass impacts PSF processes and how this varies across the
382 heterogeneous landscapes of South Texas.

383

384 **Conclusions and future directions**

385 We found evidence for strong differences in PSF as a function of invasion with negative PSF in
386 non-invaded, native grasslands and positive PSF in grasslands invaded by Guinea grass.

387 Negative PSFs in non-invaded grasslands were associated with higher species richness and
388 abundance of the native plant community possibly contributing to species coexistence in native
389 grasslands. We found evidence to suggest that positive PSFs observed in invaded grasslands are
390 due to a combination of increased nutrient availability and a release of allelopathic chemicals by
391 Guinea grass which could be reinforcing establishment of Guinea grass, although the
392 contribution of each needs to be explored further. Our results represent the first time PSF
393 processes have been studied in South Texas savannas and show how Guinea grass, an emerging
394 invasive within the southern United States, influences these processes reinforcing its own
395 invasion.

396

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406

407 **Code availability** In order to support open science and data reproducibility, all data and
408 scripts used for analyses are available in the eabowman/Bowmanetal-STexasGuineaGrass-
409 PlantSoilFeedback repository on GitHub, [Zenodo permanent link].

410

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 572

573 **Figure legends:**

574

575 **Fig. 1** Guinea grass height (a), root length (b), and biomass (c) when grown in soil collected
576 from i) grassland invaded by conspecifics and ii) non-invaded grasslands dominated by native
577 species. All data shown are non-transformed.

578

579 **Fig. 2** Seedling count (a), abundance (b), biomass (c), and species richness (d) of native plant
580 community when grown in soil from Guinea grass invaded and non-invaded grasslands. All data
581 shown are non-transformed.

582

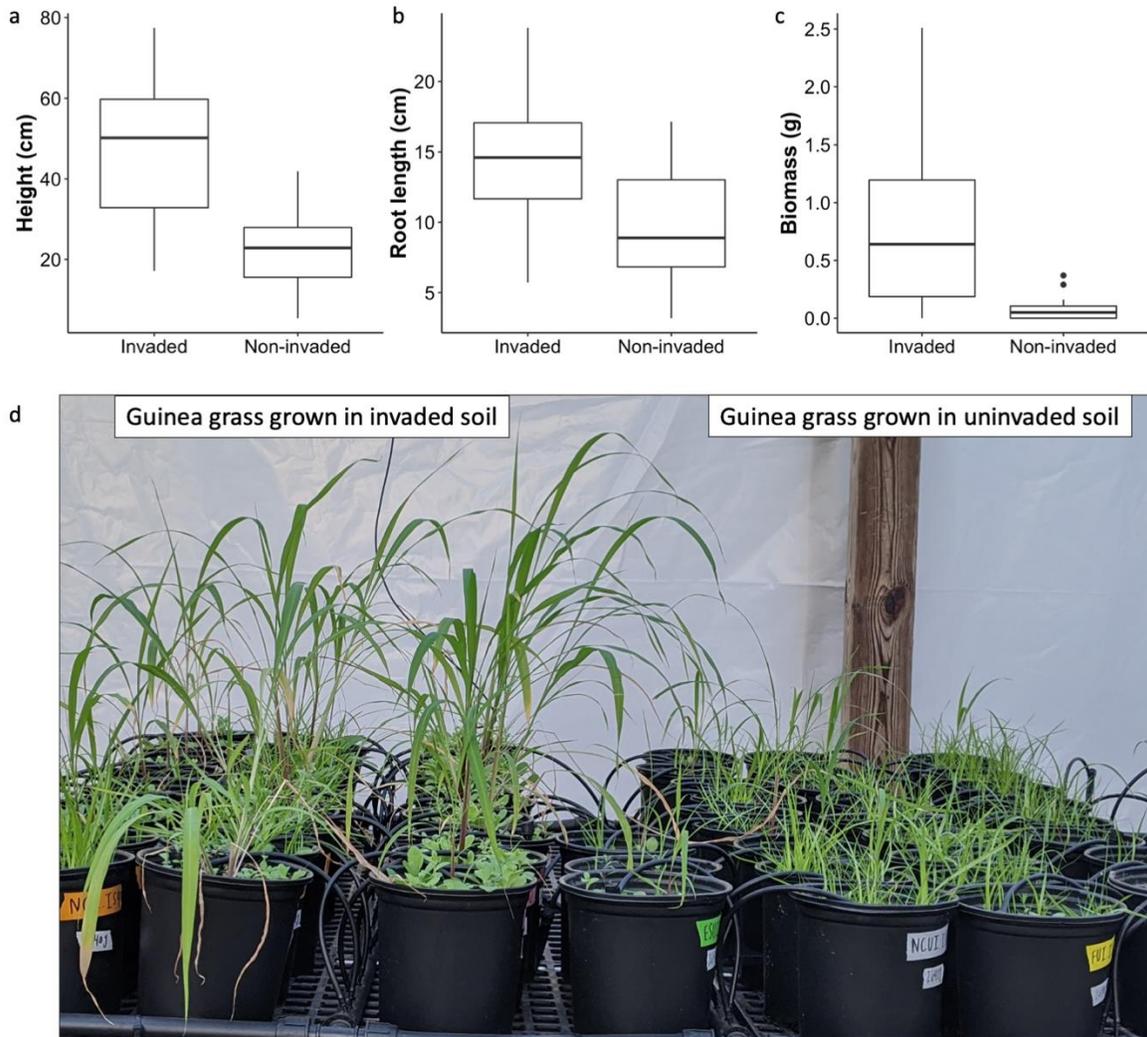
583 **Fig. 3** Plant abundance (a) and species richness (b) as a function of invasion and plant group.
584 Monocot species richness and plant abundance were significantly higher in soil from non-
585 invaded sites than invaded sites (species richness: Kruskal-Wallis $X_1^2 = 13.4$, $p = 0.0002$; plant
586 abundance: Kruskal-Wallis $X_1^2 = 18.1$, $p < 0.0001$), whereas species richness and abundance of
587 dicots showed no difference. All data shown here are non-transformed.

588

589 **Fig. 4** Soil characteristics as a function of invasion. None of the soil characteristics were
590 significantly different based on soil origin although in general soil nutrients and characteristics
591 were higher in soil from invaded sites. All data shown here are non-transformed. EC is electrical
592 conductivity.

593

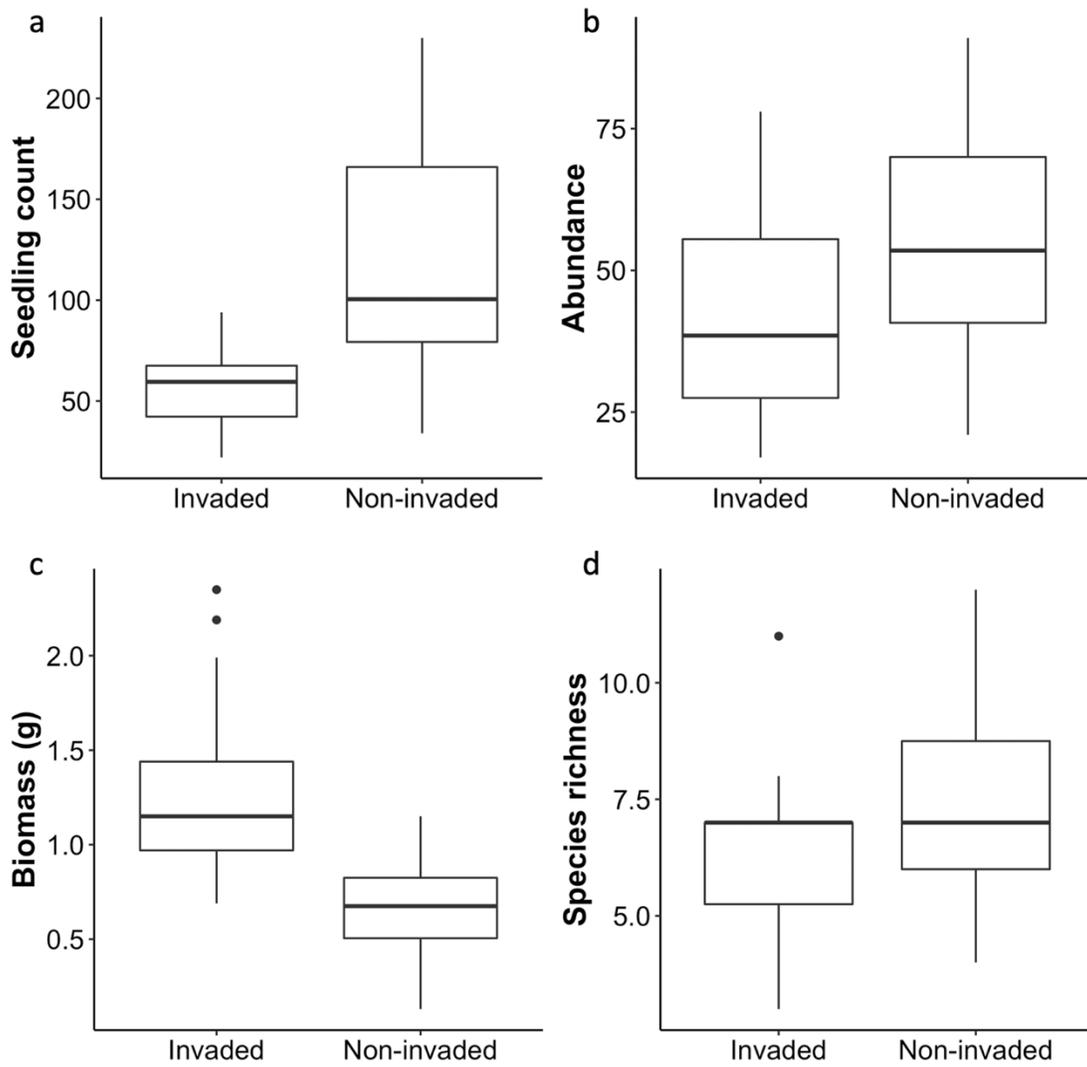
594 **Figure 1**
595



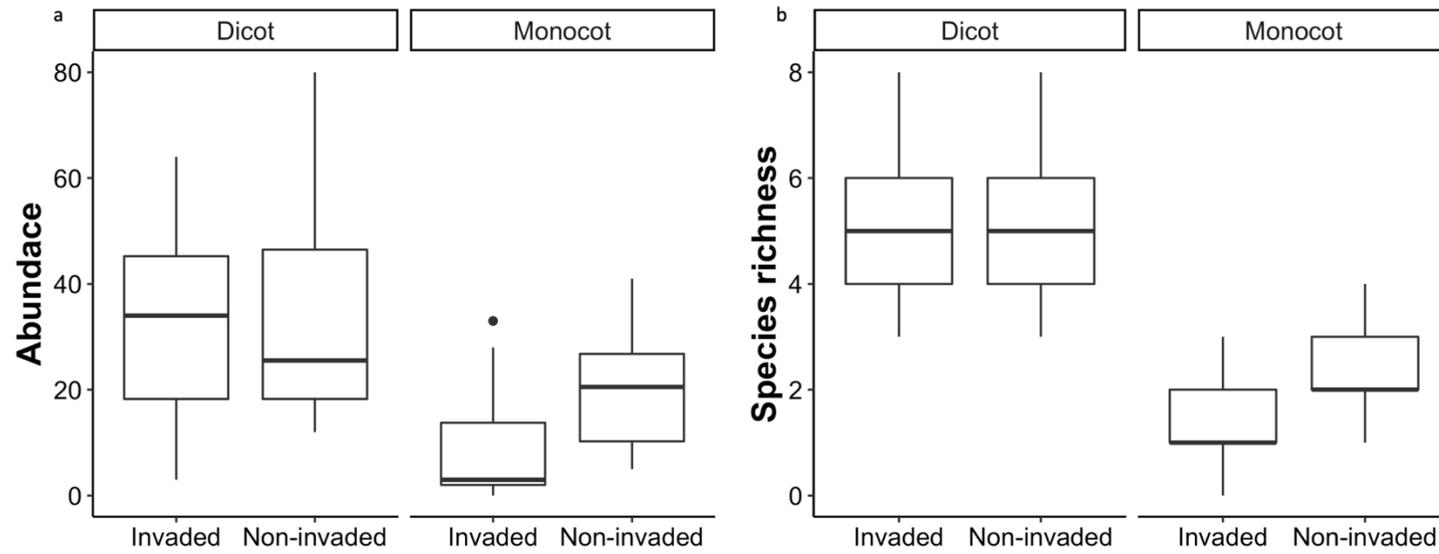
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597

598 **Figure 2**

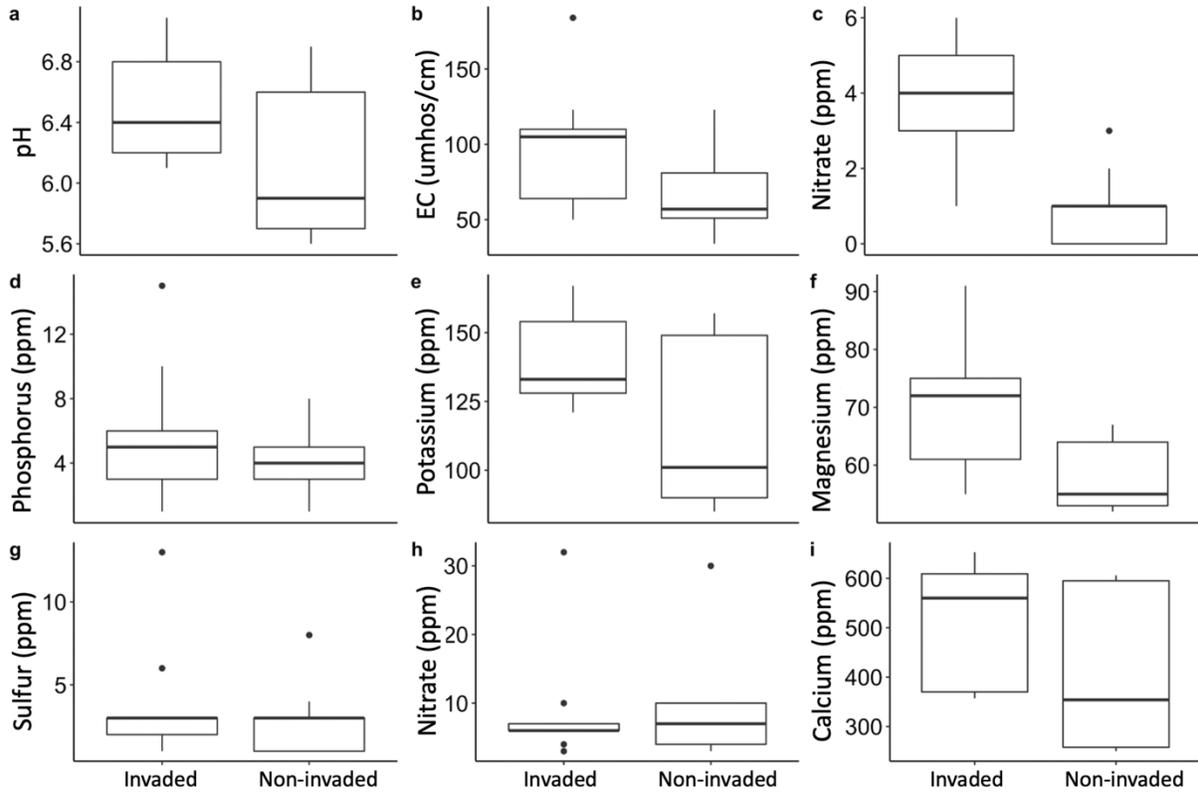


600 **Figure 3**
601



602

603 **Figure 4**
604



605

606 **Supplementary figure legends:**

607

608 **Supplementary Fig. S1** Soil sampling sites showing extent of Guinea grass patch (white
609 boundary, I) and adjacent native grassland (N) with nearby mesquite tree mottes. Google Earth
610 Imagery date 1/13/2014. Scale bar 70m.

611

612 **Supplementary Fig. S2** Initial germination of Guinea grass seed during week 1 was higher in
613 soil from invaded sites than non-invaded sites. All data shown here are non-transformed.

614

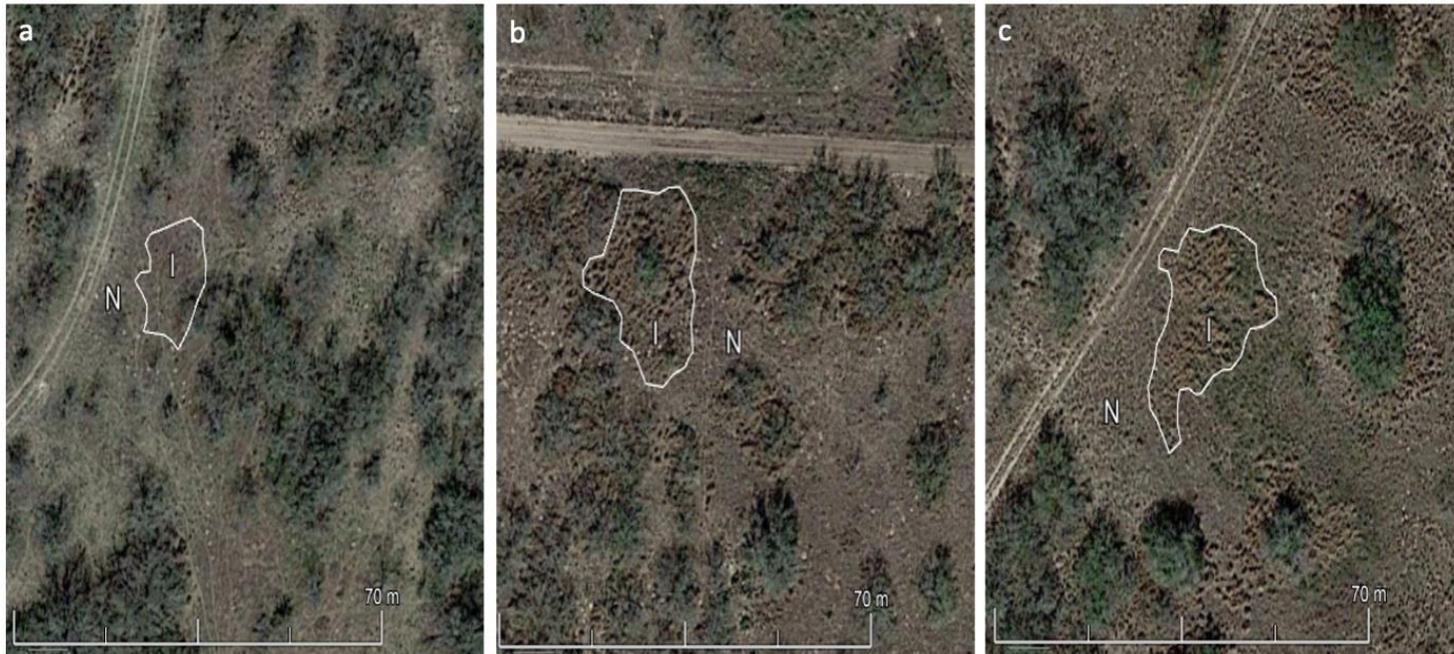
615 **Supplementary Fig. S3** Initial germination of the seed bank during week 1 was higher in soil
616 from invaded sites than non-invaded sites. All data shown here are non-transformed.

617

618 **Supplementary Fig. S4:** Effect of soil handling method on Guinea grass seedling count (a),
619 native community plant abundance (b), and native community biomass (c). MSS: mixed soil
620 sampling; ISS: individual soil sampling. All data shown are non-transformed.

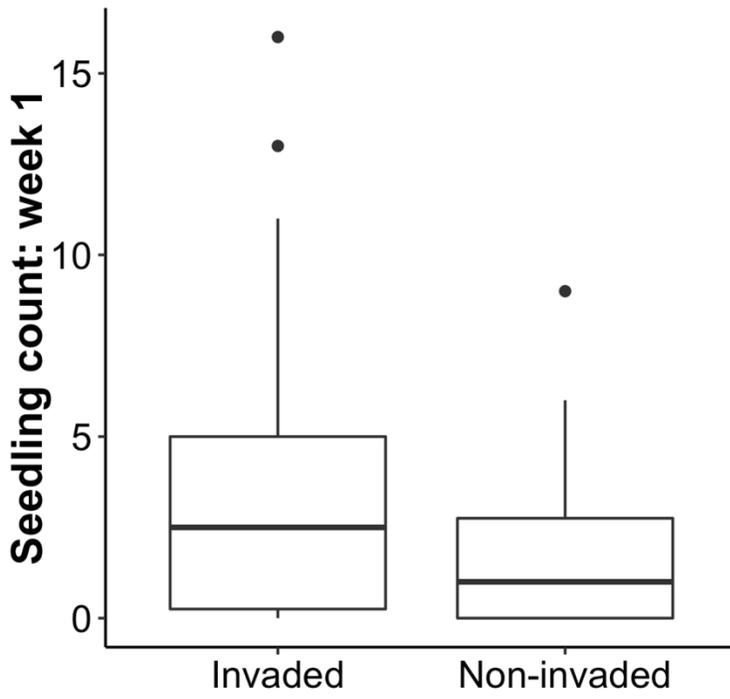
621

622 **Supplementary Figure S1**
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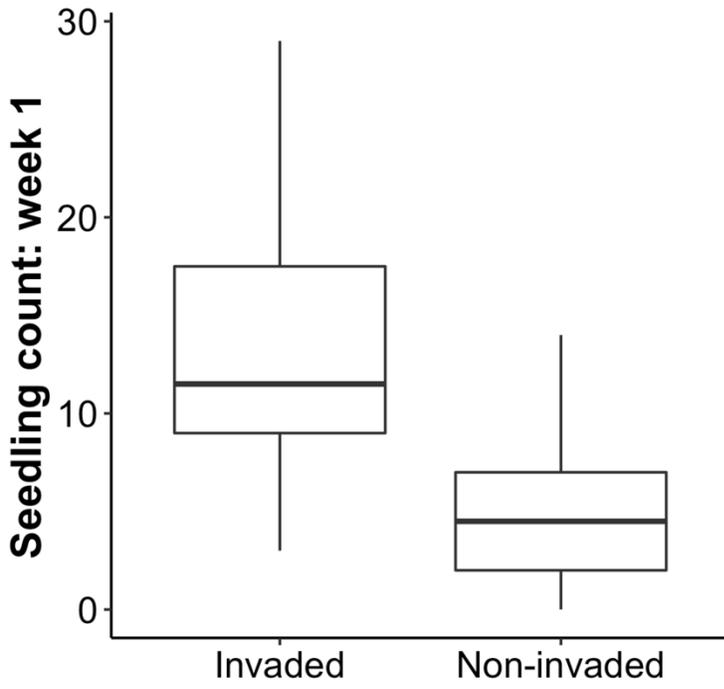
625

626 **Supplementary Figure S2**



627
628

629 **Supplementary Figure S3**

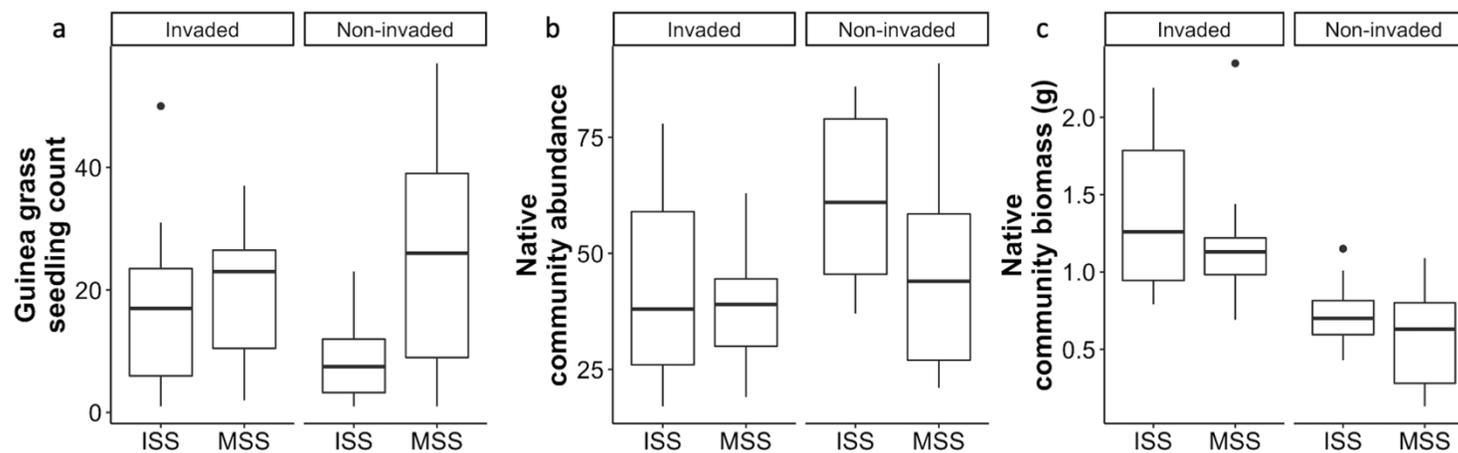


630

631 **Supplementary Figure S4**

632

633



634

635 **Supplementary tables**

636

637 **Supplementary Table S1:** Results of one-way ANOVA examining the effect of autoclave time
638 on soil characteristics. Electrical conductivity, phosphorus, and sulfur were log-transformed prior
639 to analysis.

640

641 **Supplementary Table S2:** Results of t-test examining differences in soil characteristics between
642 invaded and non-invaded sites. Electrical conductivity, phosphorus, and sulfur were log-
643 transformed prior to analysis.

644

645 **Supplementary Table S1**
646

Soil characteristics	F-statistic	<i>p</i>
pH	$F_{1,16} = 0.35$	0.5648
Electrical conductivity	$F_{1,16} = 12.08$	0.0031
Nitrate	$F_{1,16} = 0.02$	0.8937
Phosphorus	$F_{1,16} = 13.83$	0.0019
Potassium	$F_{1,16} = 0.13$	0.7279
Magnesium	$F_{1,16} = 0.92$	0.3513
Sulfur	$F_{1,16} = 29.28$	0.0001
Sodium	$F_{1,16} = 7.92$	0.0125
Calcium	$F_{1,16} = 0.01$	0.9277

647

648 **Supplementary Table S2**

649

Soil characteristics	t-statistic	df	<i>p</i>	Invaded	Non-invaded
pH	0.93	3.75	0.409	6.4 ± 0.4	6.0 ± 0.5
Electrical conductivity	1	2.72	0.3975	58.0 ± 7.6	49.0 ± 14.1
Nitrate	1.88	2.81	0.1635	3.7 ± 2.5	0.7 ± 1.2
Phosphorus	0.38	3.32	0.7288	3.0 ± 2.7	2.0 ± 1.0
Potassium	0.85	3.59	0.448	137.0 ± 25.7	115.0 ± 36.7
Magnesium	1.67	2.98	0.1945	67.3 ± 10.8	55.7 ± 5.5
Sulfur	1	2	0.4226	1.3 ± 0.6	1.0 ± 0.0
Sodium	0.71	2.56	0.5384	4.3 ± 1.5	3.7 ± 0.6
Calcium	0.87	3.91	0.4348	517.3 ± 149.5	402.3 ± 173.6

650