

**PREPRINT**

*Author-formatted, not peer-reviewed document posted on 01/06/2022*

DOI: <https://doi.org/10.3897/arphapreprints.e86933>

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

 Elizabeth Bowman, Robert Plowes, Lawrence Gilbert

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

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

Elizabeth A. Bowman<sup>\*1</sup>, Robert M. Plowes<sup>1</sup>, and Lawrence E. Gilbert<sup>1</sup>

<sup>1</sup>Brackenridge Field Laboratory, University of Texas at Austin, 2907 Lake Austin Blvd, Austin, TX 78703, United States

\*Corresponding author [eabowman@utexas.edu](mailto:eabowman@utexas.edu), Brackenridge Field Laboratory, Room 105, 2907 Lake Austin Blvd., Austin, TX 78703, United States

Elizabeth A. Bowman ORCID: 0000-0002-9631-3559

## 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

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

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

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

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

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

## Methods

To test our hypotheses, we conducted a greenhouse experiment that used soil collected in August 2020, from grasslands invaded by Guinea grass, *Megathyrsus maximus*, and non-invaded grasslands in Kleberg County, Texas (latitude: 27.433, longitude: -97.67). Here in its unmanipulated state, grasses form the matrix of a savanna punctuated by clumps or mottes of diverse shrubs and low trees dominated by mesquite (*Prosopis glandulosa*). The area receives on average 73.6 cm of rain per year (U.S. Climate Data). Sampled grasslands were located between 4 to 8 km apart spanning an area of approximately 5.5 km<sup>2</sup>. Soil from the three sites sampled in this study was composed predominantly of sand (mean 92% ± 1.8%) with minor amounts of silt and clay (mean 5.7% ± 0.8% and 2.3% ± 1.5%, respectively). Two of the sample sites were in grasslands that had remained intact at least since the 1980's, while the third site had



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

### *Sampling and experimental design*

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

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

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

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

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

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

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

#### *Germination and growth of Guinea grass*

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

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

### *Germination and growth of seed bank*

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

### *Statistical analyses*

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

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

The effect of soil origin and soil handling method on germination and growth of the seedbank plant community was also assessed using mixed-effects models as above. Here we also treated germination as the total number of seedlings that germinated as the amount of whole-soil inoculum and additional inoculum used was the same across all treatments and replicates. As above, all data were assessed to see if they met the assumptions for parametric analysis. Germination counts and plant abundance were log-transformed prior to analysis, whereas species richness and biomass were transformed using the formula  $\log(x + 1)$ .

To assess for differences in soil characteristics as a function of invasion, we used a t-test and included only data from unautoclaved soil ( $n = 6$  samples; 3 from invaded sites and 3 non-invaded sites). Electrical conductivity, phosphorus, and sulfur were log transformed prior to analysis.

## Results

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

We found a significant difference in Guinea grass growth between invaded and non-invaded sites (Fig. 1; Table 1). Height, root length, and biomass of Guinea grass were higher when grown in soil from invaded sites (height:  $46.6 \text{ cm} \pm 17.4$ ; root length:  $14.1 \text{ cm} \pm 4.2$ ; biomass:  $0.8 \text{ g} \pm 0.7$ ) versus non-invaded sites (height:  $22.1 \text{ cm} \pm 8.3$ ; root length:  $9.8 \text{ cm} \pm 4.2$ ; biomass:  $0.01 \text{ g} \pm 0.1$ ). Germination of Guinea grass did not differ in invaded or non-invaded soil (Table 1), but

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

Table 1: Results of ANOVA mixed effect model to assess the effect of soil origin (invasion status) and soil handling method on Guinea grass germination and growth. Seedling count here is 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

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

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

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

### *Effect of soil handling method*

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

### *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



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

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

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

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

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

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

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

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

### **Conclusions and future directions**

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

### **Acknowledgements**

398 L. Miksch for assistance with the experiment and comments on the manuscript; A. Leo and A.  
 399 Rhodes for comments on manuscript. The Lee and Ramona Bass Foundation for funding and B.  
 400 DuPont, J. Rutledge and E. Grahmann for arranging access and providing insights into the  
 401 Guinea grass invasion of the study area.

402

403 **Funding** Funding for this project was provided by the Lee and Ramona Bass foundation.

404

405 **Competing interests** The authors declare no competing financial interests.

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

## References

- Allison SD, Vitousek PM (2004) Rapid nutrient cycling in leaf litter from invasive plants in Hawai'i. *Oecologia* 141: 612–619. <https://doi.org/10.1007/s00442-004-1679-z>
- Ammond SA, Litton CM (2012) Competition between Native Hawaiian Plants and the Invasive Grass *Megathyrsus maximus*: Implications of Functional Diversity for Ecological Restoration. *Restoration Ecology* 20: 638–646. <https://doi.org/10.1111/j.1526-100X.2011.00806.x>
- Ammond SA, Litton CM, Ellsworth LM, Leary JK (2013) Restoration of native plant communities in a Hawaiian dry lowland ecosystem dominated by the invasive grass *Megathyrsus maximus*. *Applied Vegetation Science* 16: 29–39. <https://doi.org/10.1111/j.1654-109X.2012.01208.x>
- Anon (2021) U.S. Climate Data.
- Assessment ME (2005) Millennium Ecosystem Assessment Synthesis Report Ecosystems and human well-being: Synthesis. Washington, D.C. [https://doi.org/10.5822/978-1-61091-484-0\\_1](https://doi.org/10.5822/978-1-61091-484-0_1)
- Bárcenas-Moreno G, Rousk J, Bååth E (2011) Fungal and bacterial recolonisation of acid and alkaline forest soils following artificial heat treatments. *Soil Biology and Biochemistry* 43: 1023–1033. <https://doi.org/10.1016/j.soilbio.2011.01.019>
- Batten KM, Scow KM, Davies KF, Harrison SP (2006) Two invasive plants alter soil microbial community composition in serpentine grasslands. *Biological Invasions* 8: 217–230. <https://doi.org/10.1007/s10530-004-3856-8>
- Belnap J, Phillips SL, Sherrod SK, Moldenke A (2005) Soil biota can change after exotic plant invasion: Does this affect ecosystem processes? *Ecology* 86: 3007–3017. <https://doi.org/10.1890/05-0333>
- Bennett JA, Klironomos J (2019) Mechanisms of plant–soil feedback: interactions among biotic and abiotic drivers. *New Phytologist* 222: 91–96. <https://doi.org/10.1111/nph.15603>
- Bever JD (1994) Feedback between plants and their soil communities in an old field community. *Ecology* 75: 1965–1977. <https://doi.org/10.2307/1941601>
- CABI (2021) *Megathyrsus maximus* (Guinea grass). Available from: <https://www.cabi.org/isc/datasheet/38666>.
- Callaway RM, Thelen GC, Barth S, Ramsey PW, James E, Callaway RM, Thelen GC, Barth S, Ramsey PW, Gann JE (2004) Soil Fungi Alter Interactions between the Invader *Centaurea maculosa* and North American Natives. 85: 1062–1071.
- Callaway RM, Ridenour WM (2004) Novel weapons: Invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* 2: 436–443. [https://doi.org/10.1890/1540-9295\(2004\)002\[0436:NWISAT\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2004)002[0436:NWISAT]2.0.CO;2)
- Chou CH, Young CC (1975) Phytotoxic substances in twelve subtropical grasses. *Journal of Chemical Ecology* 1: 183–193. <https://doi.org/10.1007/BF00987867>
- Crawford KM, Bauer JT, Comita LS, Eppinga MB, Johnson DJ, Mangan SA, Queenborough SA, Strand AE, Suding KN, Umbanhowar J, Bever JD (2019) When and where plant–soil feedback may promote plant coexistence: a meta-analysis. *Ecology Letters* 22: 1274–1284. <https://doi.org/10.1111/ele.13278>
- D'Antonio C, Flory SL (2017) Long-term dynamics and impacts of plant invasions. *Journal of Ecology* 105: 1459–1461. <https://doi.org/10.1111/1365-2745.12879>
- Dogra KS, Sood SK, Dobhal PK, Sharma S (2010) Alien plant invasion and their impact on

- indigenous species diversity at global scale : A review. *Journal of Ecology and the Natural Environment* 2: 175–186.
- Ehrenfeld JG (2003) Effects of Exotic Plant Invasions on Soil Nutrient Cycling Processes. *Ecosystems* 6: 503–523. <https://doi.org/10.1007/s10021-002-0151-3>
- Ellsworth LM, Litton CM, Leary JJK (2015) Restoration impacts on fuels and fire potential in a dryland tropical ecosystem dominated by the invasive grass *Megathyrsus maximus*. *Restoration Ecology* 23: 955–963. <https://doi.org/10.1111/rec.12263>
- Fehmi JS, Rasmussen C, Arnold AE (2021) The pioneer effect advantage in plant invasions: site priming of native grasslands by invasive grasses. *Ecosphere* 12. <https://doi.org/10.1002/ecs2.3750>
- Gilbert GS, Parker IM (2006) Invasions and the regulation of plant populations by pathogens. In: Cadotte MW, McMahon SM, Fukami T (Eds), *Conceptual Ecology and Invasion Biology: Reciprocal Approaches to Nature*. Springer, Dordrecht, 289–305.
- Halbritter AH, Carroll GC, Güsewell S, Roy BA (2012) Testing assumptions of the enemy release hypothesis: Generalist versus specialist enemies of the grass *Brachypodium Sylvaticum*. *Mycologia* 104: 34–44. <https://doi.org/10.3852/11-071>
- Hawkes C V., Wren IF, Herman DJ, Firestone MK (2005) Plant invasion alters nitrogen cycling by modifying the soil nitrifying community. *Ecology Letters* 8: 976–985. <https://doi.org/10.1111/j.1461-0248.2005.00802.x>
- Hawkes C V., Kivlin SN, Du J, Eviner VT (2013) The temporal development and additivity of plant-soil feedback in perennial grasses. *Plant and Soil* 369: 141–150. <https://doi.org/10.1007/s11104-012-1557-0>
- Ho CY, Tsai MY, Huang YL, Kao WY (2016) Ecophysiological factors contributing to the invasion of *Panicum maximum* into native *Miscanthus sinensis* grassland in Taiwan. *Weed Research* 56: 69–77. <https://doi.org/10.1111/wre.12186>
- Keane RM, Crawley MJ (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* 17: 164–170. [https://doi.org/10.1016/S0169-5347\(02\)02499-0](https://doi.org/10.1016/S0169-5347(02)02499-0)
- Kulmatiski A, Beard KH, Stevens JR, Cobbold SM (2008) Plant-soil feedbacks: A meta-analytical review. *Ecology Letters* 11: 980–992. <https://doi.org/10.1111/j.1461-0248.2008.01209.x>
- Lekberg Y, Bever JD, Bunn RA, Callaway RM, Hart MM, Kivlin SN, Klironomos J, Larkin BG, Maron JL, Reinhart KO, Remke M, van der Putten WH (2018) Relative importance of competition and plant–soil feedback, their synergy, context dependency and implications for coexistence. *Ecology Letters* 21: 1268–1281. <https://doi.org/10.1111/ele.13093>
- Levine JM, Pachepsky E, Kendall BE, Yelenik SG, Lambers JHR (2006) Plant-soil feedbacks and invasive spread. *Ecology Letters* 9: 1005–1014. <https://doi.org/10.1111/j.1461-0248.2006.00949.x>
- Mehlich A (1984) Mehlich 3 Soil Test Extractant. A Modification of the Mehlich 2 Extractant. *Communications in Soil Science and Plant Analysis* 15: 1409–1416.
- Mummey DL, Rillig MC (2006) The invasive plant species *Centaurea maculosa* alters arbuscular mycorrhizal fungal communities in the field. *Plant and Soil* 288: 81–90. <https://doi.org/10.1007/s11104-006-9091-6>
- Pernilla Brinkman E, Van der Putten WH, Bakker EJ, Verhoeven KJF (2010) Plant-soil feedback: Experimental approaches, statistical analyses and ecological interpretations. *Journal of Ecology* 98: 1063–1073. <https://doi.org/10.1111/j.1365-2745.2010.01695.x>
- Van Der Putten WH, Klironomos JN, Wardle DA (2007a) Microbial ecology of biological



- 502       invasions. *ISME Journal* 1: 28–37. <https://doi.org/10.1038/ismej.2007.9>
- 503       Van Der Putten WH, Kowalchuk GA, Brinkman EP, Doodeman GTA, Van Der Kaaij RM,
- 504       Kamp AFD, Menting FBJ, Veenendaal EM (2007b) Soil feedback of exotic savanna grass
- 505       relates to pathogen absence and mycorrhizal selectivity. *Ecology* 88: 978–988.
- 506       <https://doi.org/10.1890/06-1051>
- 507       Pyšek P, Richardson DM (2010) Invasive species, environmental change and management, and
- 508       health. *Annual Review of Environment and Resources* 35: 25–55.
- 509       <https://doi.org/10.1146/annurev-environ-033009-095548>
- 510       Ramirez-Yanez LE, Ortega-S JA, Brennan LA, Rasmussen GA (2007) Use of Prescribed Fire
- 511       and Cattle Grazing To Control Guineagrass. 23Rd Tall Timbers Fire Ecology Conference:
- 512       Fire in Grassland and Shrubland Ecosystems: 240–245.
- 513       Reinhart KO, Callaway RM (2006) Soil biota and invasive plants. *New Phytologist* 170: 445–
- 514       457. <https://doi.org/10.1111/j.1469-8137.2006.01715.x>
- 515       Rhoades JD (1984) Soluble salts. In: Page AL (Ed.), *Methods of Soil Analysis: Part 2*. ASA and
- 516       SSSA, Madison, WI, 167–178.
- 517       Rhodes AC, Plowes RM, Goolsby JA, Gaskin JF, Musyoka B, Calatayud P-AP-A, Cristofaro M,
- 518       Grahmann ED, Martins DJ, Gilbert LE (2021a) The dilemma of Guinea grass (*Megathyrsus*
- 519       *maximus*): a valued pasture grass and an emergent invasive species. *Biological Invasions* 3.
- 520       <https://doi.org/10.1007/s10530-021-02607-3>
- 521       Rhodes AC, Rutledge J, DuPont B, Plowes RM, Gilbert LE (2021b) Targeted Grazing of an
- 522       Invasive Grass Improves Outcomes for Native Plant Communities and Wildlife Habitat.
- 523       *Rangeland Ecology & Management* 75: 41–50. <https://doi.org/10.1016/j.rama.2020.11.007>
- 524       Robertson SG, Hickman KR (2012) Aboveground plant community and seed bank composition
- 525       along an invasion gradient. *Plant Ecology* 213: 1461–1475. [https://doi.org/10.1007/s11258-](https://doi.org/10.1007/s11258-012-0104-7)
- 526       012-0104-7
- 527       Rúa MA, Antoninka A, Antunes PM, Chaudhary VB, Gehring C, Lamit LJ, Piculell BJ, Bever
- 528       JD, Zabinski C, Meadow JF, Lajeunesse MJ, Milligan BG, Karst J, Hoeksema JD (2016)
- 529       Home-field advantage? evidence of local adaptation among plants, soil, and arbuscular
- 530       mycorrhizal fungi through meta-analysis. *BMC Evolutionary Biology* 16: 1–15.
- 531       <https://doi.org/10.1186/s12862-016-0698-9>
- 532       Rudgers JA, Mattingly WB, Koslow JM (2005) Mutualistic fungus promotes plant invasion into
- 533       diverse communities. *Oecologia* 144: 463–471. <https://doi.org/10.1007/s00442-005-0039-y>
- 534       Sardans J, Bartrons M, Margalef O, Gargallo-Garriga A, Janssens IA, Ciais P, Obersteiner M,
- 535       Sigurdsson BD, Chen HYH, Peñuelas J (2017) Plant invasion is associated with higher
- 536       plant–soil nutrient concentrations in nutrient-poor environments. *Global Change Biology*
- 537       23: 1282–1291. <https://doi.org/10.1111/gcb.13384>
- 538       Sarmiento C, Zalamea P-C, Dalling JW, Davis AS, Stump SM, U'Ren JM, Arnold AE (2017)
- 539       Soilborne fungi have host affinity and host-specific effects on seed germination and survival
- 540       in a lowland tropical forest. *Proceedings of the National Academy of Sciences* 114:
- 541       201706324. <https://doi.org/10.1073/pnas.1706324114>
- 542       Schofield RK, Taylor AW (1955) The Measurement of Soil pH. *Soil Science Society of America*
- 543       *Journal* 19: 164–167. <https://doi.org/10.2136/sssaj1955.03615995001900020013x>
- 544       Skipper HD, Westermann DT (1973) Comparative effects of propylene oxide, sodium azide, and
- 545       autoclaving on selected soil properties. *Soil Biology and Biochemistry* 5: 409–414.
- 546       [https://doi.org/10.1016/0038-0717\(73\)90067-9](https://doi.org/10.1016/0038-0717(73)90067-9)
- 547       Suding KN, Stanley Harpole W, Fukami T, Kulmatiski A, Macdougall AS, Stein C, van der



- Putten WH (2013) Consequences of plant-soil feedbacks in invasion. *Journal of Ecology* 101: 298–308. <https://doi.org/10.1111/1365-2745.12057>
- Theoharides KA, Dukes JS (2007) Plant invasion across space and time: Factors affecting nonindigenous species success during four stages of invasion. *New Phytologist* 176: 256–273. <https://doi.org/10.1111/j.1469-8137.2007.02207.x>
- Tuominen L, Kairesalo T, Hartikainen H (1994) Comparison of methods for inhibiting bacterial activity in sediment. *Applied and Environmental Microbiology* 60: 3454–3457. <https://doi.org/10.1128/aem.60.9.3454-3457.1994>
- van de Voorde TFJ, van der Putten WH, Bezemer TM (2012) Soil inoculation method determines the strength of plant-soil interactions. *Soil Biology and Biochemistry* 55: 1–6. <https://doi.org/10.1016/j.soilbio.2012.05.020>
- Wolfe BE, Klironomos JN (2005) Breaking New Ground: Soil Communities and Exotic Plant Invasion. *BioScience* 55: 477. [https://doi.org/10.1641/0006-3568\(2005\)055\[0477:bngsca\]2.0.co;2](https://doi.org/10.1641/0006-3568(2005)055[0477:bngsca]2.0.co;2)
- Zalamea PC, Sarmiento C, Elizabeth Arnold A, Davis AS, Dalling JW (2015) Do soil microbes and abrasion by soil particles influence persistence and loss of physical dormancy in seeds of tropical Pioneers? *Frontiers in Plant Science* 5: 799. <https://doi.org/10.3389/fpls.2014.00799>
- Zhang P, Li B, Wu J, Hu S (2019) Invasive plants differentially affect soil biota through litter and rhizosphere pathways: a meta-analysis. *Seabloom E (Ed.). Ecology Letters* 22: 200–210. <https://doi.org/10.1111/ele.13181>
- Zhang Z, Liu Y, Yuan L, Weber E, van Kleunen M (2021) Effect of allelopathy on plant performance: a meta-analysis. *Ecology Letters* 24: 348–362. <https://doi.org/10.1111/ele.13627>

**Figure legends:**

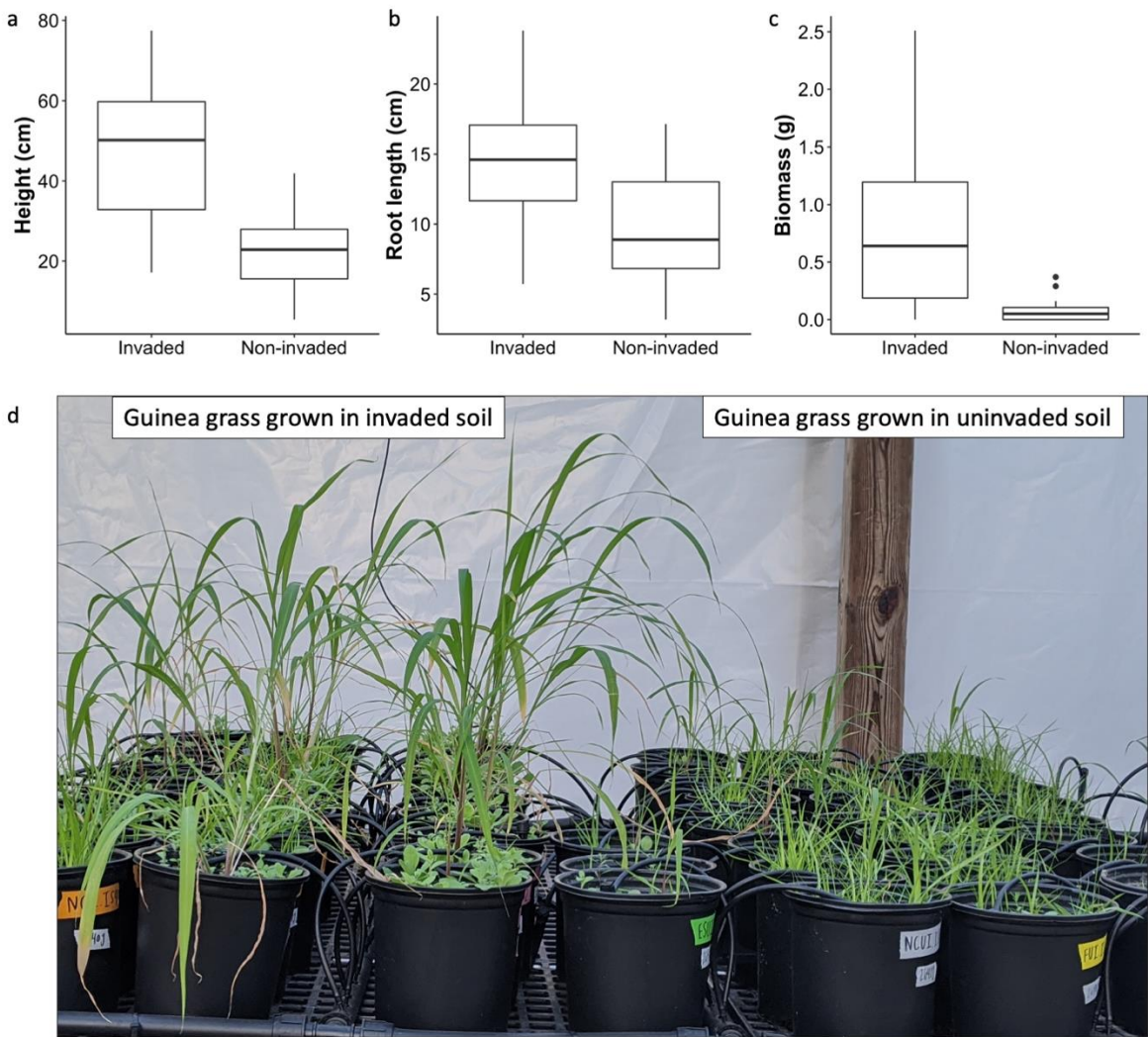
**Fig. 1** Guinea grass height (a), root length (b), and biomass (c) when grown in soil collected from i) grassland invaded by conspecifics and ii) non-invaded grasslands dominated by native species. All data shown are non-transformed.

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

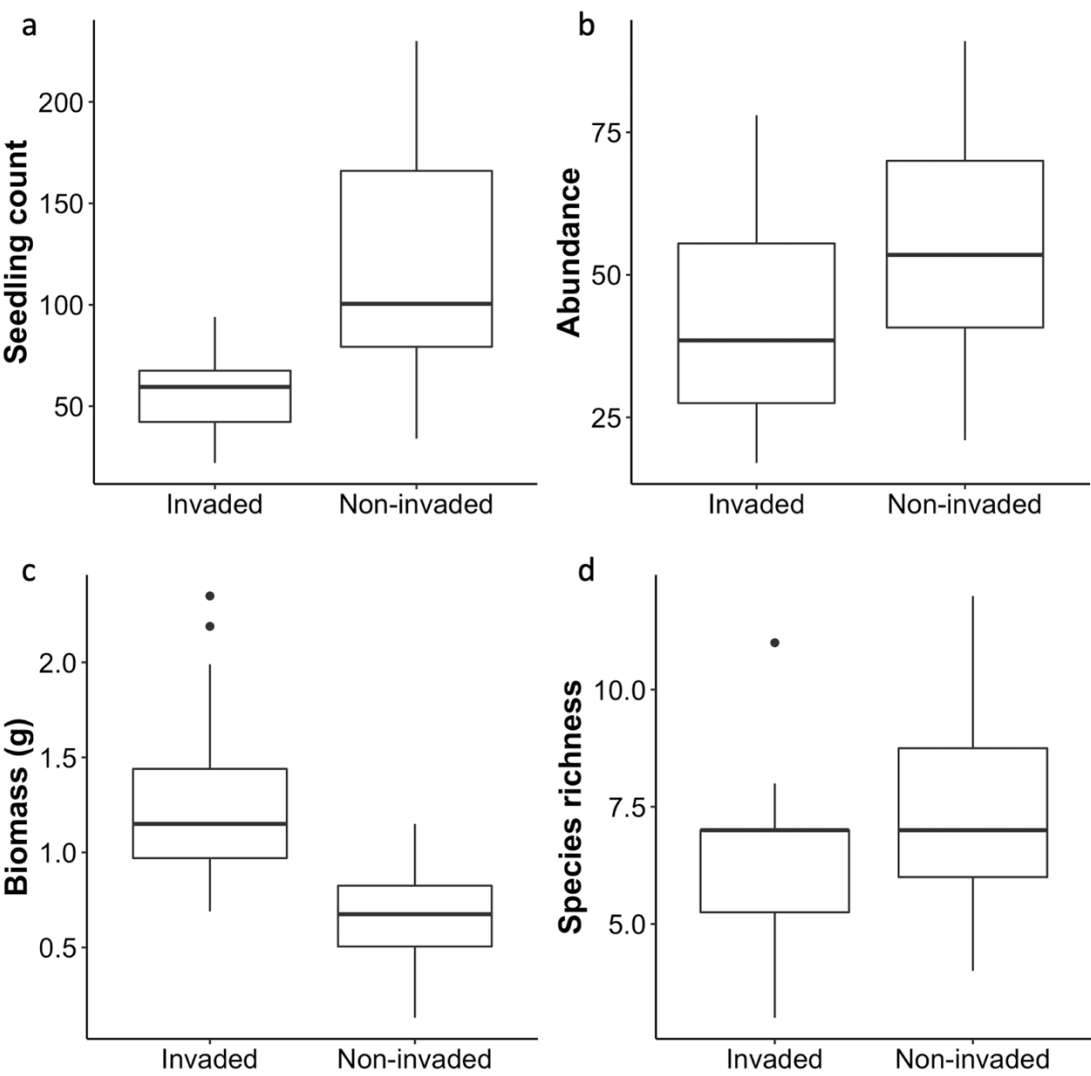
**Fig. 3** Plant abundance (a) and species richness (b) as a function of invasion and plant group. Monocot species richness and plant abundance were significantly higher in soil from non-invaded sites than invaded sites (species richness: Kruskal-Wallis  $X_1^2 = 13.4$ ,  $p = 0.0002$ ; plant abundance: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

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

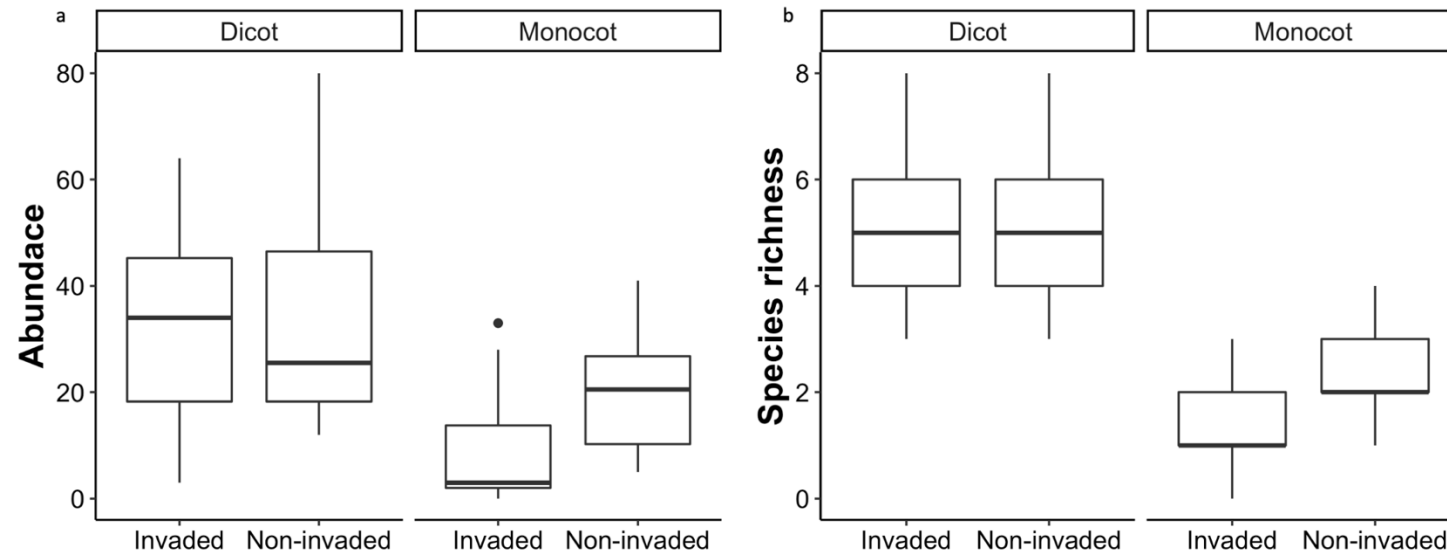
**Figure 1**



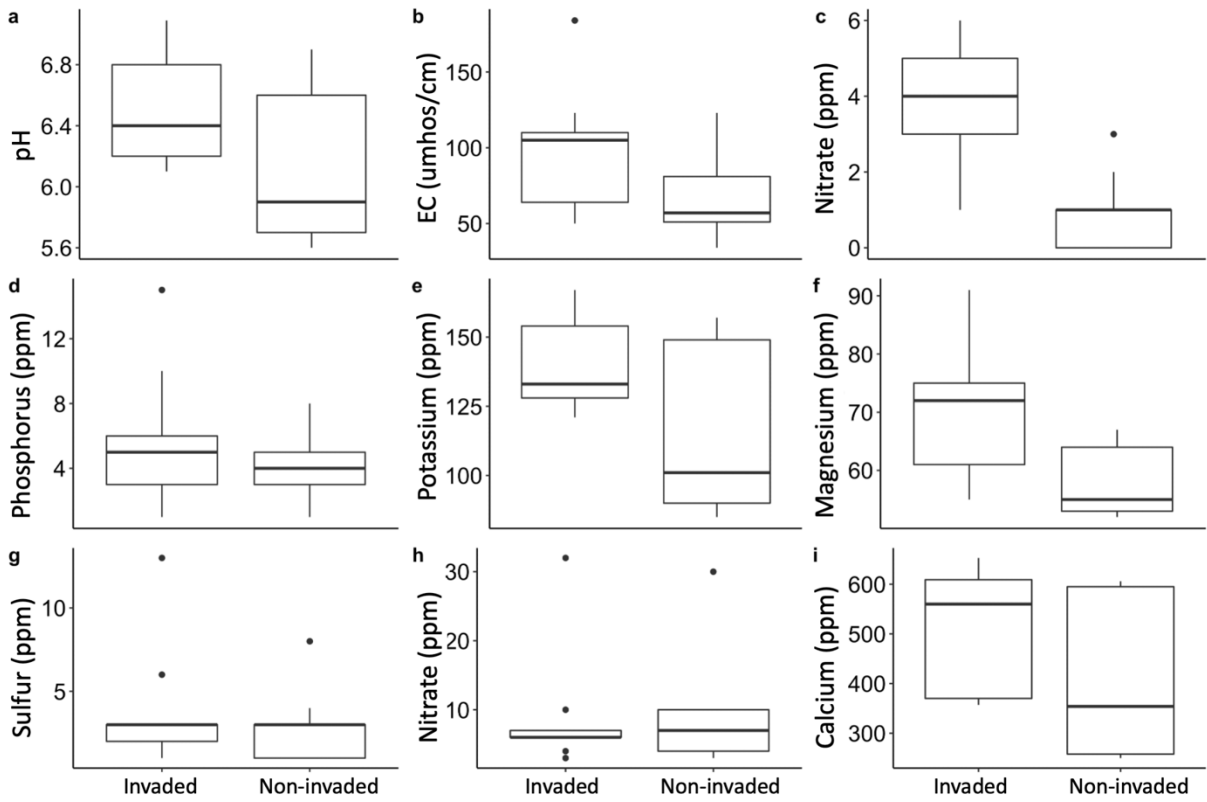
598 **Figure 2**



**Figure 3**



**Figure 4**



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.

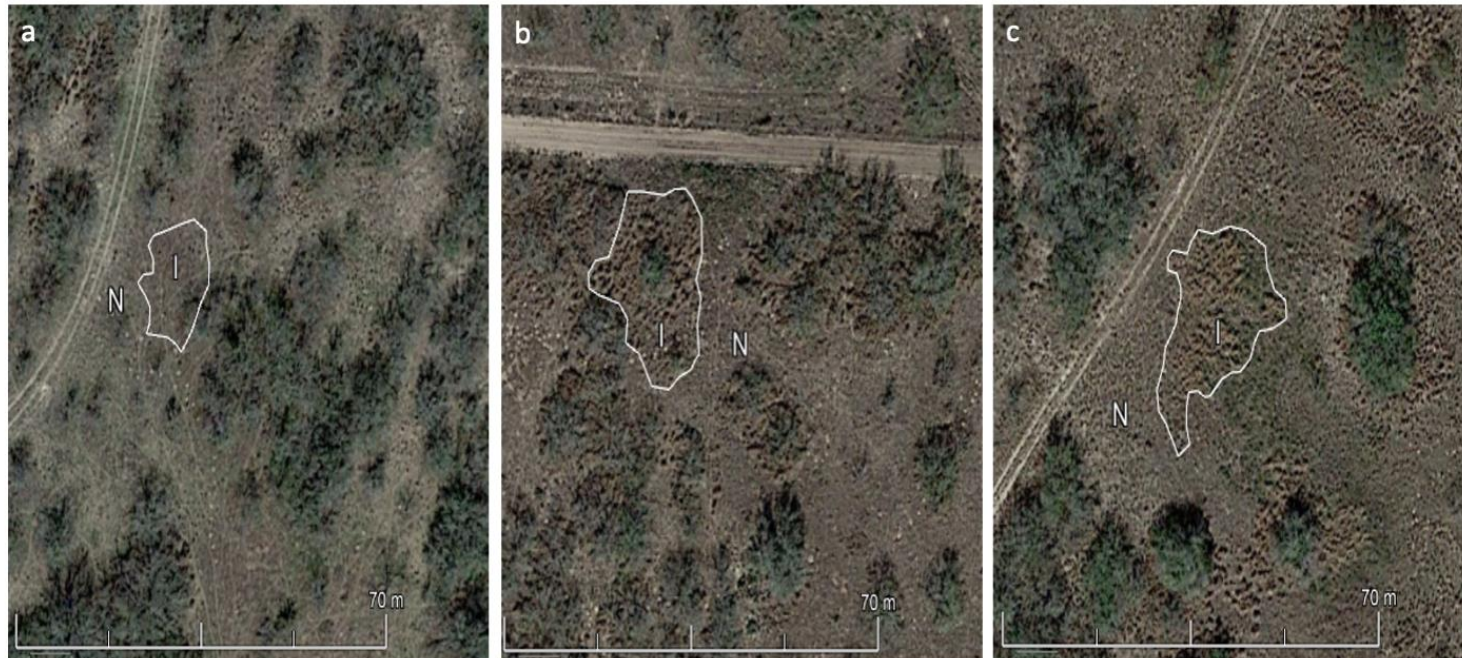
617

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

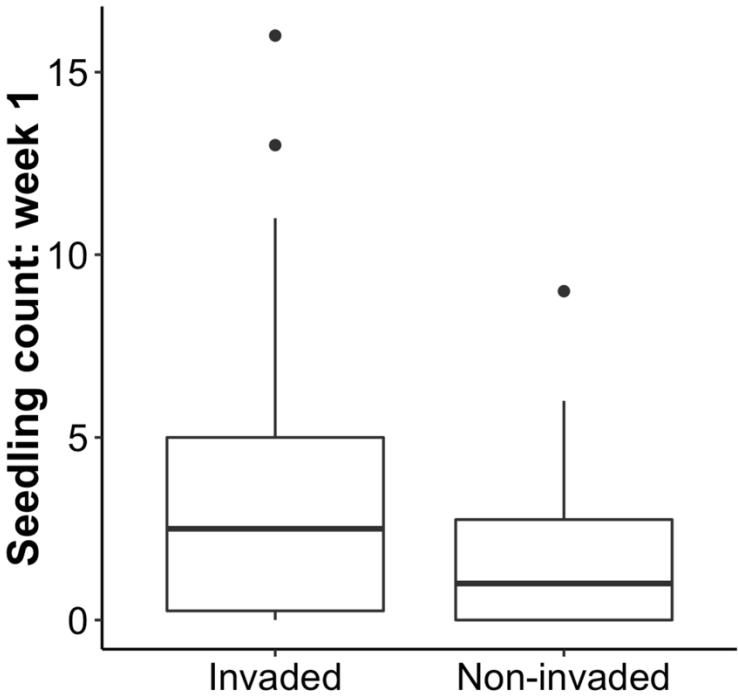


**Supplementary Figure S1**



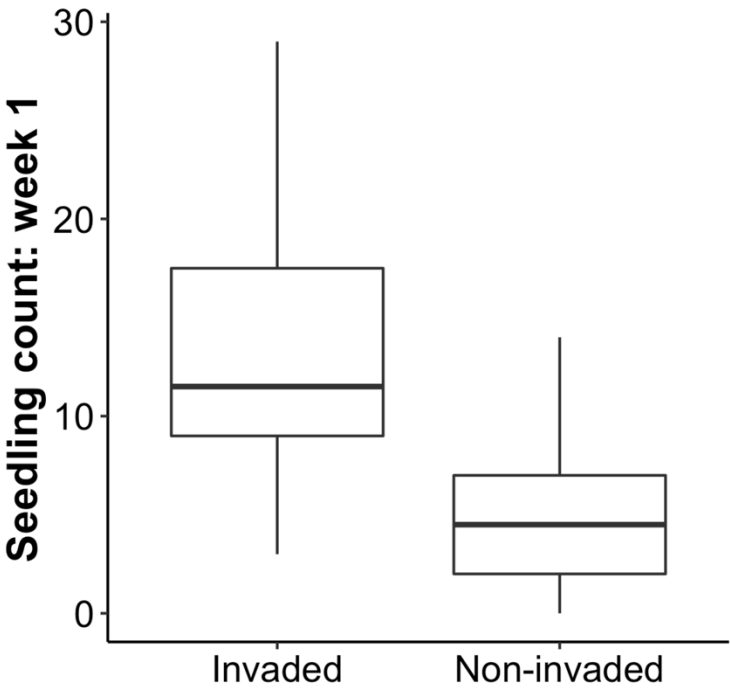


626 **Supplementary Figure S2**

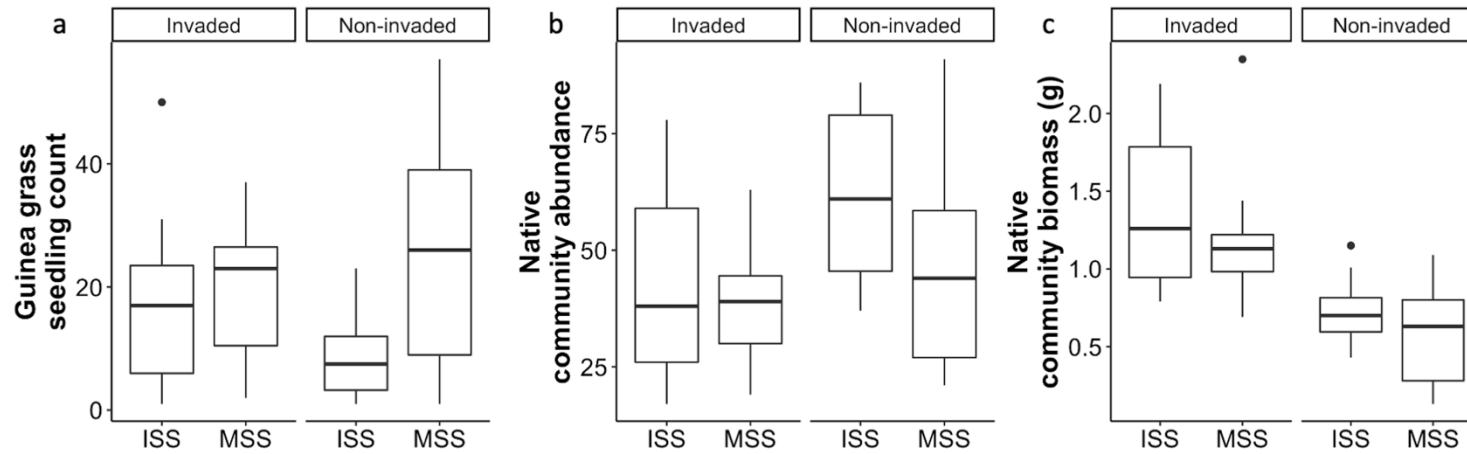


627  
628

Supplementary Figure S3



# Supplementary Figure S4



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

# Supplementary Table S1

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

**Supplementary Table S2**

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