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

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

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Abstract

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

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

In a process called plant-soil feedback (PSF), plants modify their soil environment via root exudates and litter which can impact nutrient cycling and soil microbial communities (Bever 1994, Bennett and Klironomos 2019). In native grasslands, PSF has a largely negative or stabilizing effect on plant growth which contributes to species coexistence and succession through negative-density dependent processes (e.g. competition, pathogens, herbivory) (Kulmatiski et al. 2008, Hawkes et al. 2013, Lekberg et al. 2018). Non-natives, if sufficiently distinct from established plant species, can alter root microbial communities and decomposition rates (Reinhart and Callaway 2006, Hawkes et al. 2013, Zhang et al. 2019, Fehmi et al. 2021). 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% (Ammondt 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 (Ammondt 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, Ammondt and Litton 2012, Ammondt 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 (Ammondt 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 km². 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 cm ± 17.4; root length: 14.1 cm ± 4.2; biomass: 0.8 g ± 0.7) versus non-invaded sites (height: 22.1 cm ± 8.3; root length: 9.8 cm ± 4.2; biomass: 0.01 g ± 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.

<table>
<thead>
<tr>
<th></th>
<th>Soil origin $F_{1,51}$</th>
<th>Soil handling method $F_{1,51}$</th>
<th>Interaction $F_{1,51}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seedling count</td>
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<td>7.20</td>
<td>2.44</td>
</tr>
<tr>
<td>Height</td>
<td>38.60</td>
<td>&lt; 0.0001</td>
<td>0.00</td>
</tr>
<tr>
<td>Root length</td>
<td>14.55</td>
<td>0.0004</td>
<td>0.39</td>
</tr>
<tr>
<td>Biomass</td>
<td>31.22</td>
<td>&lt; 0.0001</td>
<td>0.03</td>
</tr>
</tbody>
</table>

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 $1.3 \pm 0.4$) in soil from invaded sites than non-invaded sites (mean $0.6 \pm 0.3$) (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 $X^2_1 = 13.4, p = 0.0002$; plant abundance: Kruskal-Wallis $X^2_1 = 18.1, p < 0.0001$; 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.

<table>
<thead>
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<th></th>
<th>Soil origin</th>
<th>Soil handling method</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$F_{1,54}$</td>
<td>$p$</td>
<td>$F_{1,54}$</td>
</tr>
<tr>
<td>Seedling count</td>
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<td>$&lt;0.0001$</td>
<td>0.14</td>
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<tr>
<td>Plant abundance</td>
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<td>0.004</td>
<td>5.57</td>
</tr>
<tr>
<td>Total biomass</td>
<td>51.65</td>
<td>$&lt;0.0001$</td>
<td>4.26</td>
</tr>
<tr>
<td>Species richness</td>
<td>4.52</td>
<td>0.0382</td>
<td>0.02</td>
</tr>
</tbody>
</table>

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 ± 15) than ISS treatments (mean 12 ± 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 ± 21; total biomass: mean 1.04 ± 0.5) than MSS (plant abundance: mean 43 ± 17; total biomass 0.85 ± 0.5) (Fig. 2; Table 2).

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

Discussion

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

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

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


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Suding KN, Stanley Harpole W, Fukami T, Kulmatiski A, Macdougall AS, Stein C, van der


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 $X^2_1 = 13.4, p = 0.0002$; plant abundance: Kruskal-Wallis $X^2_1 = 18.1, p < 0.0001$), 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

(a) Height (cm)
(b) Root length (cm)
(c) Biomass (g)

(d) Guinea grass grown in invaded soil
Guinea grass grown in uninvaded soil
Figure 2
Figure 3

(a) Abundance and (b) Species richness for Dicot and Monocot plants in invaded and non-invaded areas.
Figure 4

(a) pH  
(b) EC (mhos/cm)  
(c) Nitrate (ppm)  
(d) Phosphorus (ppm)  
(e) Potassium (ppm)  
(f) Magnesium (ppm)  
(g) Sulfur (ppm)  
(h) Nitrate (ppm)  
(i) Calcium (ppm)

Invaded vs. Non-invaded
Supplementary figure legends:

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

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

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

Supplementary Fig. S4: Effect of soil handling method on Guinea grass seedling count (a), native community plant abundance (b), and native community biomass (c). MSS: mixed soil sampling; ISS: individual soil sampling. All data shown are non-transformed.
Supplementary Figure S1
Supplementary Figure S2

Seedling count: week 1

Invaded | Non-invaded
Supplementary Figure S3

![Box plot showing seedling count: week 1](https://doi.org/10.3897/arphapreprints.e86933)
Supplementary Figure S4

(a) Guinea grass seedling count
(b) Native community abundance
(c) Native community biomass (g)
Supplementary tables

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

Supplementary Table S2: Results of t-test examining differences in soil characteristics between invaded and non-invaded sites. Electrical conductivity, phosphorus, and sulfur were log-transformed prior to analysis.
### Supplementary Table S1

<table>
<thead>
<tr>
<th>Soil characteristics</th>
<th>F-statistic</th>
<th>$p$</th>
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<tbody>
<tr>
<td>pH</td>
<td>$F_{1,16} = 0.35$</td>
<td>0.5648</td>
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<tr>
<td>Electrical conductivity</td>
<td>$F_{1,16} = 12.08$</td>
<td><strong>0.0031</strong></td>
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<tr>
<td>Nitrate</td>
<td>$F_{1,16} = 0.02$</td>
<td>0.8937</td>
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<tr>
<td>Phosphorus</td>
<td>$F_{1,16} = 13.83$</td>
<td><strong>0.0019</strong></td>
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<tr>
<td>Potassium</td>
<td>$F_{1,16} = 0.13$</td>
<td>0.7279</td>
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<tr>
<td>Magnesium</td>
<td>$F_{1,16} = 0.92$</td>
<td>0.3513</td>
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<td>Sulfur</td>
<td>$F_{1,16} = 29.28$</td>
<td><strong>0.0001</strong></td>
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<tr>
<td>Sodium</td>
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<tr>
<td>Calcium</td>
<td>$F_{1,16} = 0.01$</td>
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Supplementary Table S2

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