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Native soil microbial amendments generate tradeoffs in plant productivity, diversity, and soil stability in coastal dune restorations

Running head: Soil microbe impacts on dune restoration

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Author contributions: KMC designed the research; KMC, MHB established the experiment; KMC, MHB, HL, NCL collected data; KMC analyzed the data; KMC wrote the manuscript; MHB, HL, NCL edited the manuscript.

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/rec.13073

ABSTRACT

Given the important role that soil microbes play in structuring plant communities and mediating ecosystem functions, there is growing interest in harnessing microbial communities to restore degraded ecosystems. Dune restorations, in particular, may benefit from native soil amendments because microbial diversity and abundance are very low in unvegetated areas. In an outdoor mesocosm experiment simulating Texas Gulf Coast dune restorations, we tested how native soil microbial amendments and restored diversity of foundational grasses influenced three key restoration responses: plant performance, plant diversity (including the colonization of native forbs), and soil stability. We found that native microbial amendments increased plant diversity and have the potential to increase soil stability, but this came at the cost of decreased plant biomass. Our results suggest that soil enemies in the native microbial amendments increased plant diversity by decreasing the performance of the dominant grass species and that arbuscular mycorrhizal fungi in the native microbial amendments increased the density of fungal hyphae in the soil, which can increase soil stability. Depending on the goals of the restoration, native soil microbial amendments may be a simple and inexpensive method to provide restoration benefits.

Key words: AM fungi, pathogens, plant diversity, plant productivity, sand dunes, soil stability

Implications for Practice

- Microbes in native soil amendments can increase plant diversity and may increase soil stability, but this may come at the cost of decreased plant productivity.

- Native soil microbial amendments have the potential to improve restoration outcomes, but desired restoration outcomes should be carefully considered prior to their use as trade-offs in desirable outcomes may occur.

INTRODUCTION

Coastal sand dunes are ecologically and economically important ecosystems. They sustain a diversity of plants and animals, shelter neighboring wetlands, and serve as popular tourist attractions (Patterson 2005; Everard et al. 2010). Native vegetation also plays a critical role in protecting inland development by stabilizing soils, which provide a natural buffer to storms (Nordstrom 2008; Feagin et al. 2015, 2019; Silva et al. 2016; Sigren et al. 2018, 2014). Stabilized dunes will likely play an increasingly important role in mitigating the negative impacts of climate change, including changing sea levels, more severe storms, and increasing erosion (Patterson 2005; Feagin et al. 2015). Unfortunately, coastal dunes are highly susceptible to human disturbance, and degraded and denuded dunes do not offer the same benefits as undisturbed dunes. Therefore, there has been growing interest in restoring dunes as a flexible alternative to hard structures (like levees) for protection against weather events (Barbier et al. 2008; Temmerman et al. 2013; Feagin et al. 2015). Current restoration techniques are plagued by poor plant performance and high rates of erosion. Coastal dunes are relatively harsh environments where plants face nutrient poor soils, salt spray, and sand burial (Lane et al. 2008; Miller et al. 2009). Poor plant performance in dune restorations is believed to be caused by nutrient limitation (Hannan et al. 2007; Williams & Feagin 2010), although other stressors also likely decrease plant performance.

Soil microbes strongly influence plant productivity, community dynamics, and soil development in natural systems (Crawford et al. 2019; Degens 1997; Reynolds et al. 2003; van

der Heijden et al. 2008). For example, the presence of soil mutualists that help plants uptake nutrients can more than double the amount of biomass produced in grasslands (Vogelsang et al. 2006; van der Heijden et al. 2008). At the same time, soil microbes can increase plant diversity and foster plant species coexistence by decreasing the performance of dominant plant species, for example through the accumulation of species-specific pathogens (Bever et al. 1997, 2015; Crawford et al. 2019). As the largest pool of biomass in the soil, microbes also play a key role in soil aggregation (Degens 1997; Blankinship et al. 2016). In particular, arbuscular mycorrhizal (AM) fungi, which are common plant mutualists, increase soil aggregation through hyphal binding of soil particles and hyphal deposition of glomalin (Rillig 2004; Leifheit et al. 2014). Soil aggregates increase soil stability by increasing erosion resistance because larger, heavier soil particles are more difficult to transport by wind or water (Teixeira & Misra 1997; Barthès & Roose 2002).

Despite their importance in natural systems, soil microbes have only recently been considered during ecological restorations. Severely degraded dunes that lack vegetation and dunes reconstructed from dredged sand have very low abundances of soil microbes (Koske & Gemma 1997), which may in part explain the poor performance of some restorations. Soil microbes are expected to eventually colonize these restorations, but it could take several years for this to occur naturally (Sylvia & Will 1988; Koske & Gemma 1997). Therefore, the addition of native soil microbes to initial plantings may help accelerate restoration establishment and soil development. Little work has examined how the addition of entire soil microbial communities

influence saltwater sand dune restorations (but see Sylvia 1989, Sylvia et al. 1993 for examples of restoration experiments with AM fungi), but at least one study has shown that native microbial amendments can increase the survival and performance of restored plants on freshwater dunes along Lake Michigan (Emery & Rudgers 2011).

The addition of native soil microbial amendments has the potential to positively affect restoration outcomes, but there are lingering questions that should be addressed prior to their widespread adoption. First, the effects of soil microbial communities often depend on plant species identity. The same soil microbial community can positively affect one plant species and negatively affect another (Bever 1994; Crawford & Knight 2017). Since many restorations are initiated with only a few foundational plant species, it may be important to determine how these plants respond to soil microbes. Furthermore, the effects of soil microbial communities may also depend on plant diversity (Schnitzer et al. 2011), so testing microbial effects in realistic planting scenarios is necessary. Second, there may be trade-offs in how soil microbial amendments influence ecosystem functions. For example, soil microbial amendments may promote the dominance of planted species at the expense of other native plants that may naturally colonize the restoration (Hartnett & Wilson 1999; Urcelay & Díaz 2003; Vogelsang et al. 2006; Lin et al. 2015), decreasing community diversity. Soil microbial amendments may also increase soil aggregation through microbial mechanisms, but decrease soil aggregations caused by plant-based mechanisms – for example, plant roots enmeshing soil into aggregates and the deposition of carbon into the soil (Blankinship et al. 2016) – if microbial amendments decrease belowground

plant biomass. Documenting the occurrence of such tradeoffs will help inform management decisions based on the goals of the restoration.

Here, we tested how soil microbial amendments influenced restoration outcomes under realistic restoration scenarios for Texas Gulf Coast sand dunes. In a mesocosm experiment, we added live or sterile native soil amendments collected from local dunes to three different plant diversity treatments: monocultures of two foundational grasses typically used in Texas dune restorations, *Panicum amarum* and *Uniola paniculata*, or mixtures of the two species. Following grass establishment, we added native forbs to test whether our treatments affected the ability to other plants to colonize restorations. We measured multiple above- and below-ground responses to understand how microbial amendments and plant diversity influenced restoration outcomes, including potential tradeoffs between desirable outcomes. Specifically, we asked: (1) How do native soil microbial amendments influence the performance of the restored grasses? (2) How do native soil microbial amendments and grass diversity influence the ability of native plants to colonize the restorations? (3) How do soil native microbial amendments and grass diversity influence soil stability?

METHODS

To test how native soil amendments (live or sterile) and restored plant diversity (*Panicum* monoculture, *Uniola* monoculture, mixture) influenced restoration outcomes, we established a mesocosm field experiment. We simulated dune restorations by filling round, 375 L pots (100 cm diameter × 50 cm height) with masonry sand. We purchased the sand, which was similar in

grain size and composition (96% sand, 4% clay) to sand used in restorations on Galveston Island (100% sand), from a local landscaping company (Living Earth, Houston, TX). The sand contained few AM fungal spores and little hyphae (<10 mm hyphae per 20 g soil), similar to sand on restored beaches on Galveston Island (<15 mm hyphae per 20 g soil). Each treatment combination was replicated 10 times, for a total of 60 pots. The pots were located at the University of Houston Coastal Center, which is approximately 25 km from the dunes on Galveston Island. The experiment was established in late August 2016.

Plant diversity – To initiate the restorations, we planted two grass species commonly used in Gulf Coast dune restorations, *Panicum amarum* and *Uniola paniculata*. We planted the two species in monoculture and in mixture to test how plant identity and diversity influence restoration outcomes. Following planting density recommendations for dune restorations (Patterson 2005), we planted 16 plants into each pot in a grid design that maximized spacing among plants. Mixtures contained 8 individuals of each species, and species were randomly assigned to each grid space in each pot. To replicate restoration methods, the grasses were purchased from a commercial nursery that specializes in dune restorations and frequently sells stock for restorations in Texas (Green Seasons Nursery, Parrish, FL). To determine whether fungi from the nursery had already colonized plants, we measured fungal root colonization for two individuals of *P. amarum* and *U. paniculata* using trypan blue staining and microscopy (same methods as described below). The plants were heavily colonized by AM fungi (roots of *P. amarum* and *U. paniculata* were 96% and 87% colonized, respectively). Dark septate fungi,

which can function as plant mutualists or antagonists, were present in the roots of all plants. The nursery did not explicitly add microbes to the plants, so it is likely the colonizing microbes were in the potting soil or passively colonized the pots. Therefore, our experiment is a conservative (and more realistic) test of whether adding native soil amendments can influence restoration, as stronger responses would be expected if native soil microbes were added to sterile plants.

Native soil amendments – We collected soil for the native soil amendments from vegetated dunes on Galveston Island, TX, USA (Fig. S1). The soil was collected one week prior to planting the restoration experiment and was stored in a 4 C cold room to slow microbial processes. To collect a range of soil communities, we collected soil from 15 sites, stretching 25 km of coastline (Fig. S1). At each site, soil was collected from the rooting zones of native plants, including the two grass species planted in the experiment. Prior to use in the experiment, the soil was homogenized. The inoculum contained 135 mm AM hyphae and dozens of live AM fungal spores per 20 g subsample. To generate the sterile soil amendment, we sterilized half of the homogenized soil by autoclaving it for 90 minutes at 121°C twice, with a 24-hour resting period between sterilizations. At planting, 10 cm^3 of the live or sterile native soil amendment was added to the rooting zone of each plant. While larger amounts of amendments may produce a larger effect on restoration outcomes, previous work has shown that small amounts of soil amendments can still significantly affect plant performance (Crawford et al. 2019). Furthermore, adding a small amount of amendments is more feasible and realistic for dune restorations. Mesocosms were watered for two weeks, after which they received only ambient precipitation.

Native plant colonization – In July 2018, we planted three species of native forbs (*Sesuvium portulacastrum*, *Ipomoea pes-caprae*, *Bacopa monnieri*) into each pot to simulate a colonization event. The three species are low-growing trailing vines/herbs common on Gulf Coast dunes and appear to colonize open dunes primarily through vegetative growth (Lonard & Judd 1997), although they can produce viable seeds (Devall & Thien 1989). We propagated *Sesuvium* and *Ipomoea* from cuttings taken from plants purchased from Green Seasons Nursery (Parrish, FL, USA), the same source as the grasses used in the restorations. We propagated *Bacopa* from cuttings taken from Galveston Island, TX. Cuttings were dipped in rooting hormone and planted in Metro-Mix 360 (Sun Gro Horticulture, Agawam, MA, USA). Propagules were watered as needed to prevent water limitation. They grew for 35 days before they were transplanted into the experiment. These colonists were harvested in September 2018, at the same time as total aboveground grass biomass. Colonists were dried for at least three days at 80 C prior to weighing.

Restored grass performance – In September 2018, after two years of growth, we collected total aboveground biomass of the grasses in each pot, keeping the biomass of the two species separate in the mixed plots. Biomass was dried at 80 C for five days prior to weighing. To estimate belowground biomass, we collected a single soil core (10 cm diameter × 30 cm height) from the middle of each pot. Soil cores were transported back to the lab and stored at 4 C until roots were collected. We dried belowground biomass at 80 C for three days prior to weighing.

Soil stability and microbial responses – Arbuscular mycorrhizal (AM) fungi play a key role in stabilizing soils by promoting soil aggregation. Therefore, we were interested in not only measuring soil aggregation, but also determining how native microbial amendments influenced plant colonization by AM fungi and the amount of extra-radical hyphae produced by AM fungi. From the same soil cores we used to quantify belowground biomass, we subsampled roots to measure fungal colonization and retained soil to quantify the density of extra-radical hyphae.

Root colonization by AM fungi was measured using a 0.1g sample of roots. We stained the root samples with 0.05% trypan blue using a procedure modified from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi. In brief, roots were packed in tissue cassettes, placed in hot 10% KOH for 10 minutes, rinsed, placed in 5% household bleach for 10 minutes, rinsed, acidified in 2% HCl for 15 minutes, paced in hot 0.05% trypan blue for 3 minutes, rinsed, and stored in DI water for 1 week to leach excess dye. We mounted the stained roots on microscope slides and quantified the frequency of occurrence of AM fungal structures (hyphae, arbuscules, vesicles) in 60 non-overlapping views observed at 400× magnification (Mack & Rudgers 2008; Hawkins & Crawford 2018).

To quantify extra-radical hyphae, we suspended 20 g of sand in 500 ml of DI water by stirring at 700 rpm for 2 minutes. Suspended organics were sieved through a stack of 500 μm and 212 μm sieves. Catchments on the 212 μm sieve were transferred to a beaker with 10 ml of DI water and hyphae were stained for at least 90 minutes with 20 drops of 4% trypan blue. After staining, hyphae were transferred to a 38 μm sieve and rinsed with DI water for 5 mintues.

Stained hyphae were resuspended in 200 ml of DI water by stirring at 700 rpm for 1 minute, followed by stirring at 260 rpm for 30 seconds. After counter-stirring, a syringe was used to transfer a 20 ml aliquot to a vacuum filter with a 0.45 μm nylon filter. We prepared two filters per sample and mounted the filters on a slide. The length of extra-radical hyphae on each filter was estimated using the gridline intersect method (Giovannetti & Mosse 1980) while being magnified at 100×. Hyphal lengths on the two filters were averaged prior to data analysis.

In May 2019, we collected soil cores to measure soil aggregation. After airdrying the samples for one week, soil samples were separated into seven aggregate groups (<0.25 mm, 0.25-0.5 mm, 0.5-1 mm, 1-2 mm, 2-4 mm, 4-8 mm, and >8 mm) using a vibratory sieve shaker with an amplitude of 1.00 mm/"g" for 30 s. Generally, bigger aggregates imply greater soil stability (Kemper & Rosenau 1986). To quantify soil stability, we calculated the mean weight diameter of the soil aggregates, which is the sum of the weighted mean diameter of all size class, with the weighting factor of each class being its proportion of the total sample weight (Nimmo & Perkins 2002).

Statistical analyses – We tested for treatment effects on total aboveground biomass of the grasses in each pot and subsampled belowground biomass using general linear models with the fixed effects of grass diversity (*Panicum* monoculture, *Uniola* monoculture, or both), native soil amendments (live or sterile), and their interaction. We were also interested in how *Panicum* and *Uniola* responded individually to the treatments, so we calculated average aboveground biomass of an individual *Panicum* and *Uniola* plant in each pot by dividing total biomass of each species

by the original number of individuals within the pot (16 for monocultures and 8 per species for mixtures). In May 2017, when individual plants were still distinguishable, only 16 of the 960 plants in the experiment had died, so we assumed that final mortality was low. We tested for treatment effects on average individual biomass using separate general linear models for each grass species with the fixed effects of grass diversity (monoculture or both), native soil amendments (live or sterile), and their interaction. Prior to analyses, biomass values were log transformed to improve homogeneity of variances. In pots containing both species, we used general linear models to test whether native soil amendments (live or sterile) influenced the percentage of grass biomass that was made up of *Panicum*. All analyses were conducted using Proc GLM in SAS 9.4.

Colonist survival during the colonization experiment was low. First, we tested whether treatments influenced the probability that any colonists survived using a 2×3 Fishers exact probability test (Proc FREQ, SAS 9.4) with the factors of grass diversity (*Panicum* monoculture, *Uniola* monoculture, both) and native soil amendments (live, sterile). If a pot had at least one surviving colonist, it was considered colonized. After detecting no significant treatment effects, we tested whether, in the pots with surviving colonists, the treatments influenced total colonist biomass and species richness of colonists using general linear models (Proc GLM, SAS 9.4) with the fixed effects of grass diversity (*Panicum* monoculture, *Uniola* monoculture, both), native soil amendments (live, sterile), and their interaction. We also tested whether our treatments influenced biomass of individual colonists only using the pots where each colonist survived.

Prior to analyses, biomass was log transformed to improve homogeneity of variances, and one replicate where *Bacopa* produced 30 g of biomass (the average biomass of all colonists summed together in other pots was 0.51 g) was excluded from the analyses of colonist biomass.

We tested how our treatments influenced microbial responses (length of extraradical hyphae and percent root colonization by AMF) and soil aggregation (mean weight diameter) using general linear models (Proc GLM, SAS 9.4) with the fixed effects of grass diversity (*Panicum* monoculture, *Uniola* monoculture, both), native soil amendments (live, sterile), and their interaction. Length of extraradical hyphae was log transformed prior to analysis to improve homogeneity of variances.

RESULTS

Restored grass performance – Restored grass diversity and native soil amendments had strong effects on grass biomass (Table S1; Fig. 1; aboveground: $F_{2,54} = 441.79$, $P < 0.0001$; belowground: $F_{2,54} = 14.71$, $P < 0.0001$). Across all treatments, *Uniola* produced much less biomass than *Panicum*. Mesocosms planted with only *Uniola* produced 11% of the aboveground biomass and 20% of the belowground biomass that mesocosms containing *Panicum* produced (Tukey's HSD $P < 0.0001$ for all comparisons). Communities with both *Panicum* and *Uniola* did not produce more or less biomass than communities with only *Panicum* (Tukey's HSD $P = 0.216$ and $P = 0.370$ for aboveground and belowground biomass, respectively). Despite our predictions, live native soil amendments decreased plant biomass (aboveground: $F_{1,54} = 25.84$, $P < 0.0001$; belowground: $F_{1,54} = 1.45$, $P = 0.047$). Mesocosms with live native soil amendments produced

68% of the aboveground biomass and 75% of the belowground biomass that mesocosms with sterile native soil amendments produced (Tukey's HSD $P < 0.0001$ for both comparisons). Plant diversity and native soil amendments did not interact to influence grass biomass.

The aboveground biomass of individuals within each grass species also responded to the treatments (Table S2). Individual *Panicum* plants produced 30% less biomass with live native soil amendments than sterile native soil amendments ($F_{1,36} = 20.10$, $P < 0.0001$) and 41% less biomass when growing alone than when competing with *Uniola* (Fig. 2A; $F_{1,36} = 40.70$, $P < 0.0001$). Plant diversity did not interact with native soil amendments to influence individual *Panicum* biomass. In contrast, individual *Uniola* biomass did depend on both plant diversity and native soil amendments (Fig. 2B; $F_{1,36} = 22.03$, $P < 0.0001$). When growing alone, individual *Uniola* produced 25% less biomass with live native soil amendments than sterile native soil amendments. However, when competing with *Panicum*, live native soil amendments benefited *Uniola* – individual *Uniola* produced 141% more biomass with live native soil amendments than with sterile native soil amendments. The positive effect of live native soil amendments on *Uniola* biomass when in competition with *Panicum* resulted in a small, but significant, increase in *Uniola* biomass relative to *Panicum* biomass ($F_{1,18} = 20.15$, $P = 0.0003$); mesocosms with sterile native soil amendments consisted of 99.34% *Panicum* biomass while mesocosms with live native soil amendments consisted of 97.85% *Panicum* biomass.

Native plant colonization – Across all treatments, survival of the colonists was low. Only 28 of the 60 pots had at least one colonist survive. Among colonists, *Bacopa* survived in the

most mesocosms (22 of 60), while *Ipomoea* and *Sesuvium* both survived in only 5 of the 60 mesocosms. The probability that any colonist survived was not affected by the treatments (P = 0.621, Fisher's exact test). However, within mesocosms where colonists did survive, the plant diversity treatment did affect colonist biomass (Table S3; Fig. 3; $F_{2,22} = 6.57$, $P = 0.006$). Total colonist biomass was greatest in mesocosms where only *Uniola* was initially planted. Pots with *Uniola* contained 5.8 times more colonist biomass than mesocosms with *Panicum* (Tukey's HSD $P = 0.02$) and 9.1 times more colonist biomass than mesocosms with both *Panicum* and *Uniola* (Tukey's HSD $P = 0.03$). Native soil amendments did not affect total colonist biomass. The biomass of all colonists was 0.48 g ± 0.17 SE in mesocosms with live native soil amendments and 0.53 g ± 0.23 in mesocosms with sterile native soil amendments. Individually, neither *Sesuvium* nor *Ipomoea* responded to our treatments (Table S4); however, *Sesuvium* only survived in *Uniola* monocultures (Fig. 3). *Bacopa* did respond to our plant diversity treatment (Table S4; $F_{2,20} = 5.86$, $P = 0.01$). In pots with only *Uniola*, *Bacopa* produced 7.2 times more biomass than in mesocosms with *Panicum* (Tukey's HSD $P = 0.02$) and 9.7 times more biomass than in mesocosms with both *Panicum* and *Uniola* (Tukey's HSD $P = 0.03$). Colonist richness was not affected by our treatments (Table S3).

Soil stability and microbial responses – Plant diversity influenced root colonization by arbuscular mycorrhizal (AM) fungi (Table S5; $F_{2,54} = 28.97$, $P < 0.0001$). Plant roots from mesocosms initially planted with only *Uniola* had much lower root colonization by AM fungi than mesocosms planted with *Panicum* (Tukey's HSD < 0.0001) or both *Panicum* and *Uniola*

(Tukey's HSD < 0.0001). Roots from *Uniola* only pots were 31% ± 3 SE colonized, roots from *Panicum* only pots were 57% ± 3 SE colonized, and roots from pots containing both *Panicum* and *Uniola* were 56% ± 2 SE colonized. Root colonization by AM fungi was not affected by the native soil amendments, but the addition of the live native soil amendments did increase the density of extra-radical hyphae produced by AM fungi (Table 5; $F_{1,54} = 6.37$, $P = 0.01$). Mesocosms with the live native soil amendments had 62% more extra-radical hyphae than mesocosms without the live native soil amendments (Fig. 4). Despite the effects on other belowground responses, the treatments had no effect on soil aggregate mean weight diameter (Table S5).

DISCUSSION

Microbes in native soil amendments significantly influenced multiple restoration outcomes in our sand dune mesocosm experiment, as did the identity of the restored foundational grass species. However, no single restoration treatment maximized multiple ecosystem functions. The greatest plant productivity was achieved by planting a single, dominant grass species, *Panicum amarum*, and excluding live native soil amendments. Plant diversity was greatest in restorations with the grass species that was a weaker competitor, *Uniola paniculata*, and live native soil amendments increased *Uniola*'s ability to compete with *Panicum*. Live native soil amendments increased the density of arbuscular mycorrhizal (AM) fungal hyphae in the soil, but soil aggregation was unaffected the restoration treatments. In sum, different restoration techniques yielded different outcomes, each outcome provided unique restoration benefits, and

native soil microbial amendments did not necessarily provide a quick solution for maximizing multiple ecosystem functions.

There is growing interest in using soil microbial communities to improve plant establishment in restorations (Ohsowski et al. 2012; Wubs et al. 2016), but the effects of microbial amendments likely depend on microbial community composition. Manipulations of specific groups of soil microbes, especially AM fungi, have generally found positive effects on target plant establishment and growth (Stahl et al. 1988; Sylvia et al. 1993; Allen et al. 2003; Maltz & Treseder 2015), but this may not always be the case (Hoeksema et al. 2010). While there has been debate about whether commercial or native AM fungal inocula provide greater benefits – generally native inocula is better (Maltz & Treseder 2015; Middleton et al. 2015; Koziol & Bever 2017) – there has been less research on whether whole soil amendments provide greater benefits than the addition of specific groups of microbes. Soil communities contain a diversity of microbes that can directly or indirectly increase plant performance – including AM fungi, dark septate endophytes, saprobes, plant growth promoting bacteria (Smith & Read 1998; Reynolds et al. 2003; Newsham 2011) – and that may, together, provide greater benefits to plants (Larimer et al. 2014; Afkhami & Stinchcombe 2016). Soil communities also contain a diversity of organisms that can decrease plant performance (Bever et al. 2015), which may decrease the benefit of whole soil amendments. Despite our predictions that soil amendments would generally have positive effects on plant performance, especially in the relatively stressful dune environment (Bertness & Callaway 1994), the amendments decreased plant growth. The plants

we used in our experiment were purchased from a nursery, and we observed colonization by AM fungi and dark septate endophytes in the plant roots prior to planting them in our restoration experiment. Therefore, it is possible that native mutualists provided no greater benefit for plant performance than the microbes that colonized the plants in the nursery. Our results also suggest the presence of plant antagonists in the soils we used for our amendments. It is possible that homogenizing the native soil amendments across multiple sites artificially reduced variance in soil microbe effects among our replicates (Reinhart & Rinella 2016; Rinella & Reinhart 2018). For example, homogenization could have caused a rare microbe with a negative effect on plant performance to be present in all replicates. However, our goal was to test how microbial amendments, in general, influence restoration outcomes, and this method is similar to how we would recommend practitioners apply microbial amendments in the field.

While soil amendments may have mixed effects on plant performance, they tend to enhance plant community diversity. Additions of soil fauna increased plant diversity in restored grasslands (De Deyn et al. 2003), additions of native AM fungi increased plant diversity in restored prairies (Koziol & Bever 2017, 2019), and additions of whole soil significantly changed plant community composition in a restored grassland (Wubs et al. 2016, 2019; but see Kardol et al. 2009). Soil communities may help promote diversity by keeping abundant species in check. For example, species-specific enemies can generate negative density dependence through negative plant-soil feedback, leading to coexistence among species (Bever et al. 1997). Mutualists, such as AM fungi can increase diversity by promoting late-successional species that

are normally rare in young restorations (Koziol & Bever 2017), but the boost to diversity is expected to be temporary because of positive feedbacks between the AM fungi and plant species (Koziol & Bever 2019). We found evidence that native microbial amendments increase plant diversity not through positive effects on plants, but through stronger negative effects on the dominant plant species. If enemy-mediated negative density dependence plays a role in maintaining coexistence in this system, then the introduction of enemies through native soil amendments, while counter-intuitive, may provide important restoration benefits.

Soil microbes often play a key role in promoting soil aggregation (Blankinship et al. 2016). AM fungi are expected to be particularly important for soil aggregation in systems like sand dunes where hyphae can bind sand particles together (Forster & Nicolson 1981; Sylvia, 1986; Read 1989). Interestingly, we found increased densities of mycorrhizal hyphae with the addition of native soil amendments, but increased hyphal densities did not translate into increased geometric mean weight of soil aggregates. We suspect that the lack of effect on soil aggregation may be related to the duration of the experiment. A meta-analysis of the effects of AM fungi on soil aggregation found that after 5 months the positive effect of AM fungi on soil aggregation tend to decline in pot experiments, possibly because of root growth overwhelming the effect of AM fungi (Leifheit et al. 2014). At the time we measured soil aggregation, the experiment was over two years old and plant roots were dense. In a field setting, where roots are not constrained by pot size and unlikely to be as dense, the increased hyphal density conferred by live microbial amendments may increase soil aggregation. In any case, the increase in AM fungal

hyphal density with native soil amendments suggests that the composition of the native AM fungal community is different from the AM fungal community that colonized plants in the nursery. If increased hyphal density does translate to greater soil aggregation in the field, then native AM fungi may provide superior dune stabilization benefits.

The identity of restored grasses, but not their diversity, influenced restoration outcomes. Generally, plant diversity has a positive effect on ecosystem functions (Hooper et al. 2005; Cardinale et al. 2012; Tilman et al. 2014), including plant productivity (Tilman et al. 1996) and soil aggregation (Pohl et al. 2009). However, we found that the responses of restorations with only *Panicum* were largely indistinguishable from the responses of restorations with both *Panicum* and *Uniola*. Given *Panicum*'s dominance, the overwhelming effect of *Panicum* on responses in mixtures is not especially surprising. In other systems with highly abundant foundation species, genetic diversity within a species can have stronger effects on ecosystem functions than species diversity (Hughes et al. 2008; Crawford & Rudgers 2012, 2013), but we did not account for genetic diversity within species in our experiment. Despite the lack of a diversity effect, differences in restoration outcomes in monocultures of *Panicum* and *Uniola* emphasize the importance of considering species-specific effects and the goals of restoration.

Our results highlight potential avenues for further research on the utility of soil amendments in restorations. First, the effects of soil microbes on plant responses can be very context-dependent (Hoeksema et al. 2010). Therefore, our mesocosm experiment may underestimate the effect of soil amendments if soil microbes provide benefits that only manifest

in natural dune systems (e.g., increased salt tolerance, Borde et al. 2017). Second, microbial community composition can strongly influence how microbes affect communities and ecosystems (Klironomos 2003; Koziol & Bever 2017). Additional studies that identify the microbes underlying desirable restoration outcomes could lead to the development of a commercial native microbe mix that could be used in restorations. Third, it is possible that a single application of microbial amendments does not have lasting effects. For example, one study found that inoculating *Uniola paniculata* with AM fungi caused immediate increases in plant performance, but non-inoculated plants and inoculated plants were the same size in subsequent seasons (Sylvia 1989). However, recent work has shown that a single application of a soil amendment can influence plant and soil community composition for at least 20 years (Wubs et al. 2019).

Given the important role that soil microbes play in structuring plant communities and mediating ecosystem functions, there is growing interest in harnessing them to restore degraded ecosystems. Our results show that native microbial amendments increased plant diversity and have the potential to increase soil stability, but these benefits come at the cost of decreased plant performance. Because increased plant diversity and soil stability are desirable restoration outcomes – and because the cost to plant performance was relatively small – it could be argued that native soil amendments provide a net benefit to sand dune restorations. The ease and low cost of adding soil amendments to restorations make this a feasible and easily implemented method to boost restoration outcomes. Interestingly, the positive effect of amendments on plant

diversity may be driven by plant pathogens, suggesting a novel role for enemy introduction in the restoration of native ecosystems.

ACKNOWLEDGMENTS

The authors would like to thank the University of Houston Coastal Center for providing site access and logistical support. Thanks to Claire Alverson, Sandy Berger, Yash Desai, Patricia Gianeselo, Jakob Joachin, Huda Khan, Olubunmi Oladapo, and Carlos Perez for assistance in the lab and field, and to Scott Clark and Jan Dudenhoeffer for helpful discussions. This work was supported in part by an Institutional Grant (NA14OAR4170102) to the TSGCP from NOAA, U.S. Dept. of Commerce.

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FIGURE LEGENDS

Figure 1. Effect of plant diversity and native soil amendments on (A) total aboveground biomass and (B) belowground biomass within a 300 ml soil core. Mesocosms contained either *Panicum* monocultures, *Uniola* monocultures, or equal mixes of both grasses. Bars indicate treatment means ± SE.

Figure 2. Effect of plant diversity and native soil amendments on individual aboveground of (A) *Panicum* and (B) *Uniola*. Mesocosms contained either monocultures of the grasses or equal mixes of both grasses. Bars indicate treatment means ± SE.

Figure 3. Effect of initially planted diversity on colonist biomass. Mesocosms contained either *Panicum* monocultures, *Uniola* monocultures, or equal mixtures of both grasses. Bars indicate mean total colonist biomass ± SE. The percentage of average colonist biomass composed of the three colonists (*Bacopa*, *Sesuvium*, and *Ipomoea*) is indicated by the stacked bars.

Figure 4. Effects of plant diversity and native soil amendments on density of extra-radical hyphae in soils. Mesocosms contained either *Panicum* monocultures, *Uniola* monocultures, or equal mixtures of both grasses. Bars indicate treatment means ± SE.

Figure 1.

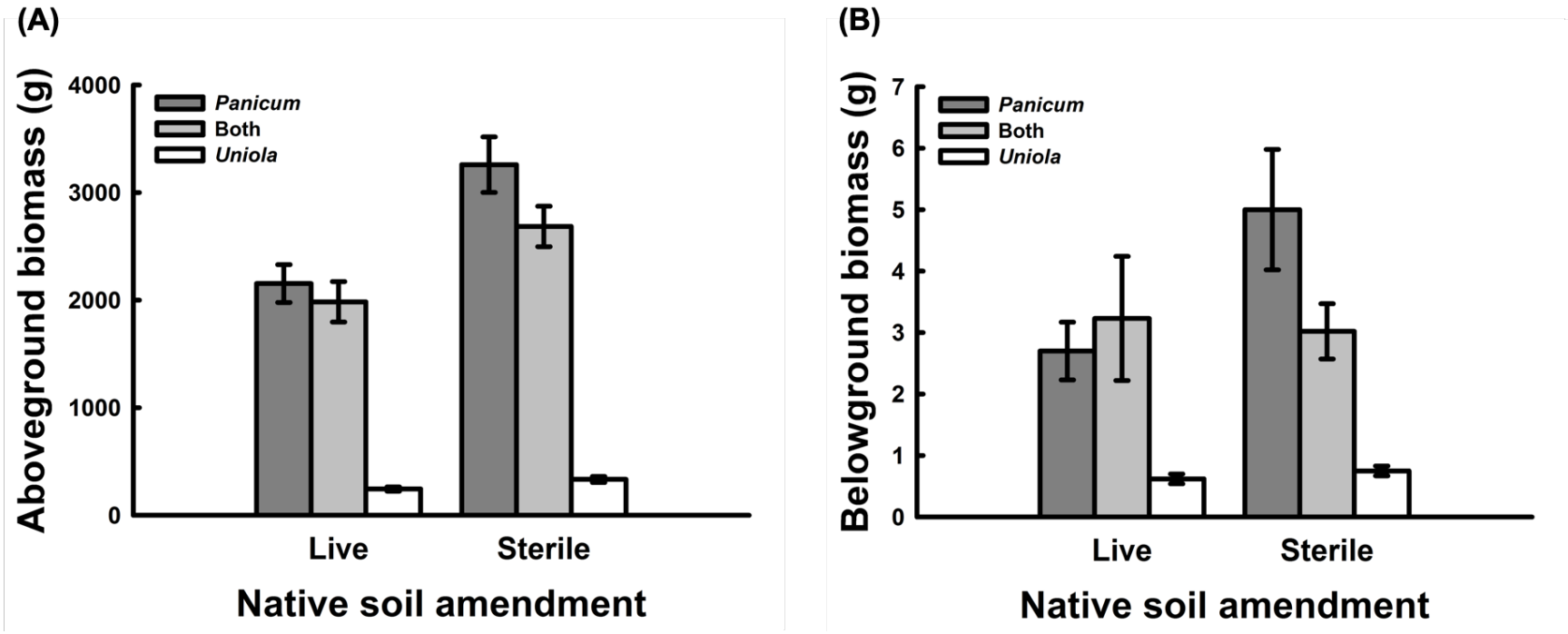

Figure 2.

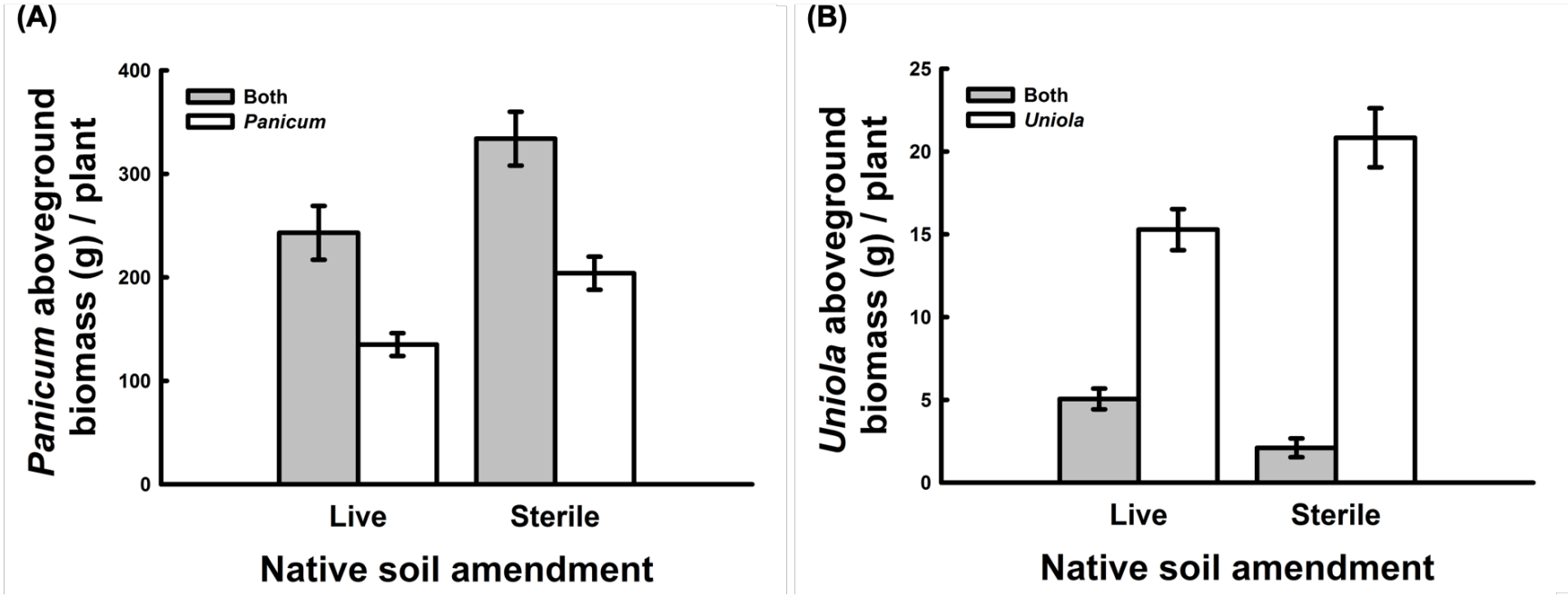

Figure 3.

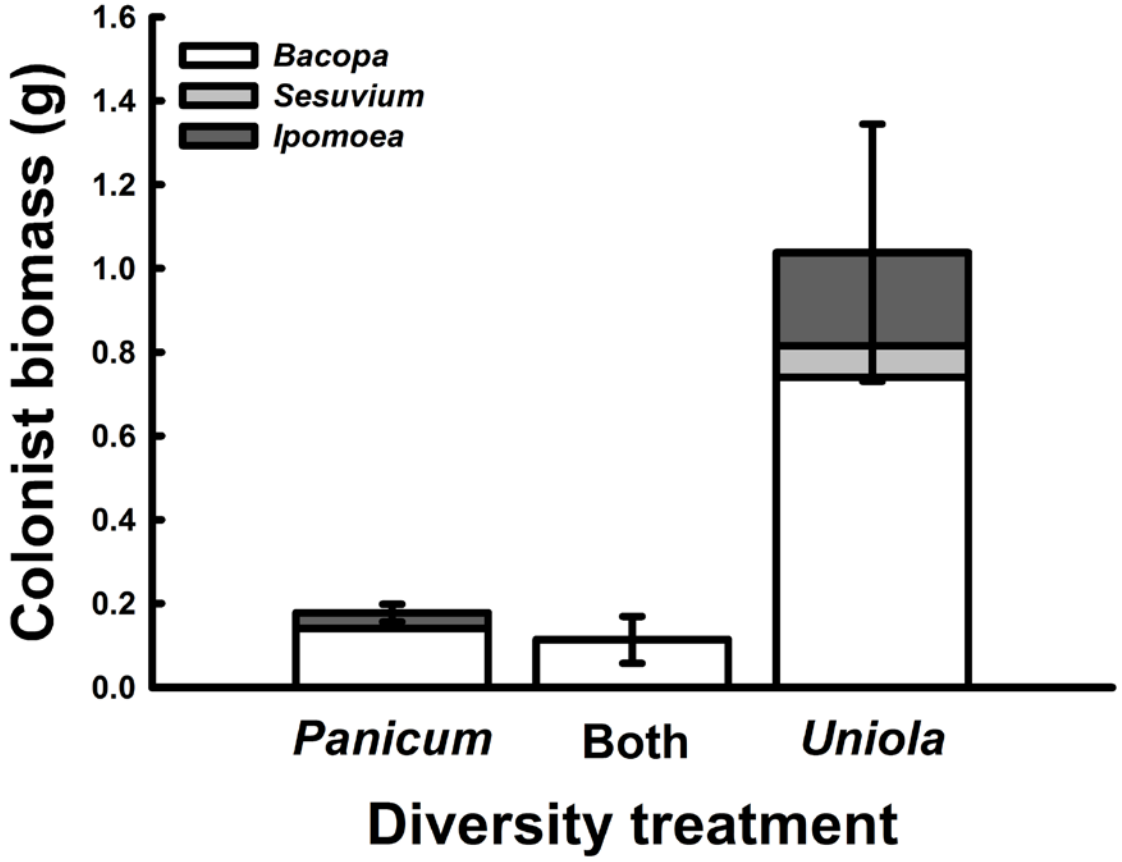

Figure 4.