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Source: Journal of Coastal Research, 37(5) : 1025-1034

Published By: Coastal Education and Research Foundation

URL: https://doi.org/10.2112/JCOASTRES-D-20-00078.1

Journal of Coastal Research	37	5	1025–1034	Coconut Creek, Florida	September 2021

Effect of Salt, Nutrients, and Native Microbe Additions on Common Dune Restoration Grasses

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ABSTRACT

Clark, G.S.; Busch, M.H., and Crawford, K.M., 2021. Effect of salt, nutrients, and native microbe additions on common dune restoration grasses. *Journal of Coastal Research,* 37(5), 1025–1034. Coconut Creek (Florida), ISSN 0749-0208.

Sand dunes are a valuable resource that shelters coastal communities and wildlife. Unfortunately, their survival is threatened by commercial development and erosion accelerated by climate change. Restoring sand dunes is challenging because of the difficulty in establishing vegetation in such a hostile environment. Restoration practitioners have responded by choosing stress-tolerant native species, adding nutrients, or incorporating native microbial communities into transplanted soils, but little is known about how these practices interact. In other systems, native microbes sometimes increase plant performance and salt tolerance. Nutrient additions promote growth but may inhibit development of beneficial fungi and disproportionately benefit some plant species at the expense of community diversity. To explore these interactions, two grasses commonly used in dune restorations—*Panicum amarum* and *Uniola paniculata*—were grown in a greenhouse and exposed to soil with and without native microbiota and to a range of nutrient and salinity treatments. Native microbial additions had little impact on live, aboveground biomass or the salt tolerance of the nursery-grown plants. Nutrient additions did not inhibit beneficial fungi. In fact, the density of extraradical hyphae in the soil increased by an average of 31% across all plants subject to a balanced, slow-release fertilizer. The two grasses responded differently to the salinity and nutrient treatments. Under a 2.5% salinity treatment, average live aboveground biomass for *P. amarum* fell 52%, compared with a 5.5% increase for *U. paniculata*. None of the nutrient additions affected *U. paniculata*; however, a combination of nitrogen and phosphorus increased *P. amarum* live, aboveground biomass by 356%. The strong response of *P. amarum* to fertilization offers a path to rapid growth of dune vegetation, but it may foster that species' dominance, which could lower the plant community's aggregate resistance to salinity.

ADDITIONAL INDEX WORDS: *Coastal ecosystems, restoration, vegetation, fungi, fertilization, salinity, community diversity, grasses.*

INTRODUCTION

Commercial development and recreational activity, along with rising seas and more frequent severe storms due to climate change, have combined to threaten the survival of coastal sand dunes (Mendoza-González *et al*., 2012; Nordstrom, 2008). Because of their importance in buffering storm surges (Silva *et al*., 2016), mitigating erosion (Feagin *et al*., 2019; Sigren *et al*., 2018), and providing a habitat for diverse flora and fauna (Defeo *et al*., 2009; Everard, Jones, and Watts, 2010), considerable effort is expended by public and private agencies to conserve and restore coastal dune systems (Lithgow *et al*., 2013). Restoration efforts concentrate on the two key ingredients of dune development: sand and vegetation. Sand is often collected and transported from remote locations (Spodar *et al*., 2018), whereas native vegetation is planted to promote and stabilize dune development (Nordstrom, 2008). Vegetation anchors the soil and creates a barrier that slows down the wind, causing it to drop its suspended sand and to build dune mass (Nordstrom, Psuty, and Carter, 1990). The long-term success of restoration depends in part on this interaction of soil and vegetation. Unfortunately, dunes present a hostile environment that often defies restoration: Coastal sands lack moisture, are poor in nutrients, and exhibit a range of salinity (Lane *et al*., 2008; Long, Fegley, and Peterson, 2013). Dune restoration practitioners sometimes moderate these stresses by choosing native plant species that are stress tolerant, by adding nutrients (Long, Fegley, and Peterson, 2013), or by incorporating soil microbial communities that help mitigate plant stress (Emery and Rudgers, 2011). Although each of these practices may foster dune vegetation establishment, little is known about how they interact.

Many studies have demonstrated the importance of soil microbiota for plant productivity in general (Crawford *et al*., 2019; Van Der Heijden, Bardgett, and Van Straalen, 2008) and for dune vegetation in particular (Emery and Rudgers, 2011; Sylvia, 1989; Sylvia, Jarstfer, and Vosátka, 1993). Dune plants develop relationships with both soil bacteria and arbuscular mycorrhizal fungi (AMF) (Corkidi and Rincón, 1997; Gemma and Koske, 1997; Lambers *et al*., 2008; Roy-Bolduc *et al*., 2016). The AMF extend the plants' root structure to absorb water and to provide accessible phosphorus to the plant in return for carbon (Nouri *et al*., 2014). Rhizobacteria fix nitrogen and make that essential nutrient available to their hosts (Will and Sylvia, 1990). Some soil microbiota also release metabolites that may promote dune plant growth (Khan *et al*., 2012; Muthezhilan *et al*., 2012; Shin *et al*., 2007). Other studies have shown that soil fungi and bacteria moderate salt stress in some plant species

DOI: 10.2112/JCOASTRES-D-20-00078.1 received 13 June 2020; accepted in revision 23 January 2021; corrected proofs received 18 March 2021.

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(Azad and Kaminskyj, 2016; Baltruschat *et al*., 2008; Hashem *et al*., 2016; Qin *et al*., 2018; Sinclair *et al*., 2013; Talaat and Shawky, 2014).

For restoration, there is evidence that native soil communities offer an advantage over microbial amendments from other sources. A meta-analysis of 28 field studies suggested that native mycorrhizal inocula produce greater root colonization than commercial supplements (Maltz and Treseder, 2015). Similarly, native rhizobacteria can enhance nitrogen fixation in common beach grasses (Dalton *et al*., 2004). However, a study directly comparing commercial and native soil inocula in dune restorations produced mixed results. At one location, native inocula fostered twice the productivity of commercial amendments, but no difference occurred between them at two other sites (Sylvia, Jarstfer, and Vosátka, 1993).

Some common restoration practices may undermine the potential benefits of native soil microbiota. For dune and beach replenishment, restoration practitioners often draw on submerged sands from offshore locations that have no recent vegetative history (Silveira, Santos, and Andrade, 2013; Spodar *et al*., 2018). Dredged soils may be convenient, but they tend to lack robust microbial communities, delaying the development of beneficial plant-microbial associations (Sylvia and Will, 1988). Other research has shown that excess nutrients, particularly phosphorus, can retard AMF growth (Camenzind *et al*., 2014; Nouri *et al*., 2014; Shantz, Lemoine, and Burkepile, 2016). In fact, the relationship between AMF and their host plants may shift from mutualistic to parasitic in response (Aleklett and Wallander, 2012; Johnson, Graham, and Smith, 1997; Kogel, Franken, and Hu, 2006). So, whereas fertilization may enhance the productivity of dune vegetation in the short term, it may inhibit development of the mutualistic relationships that are important to the plants' long-term success. Because mycorrhizal fungi are important to the salt tolerance of some species, their inhibition in turn could increase the vulnerability of some coastal species to salt exposure. Further complicating the interaction of these restoration practices is the fact plant species often respond differently to nutrient additions, abiotic stresses, and their microbial community context (Armitage, Frankovich, and Fourqurean, 2011; Biederman *et al*., 2017; Harris, Zinnert, and Young, 2017), which can lead to unexpected shifts in community composition. Abiotic stresses in dune environments include low moisture and nutrient availability, soil salinity, high temperatures, and frequent sand burial (Maun, 1994; Rajaniemi and Allison, 2009). The microbial community context refers to the makeup of the soil microbiome, including bacteria and mycorrhizal fungi. The community context may be the soil in which the plants were grown, the native soil into which they are transplanted, or the microbial community created by the addition of commercial inoculum. A difference in species response to these factors is possible because (1) zoning of plant species into microhabitats is common in dune ecosystems (Sorce, Bottega, and Spanò, 2019); (2) plant species, in general, sometimes respond differently to nutrients (Biederman *et al*., 2017); and (3) a difference in the response of these dune plant species to salinity is noted in the literature (Seneca, 1972b).

In this study, two separate experiments test how salinity and nutrient additions influence the outcome of interactions between native soil microbes and two grass species commonly used in dune restorations, *Panicum amarum* and *Uniola paniculata*. Both species are dominant perennial C_4 grasses in coastal dune systems stretching from the temperate U.S. Atlantic Coast, across the Gulf Coast, and into Mexico (Hacker *et al*., 2019). *Uniola paniculata* has leaf blades up to 80 cm in length and culms reaching 2.5 m (Lonard, Judd, and Stalter, 2011). *Panicum amarum* has leaf blades 20–40 cm in length and culms reaching up to 1.5 m (Lonard and Judd, 2010). The two species often grow together in the backshore above the high tide line, the windward side of foredunes, and on the windward and leeward slopes of the primary dune system, where they facilitate the establishment of other species (Lonard and Judd, 2010; Lonard, Judd, and Stalter, 2011).

Specifically, the experiments address five questions.

(1) Do native soil microbiota enhance the productivity of these common dune restoration grasses?
(2) Do native soil microbiota ameliorate salt stress in these grasses?
(3) Does fertilization enhance the growth of these grasses?
(4) Does the provision of essential nutrients, particularly phosphorus, retard development of beneficial mycorrhizal fungi?
(5) Do any of these factors differ significantly between *P. amarum* and *U. paniculata*?

The hypotheses are as follows. (1) Dune grasses grown in soil inoculated with native microbiota will exhibit greater productivity and will experience less salt stress than those in soil without native soil bacteria and fungi. (2) Nutrient additions will increase plant productivity but will inhibit mycorrhizal fungal development as measured by the amount of extraradical hyphae (ERH) in the soil. (3) The intensity of these responses will vary by plant species.

METHODS

To test these hypotheses, the common dune restoration grasses *U. paniculata* and *P. amarum* (Dahl and Woodard, 1977; Sylvia and Will, 1988) were grown in a greenhouse and subjected to gradients of salt stress and nutritional supplements in both sterile sand and sand amended with inoculum from existing vegetated dunes (Figure 1).

Source Plants

Uniola paniculata and *P. amarum* plants were obtained from Green Seasons Nursery (Parrish, Florida), which is a common source of plants for dune restorations along the Gulf Coast, including Texas. The *P. amarum* plants were grown by the nursery from cuttings gathered on the Louisiana Coast, whereas *U. paniculata* were grown from seed collected along the Florida panhandle (G. Clark, *personal communication*). The plants, including the soil of their root balls, were potted in conical 262-mL Deepots (Stuewe & Sons, Tangent, Oregon) lined with newspaper. Both species were planted in masonry sand that was sterilized by autoclaving it twice for 60 minutes at 121°C, with a 24-hour rest period between sterilizations.

Figure 1. *Panicum amarum* and *U. paniculata* growing in a greenhouse in randomized pots just prior to harvest after 12 weeks of treatments.

Native Soil Microbial Amendments

Native sand, with a grain size of 0.12–0.14 mm, was collected from existing vegetated dunes at 15 sites in Galveston, Texas. The sand was drawn from the rooting zones of native plants, including the two species planted in the experiments, homogenized, and then stored at 4°C until planting. Half of the plants received 40 cc of the "live" native sand in their root zone. The other half received 40 cc of native sand that was sterilized using the same method as the background soils. All of the pots were topped off with sterilized sand to avoid cross-contamination during the experiment. The plants receiving the sterile treatment lacked native dune inocula, but they were colonized by microbes during their growth at Green Seasons Nursery, a commercial greenhouse. Upon arrival, the root colonization of two *P. amarum* and *U. paniculata* plants was measured using a procedure modified from the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi, and they were found to be colonized 96% and 87%, respectively, by AMF (Crawford *et al.*, 2020). So, the experiment is a conservative test of how native microbes influence plant performance while reflecting a realistic scenario for the use of native microbial amendments during dune restorations. The plants were maintained for 4 months in the greenhouse prior to beginning the treatments to allow for development of interactions between the plants and the soil microbes. The pots were suspended randomly in Deepot support trays (Stuewe and Sons, Tangent, Oregon), with a blank cell between plants. To control for initial differences in plant size prior to initiation of the salinity and nutrient treatments, maximum stem width for each plant was recorded 1 week before the beginning of treatments. For these species, maximum stem width is highly correlated with aboveground plant biomass ($R^2 = 0.90$ for *P. amarum* and $R^2 = 0.87$ for *U. paniculata*) (unpublished data). After the treatments began, the grasses were grown in a greenhouse at ambient temperatures from March through May 2017.

Salinity Experiment

The four species-soil pairings (*P. amarum*-sterile sand, *U. paniculata*-sterile sand, *P.-amarum* with native inoculum, and *U. paniculata* with native innoculum) were subjected to a gradient of seven NaCl solutions with 9–11 replicates for each group for a total of 280 plants. The groups were watered weekly with 50 mL of the following percentage concentrations of NaCl by weight: 0.00, 0.25, 0.50, 1.00, 1.50, 2.00, and 2.50. The salinity levels are consistent with those likely experienced by the plants in the dune environment and include those known to produce stress in the two dune grasses. In previous studies, growth of *P. amarum* and *U. paniculata* were affected by salinity levels above 1.00%, and growth was significantly dampened at 2.00% (Seneca, 1972a). Seawater has an average salinity of about 3.5%, and a random sampling of soil from Galveston dunes after recent precipitation in September 2019 yielded salinity levels of 0.50% or less (unpublished data). The saline solutions were prepared in bulk using Instant Ocean (Instant Ocean Spectrum Brands, Blacksburg, Virginia) and deionized water. Treatments were applied weekly for 12 weeks. Between the treatments, plants received tap water to prevent water limitation.

Nutrient Experiment

The four species-soil pairings were subject to one of five nutrient treatments with 9–10 replicates for each treatment group for a total of 200 plants. The groups were watered weekly with 50 mL of the following nutrients in solution: no nutrients, 15% nitrogen, 9% phosphorus, and 15% nitrogen + 9% phosphorus. The nitrogen treatments were prepared using 15.5-0-0 Hi-Yield Calcium Nitrate (Voluntary Purchasing Group, Bonham, Texas) and deionized water. The phosphorus treatments were prepared using Triple Super Phosphate 0-45-0 (Bonide Products, Oriskany, New York). An additional treatment group received 1 ounce of slow-release 15-9-12 Osmocote fertilizer (The Scotts Company, Marysville, Ohio), administered to the soil surface at the beginning of the experiment. Thereafter, the commercial fertilizer group received 50 mL of deionized water, the same as the control group. Between the treatments, plants received tap water to prevent water limitation. The treatments were applied weekly for 12 weeks.

Responses

At the end of 12 weeks, the aboveground biomass for all 480 plants was harvested with live and dead tillers weighed separately. Dead tillers represent senesced biomass from the previous growing season as well as aboveground biomass that may have senesced during the experiments. To be considered alive, a tiller had to have at least 25% live (green) vegetation.

Table 1. *The table reports the degrees of freedom (d.f.), F-ratio (F), and P-value (*P*) resulting from general linear models testing the effects of salinity, native soil microbes, and plant species identity on live aboveground, total aboveground, belowground, and total plant biomass. Initial stem width was included as a covariate. Bold values are significant at* $P < 0.05$*. The d.f. differ for aboveground and belowground/total responses because belowground biomass was harvested in a subset of pots.*

		Live aboveground		Total aboveground			Belowground		Total	
Factor	d.f.	*F*	*P*	*F*	*P*	d.f.	*F*	*P*	*F*	*P*
Stem diameter	1, 270	9.06	**0.003**	22.51	<**0.0001**	1, 79	9.36	**0.003**	14.59	<**0.001**
Species	1, 270	2.09	0.1497	47.94	<**0.0001**	1, 79	52.74	<**0.0001**	15.10	<**0.001**
Microbes	1, 270	0.02	0.8997	0.27	0.6050	1, 79	1.62	0.2067	4.73	**0.03**
Salinity	1, 270	12.69	<**0.001**	0.49	0.4832	1, 79	2.38	0.1266	0.03	0.8554
Species × microbes	1, 270	0.81	0.37	0.00	0.9953	1, 79	0.28	0.5955	1.92	0.1697
Species × salinity	1, 270	4.17	**0.04**	2.28	0.1322	1, 79	0.05	0.8302	0.12	0.7304
Microbes × salinity	1, 270	0.37	0.85	0.07	0.7860	1, 79	2.98	0.0881	5.93	**0.02**
Species × salinity × microbes	1, 270	3.65	0.06	0.96	0.3271	1, 79	0.40	0.5292	1.37	0.2456

The shoots were clipped at the crown and dried in paper bags for four days at 60°C before weighing. One plant was dropped from the aboveground analysis because of a missing label. Belowground biomass was harvested, dried, and weighed for a random selection of 150 plants: 88 from the salinity experiment and 62 from the nutrient experiment. Each treatment level was represented by at least 10 plants. To measure ERH produced by AMF, 148 random soil samples were set aside and refrigerated at 4°C. The abundance of ERH in the soil is closely related to the level of root colonization by AMF (Kabir, O'Halloran, and Hamel, 1997). Each treatment level was represented by at least 10 soil samples. To quantify ERH, homogenized 20-g soil samples were suspended in 500 ml of deionized water by stirring at 700 rpm for 2 minutes. Suspended organics were filtered through a stack of 500-μm and 212-μm sieves. Catchments, which included hyphae, on the 212-μm sieve were transferred to a beaker with 10 ml of deionized water, and hyphae were stained for at least 3 hours using 20 drops of 0.4% trypan blue. After staining, hyphae were transferred to a 38-μm sieve and rinsed with deionized water for 5 minutes. The stained hyphae were then suspended in 200 mL of deionized water by stirring at 700 rpm for 1 minute, followed by stirring at 300 rpm for 30 seconds. After the suspension was counter-stirred with a glass rod, a syringe was used to transfer a 20-ml aliquot of the suspension to a vacuum filter with a 0.45-μm Nylon membrane filter. Repeating the process produced two filters for each plant. The filters were mounted in pairs on a slide and bisected with a line to produce four halves for each plant sample. The length of ERH on each filter half was estimated using the gridline intersect method (Giovannetti and Mosse, 1980) while being magnified at 100×. Hyphal lengths across the four half-filters were averaged prior to data analysis.

Statistical Analyses

To determine how the treatments influenced the responses of live aboveground, total aboveground, and belowground biomass, separate general linear models were used for the salinity experiment and the nutrient experiment. Before analysis, the biomass data were square-root transformed to improve the homogeneity of variances. For the salinity experiment, the general linear model contained the fixed effects of salinity (continuous variable), soil microbial treatment (live or sterile), plant species, their interaction, and stem width as a covariate to control for initial differences in plant size. For the nutrient experiment, the general linear model contained the fixed effects of soil nutrient treatment (C, N, P, N+P, commercial fertilizer), soil microbial treatment (live or sterile), plant species, their interaction, and stem width as a covariate to control for the difference in initial plant size. All analyses were conducted in R (version 3.6.0) using the car (Fox and Weisberg, 2019), pastecs (Grosjean and Ibanez, 2018), emmeans (Searle, Speed, and Milliken, 1980), and multcomp packages (Hothorn, Bretz, and Westfall, 2008). Post-hoc comparisons of means were conducted using Tukey's honestly significant difference (HSD). Comparisons of the trend in the response of the two dune grasses to the salinity gradient was conducted with the emtrends() function in the emmeans package in R. The analyses focused on live, aboveground biomass, which represented plant growth during the experiment. Total aboveground biomass included senesced plant material from the previous growing season, whereas belowground biomass growth was limited by the fact that most plants were root bound at harvest.

Mean ERH length was log-transformed before analysis to improve the homogeneity of variance and was evaluated in each treatment using a general linear model containing the fixed effects of salinity (continuous) or soil nutrients and soil microbial treatment, their interaction, and initial stem width as a covariate to control for differences in initial plant size. Post-hoc comparisons of means were conducted using Tukey's HSD. The effect of native soil microbes was also tested by pooling data across both experiments.

RESULTS

In the salinity experiment, increasing salinity reduced live aboveground plant biomass (Table 1; $p < 0.001$). *Panicum amarum* was less salt tolerant than *U. paniculata* (Table 1; Species identity × salinity $p = 0.04$; Figure 2). The significant species-salinity interaction persisted following elimination of three outlying data points with a biomass of more than 2 g in the 2.00% and 2.50% salinity treatments. Relative to the no salt control, live aboveground biomass for *P. amarum* was 52% lower at 2.50% salinity, whereas *U. paniculata* biomass was 5.5% higher. Native soil microbes had no independent or interactive effect on live aboveground biomass (Table 1). Total aboveground and belowground biomass were affected by species identity, with *P. amarum* consistently producing more biomass than *U. paniculata* (total aboveground: 2.70 g ± 0.10 SE *vs*. 1.09 g ± 0.04 SE, respectively; belowground: 1.14 g ± 0.07 SE *vs*. 0.22 g ± 0.02 SE, respectively). Native microbes

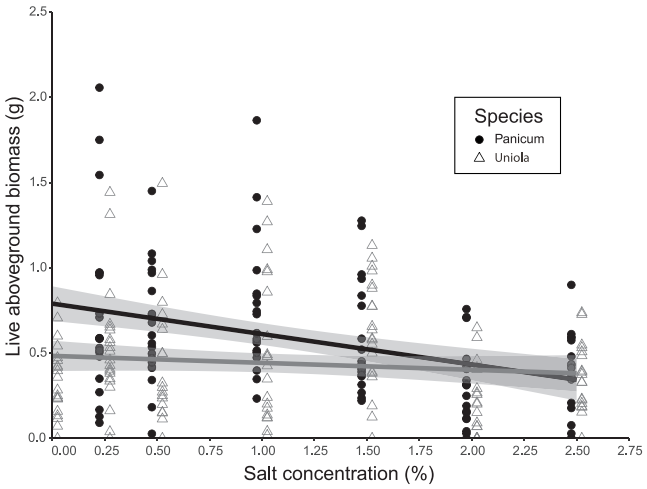

Figure 2. Live aboveground biomass for *P. amarum* (black circle) and *U. paniculata* (gray triangle) across salinity levels. Trend lines include ±SE for each species. Two outliers for *P. amarum* and one for *U. paniculata* were excluded, although they did not affect the interpretation of the results.

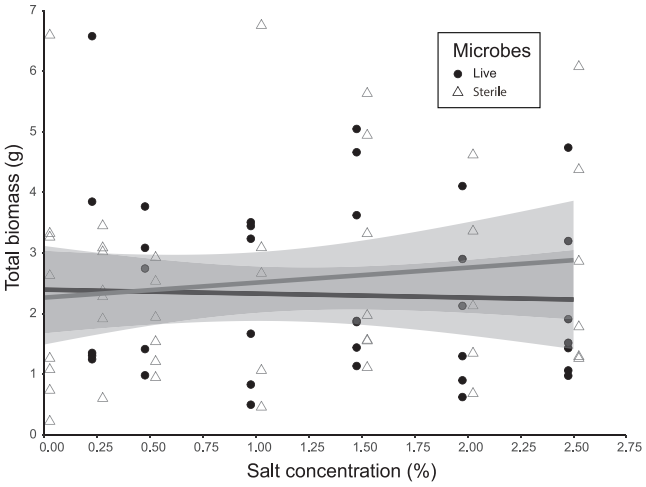

Figure 3. Total biomass for plants grown in sterilized background soil with live native microbial inoculum (black circle) and plants grown in sterilized background soil with sterilized inoculum (gray triangle), across salinity levels. Trend lines include ±SE for each soil treatment. Plants in the sterile treatment still had the microbial community they acquired from the nursery.

and salinity levels interacted significantly to affect total plant biomass (Table 1; Microbes × salinity, $p = 0.02$; Figure 3), with sterile soil producing higher plant biomass at increasing salinity levels than native soil. However, the interaction was not significant for live aboveground ($p = 0.85$), total aboveground ($p = 0.786$), or belowground biomass $p = 0.088$) considered independently (Table 1).

In the nutrient experiment, *U. paniculata* was unresponsive, with no significant change in live aboveground biomass between the control and any of the nutrient additions (Tukey's HSD, $p \geq 0.58$ for all comparisons) (Figure 4, Table 2). In contrast, *P. amarum* responded robustly to the nutrient additions across all treatments (Tukey's HSD, $p < 0.001$ for all comparisons between the control and nutrient additions). For example, *P. amarum*'s average live, aboveground biomass in the nitrogen + phosphorus treatment was 356% percent greater than in the untreated control. The response of total aboveground biomass was similar to live, aboveground biomass, but belowground and total biomass responded only to plant species identity (Table 2). As in the salinity experiment, *P. amarum* generally produced more biomass than *U. paniculata* (belowground biomass: 1.60 g ± 0.10 SE *vs*. 0.30 g ± 0.02 SE, respectively; total biomass: 5.37 g ± 0.33 SE *vs*. 1.67 g ± 0.11 SE, respectively). Microbes did not independently or interactively influence biomass (Table 2).

The treatments in the two experiments had little impact on ERH produced by AMF. Salinity did not influence ERH density (Table 3, $p = 0.21$), and nutrient additions had weak effects (Table 3, $p = 0.04$). The commercial fertilizer treatment produced 20% greater ERH density than the control and significantly more than any of the other nutrient treatments (Figure 5). In both experiments, *P. amarum* had greater ERH density than *U. paniculata* (salinity: 108.2 mm/20 g ± 12.7 SE *vs*. 36.2 mm/20 g ± 8.1 SE, respectively; nutrients: 115.9 ± 17.3 SE *vs*. 33.9 mm/20 g ± 5.0 SE, respectively). The addition of native soil microbes did not influence ERH density in either experiment (Table 3). However, if analyzed across both experiments, soils with the live native microbe inoculum had a 31% higher ERH density than those with the sterile treatment ($F_{1,143} = 10.25$, $p = 0.002$) (Table 3, Figure 6).

DISCUSSION

Native soil microbiota offered little advantage in increased plant productivity or salt tolerance beyond the microbial communities already present in the dune grasses on arrival

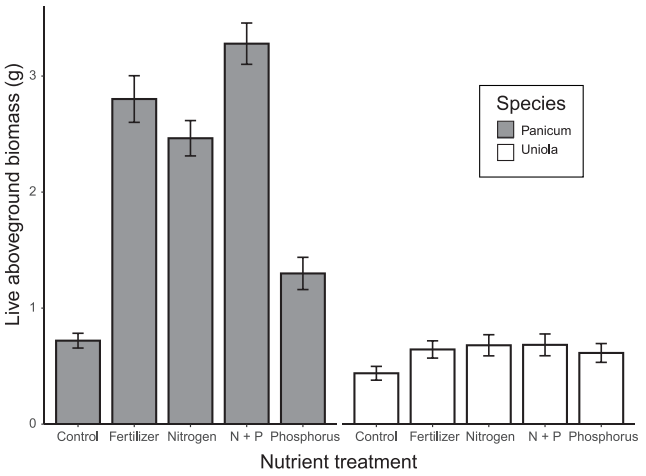

Figure 4. Average live aboveground biomass for *P. amarum* (gray) and *U. paniculata* (white) under the different nutrient treatments (no nutrients [C], slow-release commercial fertilizer [F], 15% nitrogen [N], 9% phosphorus [P], and N + P). Error bars represent ±SE.

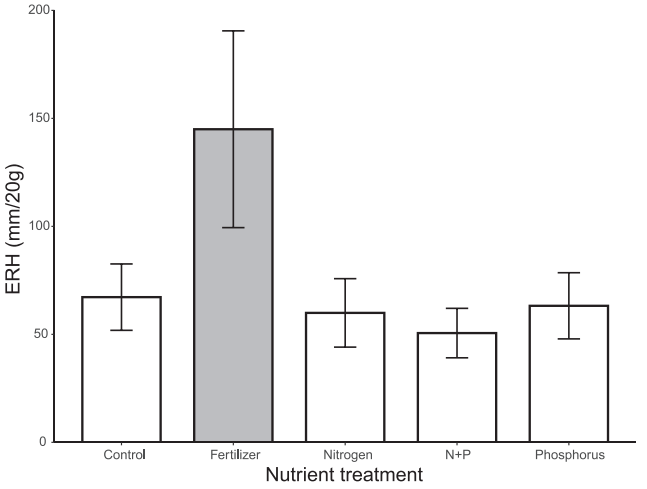

Figure 5. Average length of fungal extraradical hyphae (ERH) in the soil (mm/20 g soil) in the different nutrient treatments. Error bars represent ±SE. Gray bar indicates that the mean differed significantly from the control.

from the commercial nursery. Nutrient additions did not inhibit growth of mutualistic fungi, and, in fact, the slow-release commercial fertilizer enhanced it. A significant difference in the two species' response to nutrients and salinity, however, has important implications for dune restoration strategies. *Panicum amarum* responded much more robustly to nutrient additions, whereas *U. paniculata* was largely unaffected by rising salinity. Live aboveground biomass, representing plant growth during the experiment, was most responsive to the treatments. The general lack of response of other biomass measures likely reflected the diluting effect of senesced biomass, which was much larger for *P. amarum*, and the fact that most plants were root bound at harvest, which tended to equalize belowground biomass. The live:dead biomass ratio for *P. amarum* across all treatments was 0.609, compared with 0.815 for *U. paniculata*.

Native Soil Microbiota

The dune grasses' lack of response to the native soil inoculum was surprising, as previous comparisons have suggested a benefit in using native soil microbiota over commercial amendments (Maltz and Treseder, 2015; Middleton *et al*., 2015). In this study, the control plants lacked native Texas dune microbiota, but it is possible that the existing community that arrived with the plants was functionally similar enough to the native one to blur the distinction between the soil treatments. An experiment testing the effects of native soil amendments using plants grown from seed in sterile conditions could help resolve this question. Another possibility is that native pathogens may have offset any benefit provided by native mutualists (Bever, Mangan, and Alexander, 2015). A previous field study of soil microbial supplements across several dune systems showed that the response of dune plants to native and commercial amendments may vary by their abiotic context (Sylvia, Jarstfer, and Vosátka, 1993). So, under different conditions, native microbes may have a positive effect on plant performance. The small but significant increase in total biomass related to the interaction of microbes and salinity was likely an artifact of accumulated senesced biomass. Nonetheless, the higher ERH density in the soil of plants with the native microbial treatment suggests that the native additions exerted some influence over the structure of the soil community, even if they did not generally translate into higher plant productivity. Genetic characterization of the soil microbiome of the plants by 16S and ITS2 sequencing would provide more insight into the role of particular pathogens or functional groups in the results and into how the microbiomes from the commercial greenhouse and native locations interact.

Nutrients

Panicum amarum's much greater growth in response to nutrient additions makes it tempting to rely on that species to quickly build dune vegetation, but doing so may have other consequences. A greenhouse study by Hester and Mendelssohn (1990) suggests that the contrasting response of *P. amarum* and *U. paniculata* may stem from different nutrient requirements. Day *et al*. (2018) saw a similar difference between two other coastal grasses, where addition of nitrogen to coastal dune systems increased productivity of one species—*Ammophila breviligulata*—while reducing growth of the other—*Spartina patens*. When plants respond differently to soil nutrients, their relative abundance in the community can shift in response (Biederman *et al*., 2017). Nutrients may mediate the changes directly (Avolio *et al*., 2014) or indirectly by altering the composition of fungal and bacterial communities in the soil (Wang *et al*., 2018; Zhang *et al*., 2017). The stronger response of *P. amarum* to nutrient additions is likely to accentuate its dominance in restored dune communities. Even

Table 2. *Results from general linear models testing the effects of soil nutrients, native soil microbes, and plant species identity on live aboveground, total aboveground, belowground, and total plant biomass. Initial stem width was included as a covariate. Bold values are significant at* $P < 0.05$. *Degrees of freedom (d.f.) differ for aboveground and belowground/total responses because belowground biomass was harvested in a subset of pots.*

		Live aboveground		Total aboveground			Belowground		Total	
Factor	d.f.	*F*	*P*	*F*	*P*	d.f.	*F*	*P*	*F*	*P*
Stem diameter	1, 179	5.86	**0.02**	10.60	**0.001**	1, 41	2.43	0.13	1.78	0.19
Species	1, 179	75.97	<**0.0001**	109.33	<**0.0001**	1, 41	73.22	<**0.0001**	50.91	<**0.0001**
Microbes	1, 179	3.04	0.08	1.066	0.30	1, 41	0.24	0.63	1.05	0.31
Nutrients	4, 179	36.11	<**0.0001**	11.19	<**0.0001**	4, 41	0.63	0.64	0.64	0.64
Species × microbes	1, 179	0.16	0.69	0.05	0.82	1, 41	0.00	0.96	0.17	0.68
Species × nutrients	4, 179	19.46	<**0.0001**	3.58	**0.0078**	4, 41	1.42	0.24	1.54	0.21
Microbes × nutrients	4, 179	0.94	0.44	1.94	0.12	4, 41	0.88	0.49	1.88	0.13
Species × microbes × nutrients	4, 179	0.27	0.90	0.33	0.86	4, 41	1.36	0.27	1.34	0.27

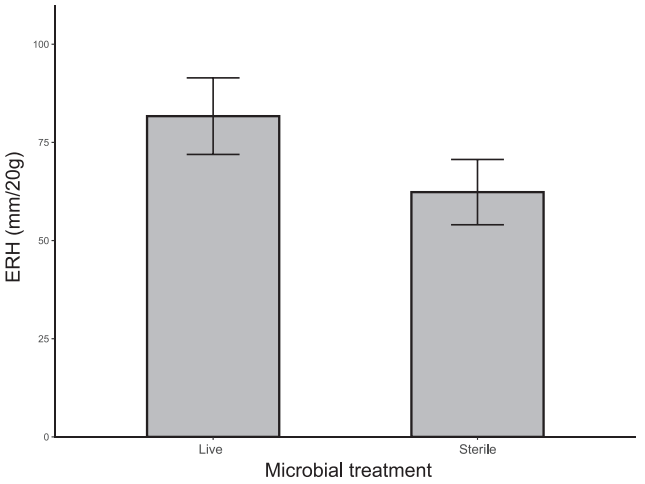

Figure 6. The effect of live and sterile native soil amendments on the average length of fungal extraradical hyphae (ERH) in the soil (mm/20 g soil). Error bars represent ±SE. The data combine both the nutrient and salinity experiments.

without fertilization, *P. amarum* tends to overwhelm associated *U. paniculata* (Crawford *et al*., 2020). Thus, fertilization may increase overall productivity of dune vegetation, but it can come at the cost of species diversity (Day *et al*., 2004; Lammerts *et al*., 1999). In a community setting with more complex interactions, the outcome may be more difficult to predict. In a study of the impact of fertilization on four common dune grasses, *P. amarum* growth was stronger in monoculture than *U. paniculata*, but when four species were grown together, *P. amarum* was no longer overwhelmingly dominant (Long, Fegley, and Peterson, 2013). Plant diversity is important to the resilience of dune communities because if plant species exhibit a range of tolerance to abiotic stresses, it is less likely that a disturbance in one will devastate the entire community.

Although most of the nutrient treatments had no effect on soil ERH density, the commercial fertilizer treatment increased ERH by 20%, contrary to the expectation that nutrients could cause a breakdown in the mutualistic relationship between AMF and their plant hosts. At least two explanations are possible: (1) The slow-release fertilizer never raised soil nutrient levels high enough to interfere with the mutualistic relationship and (2) micronutrients in the fertilizer facilitated fungal growth. In any case, the result suggests that a slow-release fertilizer may be able to foster dune plant growth and beneficial fungal mutualisms at the same time. The other, more intense and narrow nutrient treatments did not enhance ERH density, nor did they have the expected inhibitory effect. It is possible that the experimental window may not have been long enough to see a shift in the mutualistic relationship between the plants and soil fungi, although one field study of dune vegetation found significant reduction in AMF colonization in plants after only 10 weeks of fertilization (Holte, 1994). Another possibility is that the nutrient additions themselves may not have been enough to interfere with the host-mutualist relationship because of their dilution by maintenance watering between treatments.

Salinity

Differences in the salt tolerance of the two species raises another potential difficulty in capitalizing on the response of *P. amarum* to fertilization. *Panicum amarum* biomass was significantly depressed at higher salt concentrations, whereas *U. paniculata* was largely unaffected. Salt stresses plants in two ways: It inhibits their ability to take up water, and it can directly injure plant tissues, including transpiring leaves (Parihar *et al*., 2015). Previous research showed a threshold of salt tolerance for *P. amarum* and *U. paniculata* similar to that in this study, but *P. amarum* was the more salt-tolerant species (Seneca, 1972a); however, those experiments involved seeds (Seneca, 1969) and seedlings (Seneca, 1972a) rather than mature plants. A study of mature *U. paniculata* in natural conditions showed an increase in productivity in higher salinity areas near the shoreline (Gormally and Donovan, 2010), and even seedlings exhibited a similar response to moderate salt spray (Seneca, 1972b). Those results are consistent with this study in which *U. paniculata*'s live, aboveground biomass increased slightly at some salinity concentrations. Similar to sea water, the Instant Ocean formula used in the experiment included organic matter and micronutrients (Atkinson and Bingman, 1997), which may have fostered plant productivity at some concentrations, essentially acting as a weak fertilizer until overwhelmed by the negative impact of rising salinity. So, planting and fertilizing *P. amarum* may lead not only to its dominance in the dune community, but *P. amarum*'s lower salinity tolerance also could reduce the community's overall resilience in the face of more frequent saltwater inundation from severe storms associated with climate change.

Table 3. *Results from general linear models testing how extraradical hyphae (ERH) produced by arbuscular mycorrhizal fungi were affected by treatments in the salinity experiment and the nutrient experiment, as well as plant species identity and native microbial amendments across both experiments. Initial stem width was included as a covariate. Treatment refers to salinity or nutrients, depending on the experiment. Bold values are significant at* $P < 0.05$.

	Salinity experiment			Nutrient experiment			Combined		
	d.f.	*F*	*P*	d.f.	*F*	*P*	d.f.	*F*	*P*
Stem diameter	1, 81	0.12	0.73	1, 37	1.00	0.32	1, 143	0.04	0.84
Species	1, 81	11.62	**0.001**	1, 37	22.02	**<0.0001**	1, 143	46.02	**<0.0001**
Microbes	1, 81	1.66	0.20	1, 37	1.42	0.24	1, 143	10.25	**0.002**
Treatment	1, 81	1.57	0.21	4, 37	2.73	**0.04**	–	–	–
Species × microbes	1, 81	0.26	0.61	1, 37	1.49	0.23	–	–	–
Species × treatment	1, 81	0.46	0.50	4, 37	0.25	0.91	–	–	–
Microbes × treatment	1, 81	0.22	0.64	4, 37	0.79	0.54	–	–	–
Species × treatment × microbes	1, 81	0.14	0.7142	4, 37	0.93	0.46	–	–	–

CONCLUSIONS

The experimental results have several practical applications for dune restoration: (1) Both species offer important functionality to coastal dune systems and should be planted in tandem, with more *U. paniculata* in areas closer to shore because of its greater salt tolerance; (2) moderate fertilization of *P. amarum* will dramatically increase its growth and should be limited to highly stressed areas where rapid establishment of vegetation is more important than community diversity; (3) use of a balanced, slow-release fertilizer may support growth of beneficial fungi; and (4) native microbial additions may have less impact on restoration success because of the established microbial communities that accompany transplanted seedlings.

Plants obtained from commercial nurseries bring a microbial community with them that may be functionally equivalent to native communities or lacking in harmful pathogens present in native soils. Of course, the outcome may be different with other plant species, with plants from other sources, or under different abiotic conditions. Experimenting in greenhouse conditions offers greater control over these abiotic conditions but cannot replicate the complex interaction of stresses in the field. Further research should more directly compare native and imported microbial communities associated with different species, how they interact, and how they compare with plants grown from seed in sterile conditions. A recent study has suggested that native microbial amendments may not increase the productivity of dune grasses in isolation, but they still can lead to greater community diversity (Crawford *et al*., 2020).

Nutrient additions will not necessarily suppress mutualistic plant-microbial interactions in the low-nutrient environment of coastal dunes. As noted previously, steady release of a balanced fertilizer at lower levels may, in fact, facilitate plant and mycorrhizal growth. Observation of soil-nutrient interactions across a complete growing season would be useful because it may take longer for shifts in mutualist relationships to materialize. In addition, soil pH should be considered as another variable that will affect the uptake of nutrients.

Finally, nutrient amendments present other potential tradeoffs. Fertilization clearly will accelerate the establishment of *P. amarum*, which may be desirable if the only goal is to rapidly accumulate stabilizing vegetation. However, with such an advantage, *P. amarum* may overwhelm other species, such as *U. paniculata*. The results point to the need for further study of the varying impact of fertilization, microbial additions, and other restoration practices across a broader range of dune species because these variations can lead to unexpected shifts in community composition, less diverse vegetation, or less resilience to environmental disturbance.

ACKNOWLEDGMENTS

The authors thank S. Berger, L. Elizondo, L. Rodriguez, and C. Perez for their help processing belowground plant samples. This work was supported in part by an Institutional Grant (NA14OAR417012) to the TSGCP from NOAA, U.S. Department of Commerce.

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