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The Role of Biocrusts in Coastal Dune Plant Communities

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THE ROLE OF BIOCRUSTS IN COASTAL DUNE PLANT COMMUNITIES

By

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ABSTRACT

Biocrusts, microbial assemblages that form visible crusts or mats on the soil surface, have been shown to affect environmental variables, such as soil moisture, nitrogen-rich biomass additions, and soil physical properties, especially in stressful environments. While these abiotic variables are known to affect plants, the role of crusts in plant communities has received little attention. Coastal dunes are stressful environments with heterogeneous topography that experience occasional, irregular flooding, which promotes the formation of heterogeneously distributed algae-dominated microbial biocrusts. The heterogeneous distribution of biocrusts, suggests the potential for variability in abiotic factors across the dunes that affect plant growth and survival. I investigated the role of biocrusts in a coastal dune community. Particularly, I explored correlations among crusts, abiotic variables and a native coastal dune plant community, tested the effect of environmental conditions, particularly storm patterns, on interactions between crusts and common coastal dune plant species, and tested the effect of crusts on plant-plant interactions.

To explore correlative patterns between biocrusts, plants, and abiotic factors in the field, I analyzed census data from a coastal dune plant community on St George Island, FL. Species associations with biocrusts varied from negative to positive. Community analyses found plant community composition was correlated with crust, and as well as confounding abiotic factors, such as elevation and soil moisture. Surprisingly, I did not find strong correlations between biocrusts and abiotic factors, such as soil moisture or organic content, when elevation was taken into account. These data suggest the plant community does vary with biocrusts in the field; however, no causal relationship was determined.

To test the effect of crusts on plant species, I conducted a greenhouse experiment that simulated various environmental conditions, including several water and salt treatments, with and without biocrust in a factorial design. Crusts did have an effect on the growth and survival of some species, and this effect varied from negative to positive. Interesting, negative effects on survival, and positive effects on growth suggest potential for tradeoffs. Additionally, plants’ response to abiotic variables varied slightly between crust and no crust treatments; however, no significant effect of crust on variation in plant responses was detected in the analysis. These data
suggest crusts affect plant growth and survival, and that the effect of crust varies among plant species.

Lastly, I performed a target-neighbor competition experiment using four common coastal dune plant species in a full factorial design to test the effect of crusts on plant-plant interactions. The effect of crust varied between competition and no competition treatments. In no competition treatments, crusts tended to have an overall positive effect on plant growth. In competition, the effect of crusts on plant competitive effects varied among target-neighbor treatment combinations, but was often small and negative. These data suggest that, while biocrusts may have a positive effect on individual plants, they do not have a strong effect on plant-plant competitive interactions.

The combination of observational and experimental data suggest biocrusts do have differential associations and effects on individual species, which may vary in different abiotic conditions. However, competition experiments suggest that these differential responses to crust do not have a strong effect on plant-plant competitive interactions. Though, the effects of crusts on competition were tested in low stress conditions, and these interaction effects may change across gradients of stress. It may be important to investigate the effects of crusts on plant interactions in the context of stressors, and how these interactions change through succession.
INTRODUCTION

Microbial assemblages that form visible crusts or mats on the soil surface (biocrusts, or crusts) have been described in a variety of habitats, but are particularly well-known from soils that are ephemerally wet, such as deserts and coastal dunes. They affect abiotic processes such as nutrient dynamics, soil chemistry, and geomorphology (Williams et al. 1995, Belnap 2002, Smith et al. 2004, Castillo-Monroy et al. 2010). The few studies addressing the biotic effects suggest that biocrusts can alter plant-plant interactions and community composition, but that these effects are complex and poorly understood. For example, some studies have found positive effects of biocrusts on nutrients and plant growth (Smith et al. 2004, Thiet et al. 2014), while others suggest biocrusts decrease light and reduce seedling survival (Kidron 2014). Understanding the role of biocrusts on plants may be important for understanding coastal dune plant community dynamics, composition, and biogeochemistry.

Biocrusts have been found to occur on many on coastal dunes. Coastal dune communities are characterized by a number of abiotic stressors, including as low water and nutrient contents, salt spray inputs, sand bombardment, and intense storm disturbances (Wilson and Sykes 1999, Feagin et al. 2005, Gornish and Miller 2013, Miller 2015a). These disturbances and stressors make coastal dunes a particularly interesting system for studying the role of biocrusts on plant communities, and how their role might change with fluctuating environmental conditions. Previous studies have investigated the abiotic effects of biocrusts on coastal dunes, which suggest that these structures can retain moisture, increase total nutrients, and reduce the movement of sand (Williams et al. 1995, Smith et al. 2004). Fewer studies have investigated the effects of crusts on coastal dune plants (but see Thiet et al. 2014) and none have looked at the effects of crusts on plant-plant interactions. In this thesis, I investigated the role of biocrusts on coastal dune plant communities through the use of census data from a barrier island in the Gulf of Mexico coupled with greenhouse experiments testing the effect of crusts of plant growth and survival in various abiotic, and biotic conditions.

In chapter 1, I describe analyses of the patterns of vegetation and biocrusts in the field using a coastal dune plant community census data from St George Island, FL. These analyses aimed to confirm associational patterns between individual plant species, the plant community as
a whole, and abiotic effects in the field, as well as identify plant species for experimental manipulation.

In chapter 2, I describe a greenhouse experiment which simulated various environmental conditions, including several water and salt treatments, with and without biocrust in a factorial design to test the effects of abiotic stressors on growth and survival in six dune plant species. This experiment was designed to characterize the interaction effects of plant species and biocrusts, and test whether plant-crusts interactions change under various environmental contexts.

In chapter 3, I describe a competition experiment which tests the interaction effects of four dune plant species in a full factorial design, including all possible of individual and target-neighbor combinations in the presence and absence of biocrusts. This experiment provides insight on whether crusts affect plant-plant interactions.
CHAPTER 1
LONG-TERM CENSUS DATA

Introduction

Biocrusts are combinations of microbial and macrophyte organisms such as algae, mosses and lichens that form a layer over the soil surface and are often regarded as ecosystem engineers for their ability to affect their local abiotic environment. The majority of research on biocrusts has focused on desert habitats, though biocrusts and related structures are found across a number of environments. Desert biocrusts, primarily consisting of algae and cyanobacteria, have been associated with a variety of effects, including changing soil pH (Flechtner et al. 1998), stabilizing soil (Kutiel 1998), and buffering nutrient ratios (Delgado-Baquerizo et al. 2013). These effects can favor the growth of plants in otherwise inhospitable conditions, as well as affect the biogeochemistry and ecosystem function of the area. Periphyton, a generally aquatic floating mat composed primarily of algae, cyanobacteria, heterotrophs, and detritus, is considered to be ecologically important in wetland systems, such as the Everglades (Gaiser et al. 2011). These microbial assemblages are known to contribute to carbon cycling and biogeochemistry. In each case, changes in soil or water biogeochemistry correlated with algae or cyanobacteria-dominated mats or crusts have been shown to affect plant growth and survival, and hence, affect community composition.

Biocrusts are often associated with stressful ecosystems, such as the arid and semi-arid conditions found in desert systems. Coastal dunes face a number of abiotic stressors similar to other semi-arid systems. Like deserts, coastal dunes tend to be relatively nutrient poor as a result of low organic input and sandy soils. Likewise, sandy soils with little silt or organic material percolate rainwater quickly, thereby reducing the amount of water available in the system. Dune plants are exposed to a number of stressors, including salt inputs from spray and sand movement. Topography, wind, salt spray, soil factors, precipitation, storm surge, and temperature are considered major forces driving differences in vegetation in coastal dune ecosystems (Miller et al. 2009, Mallinger 2014a). Previous work suggests that coastal dune biocrusts have the potential to mitigate these stressors, or potentially affect plant interactions in the context of some of these different stressors (Thiet et al. 2014). Biocrusts in coastal dune systems have also been found to stabilize sandy soils, and have been associated with increasing moisture retention as well as
organic content, both of which are known to affect the establishment of dune plant species (Smith et al. 2004).

While the effect of biocrusts on coastal systems is less well known compared to desert crusts, at least one previous study has investigated the role of crusts in these systems. Coastal sand dune biocrusts of Cape Cod, consisting of cyanobacteria, algae, bryophytes, and lichens, have been associated with effects on soil nutrients, moisture and chemistry, the direction and magnitude of which may differ depending on crust composition (Thiet et al. 2014). This can result in positive or negative effects on seedling growth and establishment. Dense crusts may block sunlight, alter soil chemistry through the addition of allopathic or stress-induced secondary chemicals, and even provide a physical barrier for seedling growth. Such effects of biocrusts were investigated by Thiet et al. (2014) on a bunchgrass (Deschampsia flexuosa) and shrub (Morella pensylvanica) on Cape Cod, Massachusetts, US, who found that soil moisture, percent survival, and vigor in both species were consistently higher in algae-dominated crust treatments compared to controls and moss-dominated crusts, although the difference was only significant in the percent survival of M. pensylvanica.

Biocrusts may also affect the movement of sand, which could contribute to the stability and topographic homogeneity of the low-lying interdune area. Sand supplies in the interdune, where crusts are more abundant, appear to be relatively stable (Miller 2015). Smith et al. (2004) has shown that areas with crust experience less sand loss in coastal dunes on Cape Cod. Van Ancker et al. (1985) suggest that crusts may form a physical barrier that captures local sand and prevents dune blowouts and sediment loss. Additionally, coastal dune plant communities have been shown to be affected by erosion, sand bombardment, and changes in elevation over time (Wilson and Sykes 1999, Maun and Perumal 1999, Miller 2015). Hence, the effect of biocrusts on sand movement may affect vegetation patterns seen in the field.

Biocrusts have also been shown to affect nutrient dynamics in coastal dunes. Past studies in other arid and semiarid habitats suggest that biocrusts, particularly those dominated by green algae and cyanobacteria, may affect nitrogen availability for plants through nitrogen fixation or inhibition of fixation (Belnap 2002, Castillo-Monroy et al. 2010, 2011a, 2011b), and this relationship may change from positive to negative given other environmental variables. For instance, the effect of crusts on plant germination and growth has been shown to shift from positive in arid conditions to negative in extreme drought conditions owing to enhanced
evaporation caused by the lower albedo of crust (Kidron 2014). Biocrusts in deserts have also been shown to buffer soil nutrient ratios (Delgado-Baquerizo et al. 2013). Likewise, biocrusts can increase the organic material in the soil, which in addition to other effects, generally increases nutrient availability for plants. Crusts have been found to increase total carbon, nitrogen, and phosphorus in soils compared to soils without crusts in coastal dunes (Smith et al. 2004).

Biocrusts communities themselves can change in composition across successional and salinity gradients in coastal dunes (Bolhuis et al. 2013), which in turn may affect their effects on soil chemistry. While work in other arid systems suggests that crusts contribute to changes in abiotic factors, past studies also point to biocrusts themselves being affected by abiotic factors, such as elevation, pH, and soil texture (Castillo-Monroy et al. 2016). It is possible that these interactions between biocrusts and the abiotic environment create a feedback loop; wherein, environmental factors must be suitable for crust establishment and growth, and once the crust establishes, it affects environmental factors, such as soil moisture and nutrient conditions, which favor the growth of crusts, as well as some plant species.

Finally, each of these abiotic factors that have been shown to be affected by biocrusts, such as soil moisture and nutrient availability, are known to affect plant growth and survival and plant community composition. Because of this, biocrusts are thought to be a good model system for examining the relationship between diversity and ecosystem function (Bowker 2007, Bowker et al. 2010). Differences in the microbial crust community diversity have been shown to signal differences in abiotic soil characteristics, such as soil nutrient content, moisture retention, and pH, which in turn affect wider scale community and ecosystem function. Despite the significant amount of research on biocrusts in arid, mostly desert, environments, the relationship between biocrusts and the coastal dune plant community has not been well investigated. And, while there are many similarities between coastal and more inland desert communities, coastal dunes have a unique set of conditions, such as salt inundation, both wet and dry moisture extremes, and disturbances from storms. Understanding the relationships between biocrusts, plants, and the abiotic environment will allow us to better manage biotic and abiotic factors in coastal dunes to maintain and maximize ecosystem function.

Here, I used census data from a long term coastal dune research site in the Northern Gulf of Mexico to investigate correlative relationships between biocrusts, the coastal dune plant
community, and associated abiotic factors. Specifically, I investigated the correlative relationships between crust abundance and plant species occurrence, plant community composition, and abiotic factors (soil moisture and organic content), in order to determine (1) if particular plant species tend to co-occur with crusts, (2) if plant community composition varies with crust, and (3) what abiotic conditions are associated with crusts. I predict that plants associated with wetland environments will be more likely to have a positive association with crusts due to potential for moisture retention. If crusts affect species interactions, then community composition per plot will also change as a function of crust. Finally, crust presence was expected to be associated with greater soil moisture and organic content. While this information is strictly correlative, the patterns described from the field provide a foundation for future work on biocrusts in coastal dune systems.

Methods

Field Site – St George Island

St George Island is a typical Holocene barrier island fronting Apalachicola Bay in the Northern Gulf of Mexico, approximately 5 km off the coast of Florida, USA. The topography of this 45 km long island is common to many barrier islands in the southeastern US, with three distinct habitats – foredune, interdune, backdune, and in older middle sections, a maritime forest. The eastern 14 km of the island is occupied by the St. George State Park and the eastern-most tip of the park is a limited-access area with relatively minimal human traffic on the beach, and especially in the dunes. A long-term research site was established here in 1999 by Dr. Thomas Miller to document changes in vegetation and dune morphology through time. The site consists of nine 6760 x 6760 m grids, three in each in the foredune, interdune, and backdune habitats. Each grid consisted of a 7 x 7 set of 1 m² quadrats located 10 meters apart. Vegetation abundance and species identity in these plots have been censused each Fall since 1999 by students and volunteers, documenting the effects of storms and other disturbances on plant communities and restoration (Miller et al. 2009, Gornish and Miller 2010, Monge and Gornish 2015, Miller 2015). Additionally, relative elevation has been measured at each of the points in each plot over time using a rotating laser level. These analyses use only the 2015 data.
Soil samples were taken in February 2016 from each of the 441 points across all nine plots and placed in sealed plastic bags. Wet mass for each sample was collected within 24-hours of field collection. Samples were subsequently dried in an oven at 60 °C for over 48 hours, and re-weighed in order to calculate percent moisture content for each sample. Approximately 10 g subsamples were then taken from the dried samples, and combusted in a muffle furnace at approximately 600 °C for 8 hours; changes in mass were attributed to organic biomass in the soil.

Data analysis

Abundance patterns were investigated for individual species found in greater than 5% of the plots (32 out of the original 71 species); this arbitrary cutoff was used to minimize the effects of rare species in the analyses. Relationships between crust percent cover and species presence or absence were tested with generalized linear mixed models (GLMMs) using the “lme4” package in R (R Core Team 2015, Kuznetsova et al. 2016). Elevation and crust are confounded, as lower elevation areas are more prone to flooding and inundation conditions that promote crust growth. I used the residual values for each data point (representing a 1 m² plot) from the crust percent cover by elevation generalized linear mixed model regression (hereafter crust-elevation residuals) as the predictor value in the model to account for collinearity between crust and elevation (Figure 1.1). The generalized linear mixed model also accounted for spatial autocorrelation at the largest scale by nesting the data by replicate plot. A binomial distribution was used as a log link function for the species presence/absence response variable. Community characteristics, such as plant diversity and density (cumulative percent cover per plot), and soil moisture, were analyzed using the crust-elevation regression residual values \( y_i - \hat{y}_i \) in linear mixed models nested by habitat replicate or “plot” (LME; Equation 1.1).

\[
(1.1) \quad (y_i - \hat{y}_i) + (y_i - \hat{y}_i)|plot
\]

The relationship between percent organic content of the soil and biocrust percent cover was analyzed using a linear mixed model with plant density \( S_i \) measured as cumulative percent cover per quadrat) as a covariate, nested by habitat replicate (Equation 1.2). Logistic regressions
were plotted for each plant species with significant effects, and linear regressions were plotted for community characteristics and abiotic variables.

\[
(1.2) \quad (y_i - \hat{y}_i) + S_i + (y_i - \hat{y}_i) | \text{plot}
\]

Non-metric multi-dimensional scaling (NMDS) was used to visualize the relationships among the plant communities in all 441 – 1 m\(^2\) plots in the long term research site on St George in 2015 using the most abundant 32 species where crust was either present or absent. Permutational analysis of variance (PERMANOVA) was calculated based on the plant community data to test for differences in the plant community composition as a function of crust presence/absence, habitat type (foredune, interdune or backdune), and crust by habitat type interaction. The relationships between NMDS ordination values and environmental variables (crust, elevation, moisture, and organic content) were analyzed using “envfit” in the R vegan package (Oksanen et al. 2015).

**Results**

Crusts occurred in 60.5% of the 441 plots on St George in 2015. In general, crusts tended to occur in lower elevation plots, with the greatest percent cover of crusts below 1 meter in elevation (Figure 1.1). Occurrence varied among habitats, with crusts in 67% of foredune, 79% of interdune, and 35% of backdune plots. Percent cover of crust per plot varied significantly by habitat, and was far greater in the interdune plots compared to foredune and backdune plots (ANOVA, df = 2, F = 71.2, P << 0.001, n = 441; Tukey HSD, foredune-backdune, P = 0.705; interdune-backdune, P << 0.001; interdune-foredune, P << 0.001; Figure 1.2); the elevations of interdune plots were generally lower, as well (ANOVA, df = 2, F = 99.6, P << 0.001, n = 147; Tukey HSD, each P << 0.001).

The full vegetation dataset for 2015 included 71 species: 36 species in the foredune, 41 species in the interdunes, and 50 in the backdunes, with 31 species occurring in all three habitats. Of the 71 species present, only 32 species occurred in more than 5% of the plots and only these species were used to explore species-specific patterns of association with crusts.
The full results of the linear mixed models of individual species are reported in Table 1.1. Of the 32 species incorporated in the analysis, six were significantly positively correlated with increasing crust percent cover (Centella asiatica, Hydrocotyle bonariensis, Limonium carolinianum, Paspalum distichum, Phyla nodiflora, and Spartina patens), and five were significantly negatively related (Heterotheca subaxillaris, Muhlenbergia capillaris, Oenothera humifusa, Panicum amarum, and Polypremum procumbens), as shown in Figure 1.3.

![Figure 1.1](image)

Figure 1.1. Relationship between biocrust percent cover (measured as percent cover per 1 m$^2$ plot) and elevation (m). Habitat types are denoted as foredune (square), interdune (triangle), or backdune (circle). Poisson GLM, $z = -64.61$, $P < 0.0001$.

NMDS of the plant community data, based on plant species presence/absence is shown in Figure 1.4 (binomial distance, $k = 3$, stress = 0.09). Each point reflects the vegetation community censused in a 1 m$^2$ plot in 2015. An analysis of fit of environmental vectors to ordination values using the vegan package function “envfit” in R suggests significant relationships between plant community composition and crust percent cover, elevation, soil moisture and soil organic content (Table 1.2). However, these factors are clearly confounded; NMDS 1 is positively associated with elevation, and negatively correlated with soil moisture, percent crust, and percent organic material (vectors in the biplot in Figure 1.4). It is interesting that NMDS 2 does not appear to be
strongly associated with any observed environmental factors. Biocrust percent cover, habitat type, and their interaction demonstrated significant effects on community composition (PERMANOVA; Table 1.3).

The cumulative percent cover of all species found within a 1 m$^2$ quadrat increased significantly with increasing crust-elevation residuals (Figure 1.5A; df = 1, F = 21.361, P = 0.001). Species diversity, as indicated by Shannon diversity indices per 1 m$^2$ quadrat, was not significantly correlated with crust-elevation residuals (df = 1, F = 1.783, P = 0.232). Soil moisture, analyzed per habitat, was also not correlated with crust-elevation residuals (foredune: df = 1, F = 0.513, P = 0.689; interdune: df = 1, F = 5.031, P = 0.148; backdune: df = 1, F = 5.637, P = 0.115; Figure 1.5B). Soil organic content was not significantly positively correlated with crust-elevation residuals (crust residuals: df = 1, F = 3.40, P = 0.082).

Figure 1.2. Percent cover of crust per 1 meter$^2$ plot on St George Island as a function of habitat type: foredune (F), interdune (I), and backdune (B). ANOVA, df = 2, F = 71.2, P << 0.001, n = 441; Tukey HSD, foredune-backdune, P = 0.705; interdune-backdune, P << 0.001; interdune-foresdune, P << 0.001.

The cumulative percent cover of all species found within a 1 m$^2$ quadrat increased significantly with increasing crust-elevation residuals (Figure 1.5A; df = 1, F = 21.361, P = 0.001). Species diversity, as indicated by Shannon diversity indices per 1 m$^2$ quadrat, was not significantly correlated with crust-elevation residuals (df = 1, F = 1.783, P = 0.232). Soil moisture, analyzed per habitat, was also not correlated with crust-elevation residuals (foredune: df = 1, F = 0.513, P = 0.689; interdune: df = 1, F = 5.031, P = 0.148; backdune: df = 1, F = 5.637, P = 0.115; Figure 1.5B). Soil organic content was not significantly positively correlated with crust-elevation residuals (crust residuals: df = 1, F = 3.40, P = 0.082).
Table 1.1. Summary of the species relationships with crust percent cover based on binomial GLMMs of species presence/absence as a function of the residual values from the crust-elevation regression. Species are sorted by $z$ value, and significant relationships with crust-elevation residuals are bolded. Positive $z$ values indicate positive plant-crust relationships.

| Species                     | Authority                       | Family               | $z$ value | Pr(>|z|) |
|-----------------------------|---------------------------------|----------------------|-----------|----------|
| *Spartina patens*           | (Aiton) Muhl.                   | Poaceae              | 3.299     | 0.001    |
| *Phyla nodiflora*           | (L.) Greene                     | Verbenaceae          | 3.119     | 0.002    |
| *Ludwigia maritima*         | (Walter) Britton                | Onagraceae           | 2.852     | 0.004    |
| *Centella asiatica*         | (L.) Urb.                       | Apiaceae             | 2.672     | 0.008    |
| *Paspalum distichum*        | L.                              | Poaceae              | 2.204     | 0.027    |
| *Scleria verticillata*      | Muhl. ex Willd.                 | Cyperaceae           | 1.854     | 0.064    |
| *Juncus megacephalus*       | M. A. Curtis                    | Juncaceae            | 1.324     | 0.186    |
| *Dichanthelium aciculare*   | (Desv. ex Poir.) Gould & C.A. Clark | Poaceae              | 1.021     | 0.307    |
| *Bulbostylis ciliatifolia*  | (Elliott) Fernald               | Cyperaceae           | 0.994     | 0.320    |
| *Fuirena scirpoidea*        | Michx.                          | Cyperaceae           | 0.884     | 0.377    |
| *Limonium carolinianum*     | Harper                          | Plumbaginaceae       | 0.630     | 0.529    |
| *Cynanchum*                 | Pers.                           | Asclepiadaceae       | 0.189     | 0.850    |
| *Sporobolus virginicus*     | (L.) Kunth.                     | Poaceae              | -0.163    | 0.870    |
| *Rhynchospora colorata*     | (L.) H. Pfeiffer                | Cyperaceae           | -0.188    | 0.851    |
| *Paronychia erecta*         | (Chapm.) Shinners               | Caryophyllaceae      | -0.720    | 0.472    |
| *Ipomoea imperati*          | (Vahl) Griseb.                  | Convolvulaceae       | -0.807    | 0.420    |
| *Chamaesyce maculata*       | (L.) Small                      | Euphorbiaceae        | -0.930    | 0.352    |
| *Cenchrus spinifex*         | Cav.                            | Poaceae              | -1.142    | 0.253    |
| *Schizachyrium maritimum*   | (Chapm.) Nash                   | Poaceae              | -1.239    | 0.215    |
| *Cyperus croceus*           | Vahl                            | Cyperaceae           | -1.267    | 0.205    |
| *Fimbristylis sp.*          | Vahl                            | Cyperaceae           | -1.562    | 0.118    |
| *Uniola paniculata*         | L.                              | Poaceae              | -1.590    | 0.112    |
| *Smilax auriculata*         | Walter                          | Smilaceae            | -1.667    | 0.096    |
| *Eragrostis lugens*         | Nees                            | Poaceae              | -1.873    | 0.061    |
| *Hydrocotyle bonariensis*   | Comm. ex. Lam.                  | Apiaceae             | -2.103    | 0.035    |
| *Heterotheca subaxillaris*  | (Lam.) Britton & Rusby         | Asteraceae           | -2.722    | 0.006    |
| *Panicum amarum*            | Elliot                          | Poaceae              | -2.823    | 0.005    |
| *Polypreum procumbens*      | L.                              | Buddlejaceae         | -4.399    | >>0.001  |
| *Muhlenbergia capillaris*   | (Lam.) Trin.                    | Poaceae              | -11.283   | >>0.001  |
| *Oenothera humifusa*        | Nutt.                           | Onagraceae           | -17.764   | >>0.001  |
| *Physalis angustifolia*     | Nutt.                           | Solanaceae           | -30.072   | >>0.001  |
Figure 1.3. Summary of the species with significant relationships with crust percent cover based on binomial GLMMs of species presence/absence as a function of residual values from the crust by elevation regression. Species are sorted into those positively associated with crust percent cover (Centella asiatica, Ludwigia maritima, Paspalum distichum, and Spartina patens), and those negatively associated with crust percent cover (Heterotheca subaxillaris, Hydrocotyle bonariensis, Muhlenbergia capillaris, Oenothera humifusa, Panicum amarum, and Polypremum procumbens). Dashed horizontal lines refer to the expected value of crust percent cover for a given elevation based on crust-elevation GLM.
Figure 1.4. NMDS of plant community using presence/absence of the 32 most abundant species per 1 meter$^2$ plot. Habitat types are denoted as being foredune (square), interdune (triangle), or backdune (circle), as well as crust presence (green), or absence (tan). Environmental variables were fitted using envfit in the vegan package in R.

Table 1.2. Summary of fit of environmental vectors (crust percent cover, elevation, percent soil moisture, and percent organic content) per 1 m$^2$ plot to NMDS values in the ordination biplot.

<table>
<thead>
<tr>
<th></th>
<th>R</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crust</td>
<td>0.49</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Elevation</td>
<td>0.37</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Moisture</td>
<td>0.62</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Organic</td>
<td>0.17</td>
<td><strong>0.001</strong></td>
</tr>
</tbody>
</table>

Table 1.3. Summary of the PERMANOVA of relationships between crust percent cover and habitat types using the 32 most abundant species.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crust</td>
<td>1</td>
<td>154.44</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Habitat</td>
<td>3</td>
<td>23.46</td>
<td><strong>0.001</strong></td>
</tr>
<tr>
<td>Crust*Habitat</td>
<td>2</td>
<td>11.23</td>
<td><strong>0.001</strong></td>
</tr>
</tbody>
</table>
Figure 1.5. Association between crusts, expressed as the residual of the crust-elevation regression, and (A) plant density (measured as the cumulative cover per 1 meter$^2$ plot, and (B) moisture, measured as percent weight lost after drying. Habitat types are denoted per plot by shape (foredune – square, interdune – triangle, or backdune (circle). Crust presence is indicated by green symbols, while plots with no crust are tan. Moisture was analyzed separately per habitat as denoted by trend lines: foredune (solid), interdune (dotted), and backdune (dashed). Density LME, df = 1, F = 21.361, P = 0.001. Moisture LME, foredune: df = 1, F = 0.513, P = 0.689; interdune: df = 1, F = 5.031, P = 0.148; backdune: df = 1, F = 5.637, P = 0.115.

**Discussion**

This study investigated the relationship between biocrusts, plant species, community composition, and environmental factors in coastal sand dunes. Some species in this habitat have positive associations with the percent cover of crust, while others have negative associations, independent of changes in elevation. Plant community composition, measured by NMDS relationships and density, demonstrated a strong correlative relationship with crust presence/absence. Environmental factors, such as soil moisture and organic content, did demonstrate significant relationships with plant community composition. However, these correlations were largely driven by differences in elevation among plots and were not significantly associated with crust cover per plot independent of elevation.
The analysis of the plant community highlights a spectrum of negative to positive relationships between plant species occurrence, biocrust percent cover, and elevation. Most dune species have a significant relationship, positive or negative, with elevation, which is to be expected as increasing elevation is correlated with decreased soil moisture (Miller et al. 2009) and sand stability (Moreno??, Miller 2015). However, elevation is also strongly correlated with crust occurrence and percent cover. After accounting for elevation, I still found significant positive relationships between biocrusts and plant species that tend to be more hydrophilic, or flood tolerant, and occur at lower elevations (e.g. *C. asiatica, P. distichum*, and *P. nodiflora*) and are often associated with wetland environments (Devkota and Jha 2011, Lonard et al. 2015). Conversely, I found significant negative relationships between biocrusts and plant species that tend to occur in drier soils and at higher elevations (e.g. *Heterotheca subaxillaris, Ipomoea imperati, Panicum amarum*, and *Schizachyrium maritimum*). Interestingly, some species that are generally associated with wetter soils and lower elevation also demonstrated a negative relationship with crust (e.g. *Muhlenbergia capillaris*). This negative association with crust in a generally wet species suggests the potential for crusts to affect competitive interactions among species. These sorts of relationships may be elucidated using time series data to investigate changes in crust abundance, species occurrence, and community composition over time.

Vegetation community composition was also correlated with a number of environmental variables. NMDS results illustrated significant relationships with environmental variables crust, elevation, moisture, and organic content (Table 1.2); wherein, the direction of the effect of crust, elevation, and soil moisture were opposite the effect of elevation. Interestingly, NMDS axis 1 has a significantly positive correlation with elevation, suggesting that clustering or distribution of points on the NMDS axis is largely driven by elevation (GLM, P < 0.001); similar relationships between plant community composition and elevation were described by Miller et al. 2009 for this same site.

Additionally, vegetation and elevation vary by habitat type. I found significant main effects of crust and habitat type, as well as an interaction between crust presence and habitat, on community composition at the 1 m² scale. While there is a strong effect of elevation on community composition, there still appears to be clustering based on habitat type and biocrust presence/absence. In Figure 1.4, there is evident clustering of plots by habitat and by crust presence/absence along NMDS 1. Interdune plots, commonly containing biocrusts, cluster at
lower elevations, and lower NMDS 1 axis scores. Backdune plots tend to be somewhat variable with regards to elevation, though mainly occurring at around 1 m in elevation, and likewise, are more variable with respect the presence/absence of biocrusts. Foredune points are much more variable; and occur at a wide range of elevations, with and without biocrusts. Foredune variation may be due in part to disturbance and successional processes. One of the foredune plots on St George Island is in the process of becoming an interdune.

Local plant density was positively correlated with biocrust percent cover (Figure 1.5A), but plant diversity was not. This pattern of greater plant density at lower elevation environments may be due to soils with greater moisture and organic content being more hospitable, resource rich environments; and hence would support greater primary production. Likewise, previous studies suggest crusts may facilitate more hospitable conditions through moisture retention and nutrient addition (Belnap 2002, Castillo-Monroy et al. 2010, Thiet et al. 2014). If so, ephemeral inundation of plots due to pulses in rain and relative differences in elevation, which facilitate the growth of crusts, may promote a positive feedback between crust percent cover and abiotic factors affecting plant growth and reproduction. The fact that diversity did not demonstrate a significant relationship with crust-elevation residuals suggests this effect is not species-specific and so does not change patterns of relative abundance.

Coastal ecosystems have long been studied by ecologists (Cowles 1899) and are known to contain conflicting gradients of potential environmental drivers, many of which I did not measure in this study. For instance, distance from the ocean and wind direction may influence which plots are affected by salt spray (Oosting 1945, Wilson and Sykes 1999, Gornish and Miller 2010, Bitton and Hesp 2013). Further, while absolute elevation is a good metric to investigate overall trends, the elevation of each plot relative to plots directly surrounding might provide further information, and might explain some of the variation seen in the relationship between crust and elevation. For example, pooling of water resulting in higher soil moisture associated with the formation of crusts, may occur at higher elevations if the areas surrounding it are relatively higher.

Previous studies suggest that biocrusts increase organic content and retain soil moisture (Kutiel 1998, Thiet et al. 2014). However, in this study, neither organic content nor soil moisture were significantly associated with crust. Organic content was positively affected by increased density, likely due to greater litter input, and not biocrusts. Soil moisture differed across dune
habitats, and was particularly low in foredune habitats (Figure 1.5B). However, soil moisture is also confounded with elevation. These analyses, which account for the effects of elevation using residuals from the regression of crusts percent cover by elevation, do not reflect any significant relationship between biocrusts and greater soil moisture beyond what would be expected based on their common covariance with elevation.

It is important to note that soil moisture changes with factors like rain and temperature that vary significantly through time. Soil moisture data analyzed here reflect samples collected in winter conditions with infrequent rain patterns. Associations between soil moisture and crust may vary seasonally, and with varying rain conditions. For instance, crusts may confer a positive effect on soil moisture by providing a barrier to evaporation in hot summer months. Additionally, crusts have been shown to affect other abiotic factors which affect plants, such as nitrogen and erosion (Smith et al. 2004), which were not measured in this study. These factors may explain patterns of association observed between crusts and plant communities in the field.

Crusts, vegetation, and dune elevation vary through time as a function of storm frequency (Miller et al. 2009, Gornish and Miller 2010, 2013, Miller 2015). Given temporal variation of moisture and rain patterns within and between years at this site, it is also possible that the direction of cause and effect between these variables may vary. If low elevation sites experience a pattern of ephemeral inundation and drying, crusts may be promoted by wet conditions during the wet season, and retain moisture in the dry season. It is probable that soil organic matter could affect biocrust presence; however, soil organic matter is also positively correlated with plant density, likely due to increased litter inputs. Plant density may also be a function of soil moisture if high moisture environments prone to flooding favor species that tend to grow in dense monocultures, such as *P. distichum*. It is not possible to parse out the direction of cause and effect for these variables with the data currently available. In order to determine the direction of cause and effect, experimental studies directly manipulating these variables are needed.

Previous studies suggest crusts affect abiotic factors that affect plant growth, such as moisture and organic content. This study demonstrates that associations with crusts vary among common plant species, and overall community composition varies as a function of crust percent cover, and abiotic variables like elevation, soil moisture and organic content. However, surprisingly, the biotic variables measured in this study did not illustrate significant associations with crust. Hence, this study does not elucidate potential mechanisms to explain the associations
found between crusts and the plant community. It is possible that the relationships detected are not causal, but rather, conditions favoring particular plant species also promote crust growth. Likewise, a number of other factors, such nitrogen availability and erosion, have been shown to be affected by crusts, and to affect plant growth. Understanding the causal mechanisms behind the relationships among crusts, environmental factors, and plant community composition will allow us to better understand community dynamics, and optimize management regimes to increase ecosystem services and function, particularly in these socially and economically important coastal dune systems.
CHAPTER 2

CONTEXT DEPENDENCE AND STORM EFFECTS

Introduction

The direction and magnitude of pairwise species interactions may depend on the biotic and abiotic context; changes in these pairwise interactions have the potential to affect community dynamics. In systems that experience periodic disturbances such as strong storms, and variation in daily stressors, such as low nutrients or soil moisture, and tolerance among species, the abiotic context experienced by individuals has the potential to dramatically change the way individuals and populations interact, especially if species exhibit different responses to the abiotic change. In these types of stressful environments, interactions between organisms that are otherwise competitive may become more facilitative (Bertness and Callaway 1994), changing not only the magnitude, but also the direction, of pairwise interactions. For example, plant-mycorrhizae interactions have been shown to fluctuate between positive and negative as a function of environmental stress, such as nutrient depletion (Mandyam and Jumpponen 2015).

Coastal dunes provide an interesting system in which to study the effects of abiotic context dependence on species interactions. They are very dynamic environments that experience a variety of stressors, including changes in topography, extreme winds and temperatures, salt spray, and nutrient poor sandy soils. In particular, they experience periodic stress from storms such as hurricanes, which have been shown to affect coastal dune plant communities and topography (Miller et al. 2009, Seabloom et al. 2013, Mallinger 2014a, Miller 2015a). Some studies suggest that the salt spray is the main abiotic stressor that creates zonation on the dunes (Wilson and Sykes 1999). The harsh factors found on coastal dunes, such as limited soil moisture, low nutrients, and increased salt, may be associated with increased facilitative interactions between plants in these environments (Martinez and Garcia-Franco 2003). Subtle changes in abiotic factors in these systems have the potential to substantially affect the growth and survival of individual plants. Previous research on coastal dunes has also shown facilitative responses in plant species that resulted in changes in community composition, such as increased plant density and species richness (Monge and Gornish 2015).

Ephemeral inundation of the low-lying habitats of coastal dunes promotes the formation of microbial mats on the soil surface (hereafter biocrusts, or crusts) comprised primarily of
filamentous algae and cyanobacteria. During the rainy season, or after a storm, low-lying dune habitats remain inundated for anywhere from days to months, especially in interdunes and backdune troughs. This flooding may be saltwater from storm surge over foredunes, but is more often freshwater from precipitation (Miller, personal communication), as most storms are accompanied by heavy rain. Hence, plant communities in these low lying areas are generally more flood tolerant; however, less flood tolerant species may become more common in these areas during extended periods of low rain or drought (Gornish and Miller 2013). Similar crusts found in arid and semi-arid environments such as deserts are known to affect local biogeochemistry by promoting or inhibiting plant growth (Thiet et al. 2014), affecting sand movement and erosion (Kutiel 1998), and contributing to nutrient flux (Belnap 2002, Smith et al. 2004, Castillo-Monroy et al. 2010, Delgado-Baquerizo et al. 2013). Hence, the presence of crust may affect the way individual plants respond to different environmental conditions, such as storm or drought.

Relatively little is known about biocrusts or crusts in coastal dune ecosystems relative to what is known about their counterparts in other systems. Desert biocrusts consisting of algae and cyanobacteria are well studied (see reviews in Belnap 2003, Pointing and Belnap 2012) and have been associated with mediating gas, nutrient, and water flux (e.g., Eldridge and Greene 1994) and changing soil pH (Flechtner et al. 1998). Periphyton — a biocrust composed of algae, cyanobacteria, heterotrophs and detritus — is considered to be ecologically important in wetland systems, such as the Everglades (Gaiser et al. 2011), especially due to their contribution to carbon cycling and biogeochemistry.

Like their desert counterparts, coastal dune biocrusts may promote nutrient addition. One of the few studies of coastal sand dunes investigated biocrusts on Cape Cod. These crusts consist of cyanobacteria, algae, bryophytes and lichens, and have been shown to affect soil nutrients and chemistry, as well as to inhibit seedling growth and establishment; these effects differ as a function of crust composition (Thiet et al. 2014). Coastal dunes tend to be relatively nutrient poor as a result of low biomass inputs and sandy soils. It seems likely that, through the cyclical process of flooding and drying, nitrogen-fixing, algae-dominated crusts will develop, dry out and break down, and then form once again. The biomass accumulated through the algae breaking down will contribute organic material to the sandy soil. Additions of nitrogen and organic content in the ecosystem will affect soil chemistry and composition, which in turn may
affect plant community composition and ecosystem function (Chapin 1997). The magnitude and mechanism, whether through nitrogen fixation or biomass addition, of nutrient additions are dependent on abiotic conditions, particularly moisture.

Biocrusts may also affect ecosystem dynamics through soil moisture retention. Sandy soils percolate rainwater quickly, resulting in generally low water availability. Previous work from other arid dunes has shown that soils containing biocrusts contain more moisture than those without crusts (Kutiel 1998, Thiet et al. 2014). Biomass contributions to the soil may increase its ability to retain moisture or can create a barrier to retain moisture that might otherwise seep through the sand or evaporate in hot, dry conditions. Hence, the presence of crusts may increase the water availability for plants, which is particularly important during drought conditions. Additionally, crusts may contribute to plant community diversity by facilitating more hospitable conditions for drought intolerant species.

Finally, crusts may also have negative effects on plants, especially on seedlings or new growth. Dense mats may block sunlight, alter soil chemistry through the addition of allopathic, or stress induced secondary chemicals, and even provide a physical barrier to growth. For instance, algae-dominated coastal dune crusts have been shown to inhibit seedling survival of a coastal bunchgrass (*Deschampsia flexuosa*) and shrub (*Morella pensylvanica*) on Cape Cod (Thiet et al. 2014). It is unclear if these negative effects are important for clonal species, such as the grasses and forbs commonly found in interdunes.

Given the differential associations of crusts with species on St. George Island (Chapter 1), plant species are expected to demonstrate different responses to crust treatments. Particularly, those species generally associated with moist conditions and lower elevations are expected to exhibit positive effects of crusts. In general, moisture and salt tolerances vary across species; however, in general, species tend to respond positively to increasing moisture, and negatively to salt inputs. Additionally, effects of crust and abiotic conditions may interact and change the direction or magnitude of plant responses. For instance, if crusts are conferring moisture benefits to plants, plants grown with crusts may have greater growth in drought conditions compared to no crust counterparts. In this study, I used greenhouse experiments to test the effects of biocrusts on coastal dune plant species, and the interaction effects of crusts and abiotic factors. Specifically, this study tested (1) the effect of biocrusts on individual plant growth and survival, (2) the effects of various storm condition manipulations (rain periodicity and salt spray
simulations) on individual plant growth and survival, and (3) the interaction effects of crusts and
storm condition manipulations on plant growth and survival. These results provide insight on the
role of biocrusts in coastal dune plant communities, as well as contribute to our understanding of
the importance of considering abiotic stress when measuring interaction effects between
individuals in complex systems.

Methods

Field Site – St George Island

Plant materials were collected from St George Island, a typical Holocene-formed barrier
island fronting Apalachicola Bay in the Northern Gulf of Mexico off the coast of Florida, USA.
The island geology is consistent with the standard coastal dune morphology found elsewhere in
the world, with three distinct habitats – foredune, interdune, and backdune (Psuty 2008). At the
eastern third of the island is St. George State Park and the eastern-most portion of the Park is a
limited access area with relatively minimal human traffic on the beach, and especially in the
dunes. A long-term research site was established here in 1999 by Dr. Thomas Miller, and the
distribution of crusts across the dunes at this site is described in Chapter 1 of this thesis.

Experimental design

The experiment used six plant species, each collected from St George Island and cloned
in a greenhouse to produce a sufficient number of individuals. A full factorial design was used,
which incorporated the six plant species grown with and without biocrusts and with and without
a simulated saltwater overwash (Figure 2.1). Further, a fourth treatment manipulated the volume
and periodicity of rain: (1) heavy rain (simulating a storm event), (2) average rainfall, or (3)
drought. Hence, 12 treatments for 6 plant species, conducted with 5 replicates each in a
randomized block design for a total of 360 pots. All the research was conducted using 15 x 15 x
17 cm plastic pots in a greenhouse at the Mission Road Research Facility in Tallahassee Florida.
The greenhouse uses a “pad and fan” cooling system, which generally keeps temperatures similar
to ambient, outdoor temperatures.
Figure 2.1. Full factorial experimental design including dominant coastal dune plant species associated with dry (Ipomoea imperati, Schizachyrium maritimum, Uniola paniculata) or wet (Centella asiatica, Paspalum distichum, Fimbristylis caroliniana) conditions. Three levels of rain conditions (drought, average, and storm); a salt treatment (salt water input or fresh water control); and a crust treatment (crust inoculation or water control).

Plant selection, collection and rearing

I selected six plant species that occur commonly across the coastal dunes of St George Island for this experiment, including four graminoid species (Uniola paniculata, Schizachyrium maritimum, Paspalum distichum, Fimbristylis caroliniana) and 2 forbs (Centella asiatica and I. imperati). While all the selected species occur broadly across the dunes, they have been described by Miller et al. (2009) as being either being associated with relatively wet (P. distichum, F. caroliniana, and C. asiatica) or dry conditions (U. paniculata, S. maritimum, and I. imperati), driven by relative elevation. Wet species tend to occur in lower elevation areas where they may have been more likely to co-occur with biocrusts, such as interdune habitats or troughs between back dunes (see Chapter 1); whereas, drier species tend to occur on the tops or sides of dunes, and predominantly in foredune and backdune habitats where crusts are rare. Each of these species is either positively (C. asiatica, F. caroliniana, and P. distichum) or negatively (I. imperati, S. maritimum, and U. paniculata) correlated with crusts in the field; however, this relationship is confounded with elevation. In Chapter 1, when elevation is taken into account, C. asiatica and P. distichum have significantly positive relationships with crust percent cover on St George Island. All of the selected species are perennial, have the capacity to reproduce vegetatively, and all are considered to be salt tolerant or halophytic, except C. asiatica, whose germination has been shown to be negatively affected by salt (Devkota and Jha 2010).
Approximately 20 individuals of each species were collected from St George Island State Park in the winter of 2014 at a variety of locations on the easternmost tip of the island (under Florida DEP permit 10211410 to T. E. Miller). Individuals were allowed to grow clonally in the greenhouse until 60 or more ramets of each species were produced, for a total of 360 plants used in the experiment. During this period, plants were grown in a 1:1 ratio of fine sand and potting soil in the greenhouse. A minor amount of fertilizer was added at the beginning of the rearing process to encourage vegetative growth during the rearing period.

Experiments with these plants were initiated on May 5 and 6, 2015. To initiate the experiments, single ramets (forbs) or clumps of grasses were separated, cleaned, and weighed, and then transplanted to individual pots, or mesocosms, consisting of a 15 x 15 x 17 cm square pot filled with sand, and lined with thick plastic (6 mil) sheeting to contain the biocrust inoculations. Plants were given 1 week to recover from transplanting stress before any treatments were initiated.

**Biocrusts: collection, cultivation, and treatment**

Natural biocrusts on St. George Island are often initiated in standing freshwater in interdune and trough areas. To create similar conditions in the greenhouse, biocrusts were collected from multiple sites across the long-term research site at the eastern tip of St George in the winter of 2014. Collected crusts were dry due to a lack of rain and storms on the island during this period. Crust samples from across the dunescape were combined and homogenized, and then added to a glass 5 gal tank of fresh water with approximately 15 mL of 20-20-20 NPK fertilizer. Given the low concentration of fertilizer, and the long growth period, the initial fertilizer addition was unlikely to be directly available for the plants used later in the experiments. As the slurry grew, the mixture was divided into multiple tanks diluted with fresh water. Approximately 8 gallons of crust slurry were cultured in this fashion.

On May 14, algae in tanks were combined into a larger tank, and thoroughly mixed to create a homogeneous slurry prior to biocrust treatment application. Half of all the plants in the experiment were then treated with approximately 120 mL of biocrust slurry, while half received an equal amount of fresh water as a control. After being treated with either the biocrust slurry or the water control, biocrusts were given two weeks to establish and simulate an environment with
an established crust. During this two-week period, plants were watered daily. Algae-dominated crusts formed which bound the top 0.50 cm of sand and resembled drier crusts found in intermediate elevations in the field (Figure 2.2), as opposed to the more mat like crusts that form at lower elevations.

Figure 2.2. Picture of greenhouse cultivated crust at the conclusion of the storm simulation experiment. Crusts were largely dominated by filamentous algae and cyanobacteria, and also included some microbial heterotrophs.

Storm simulations

Three different rainfall treatments were implemented that span the observed conditions of interdunes on St George Island: storm conditions (heavy rainfall with inundation), moderate rainfall (short regular rain; moist, but no inundation), and drought conditions (dry, very occasional rain). The storm condition treatment simulated a major storm event, wherein plants were inundated with fresh water at the start of the experiment, followed by average rain conditions. Individuals in this treatment group were inundated with water until the aboveground water depth was approximately 2 centimeters above the sand surface. After the initial 2-week storm water treatment, plants from this treatment group were watered according to average rainfall treatment. The average condition treatment attempted to simulate typical conditions on St. George Island (relatively dry soil with occasional rain). Average condition pots were watered once a week with approximately 120 mL fresh water, which allowed the sand in the pots to dry between rain treatments. The drought condition treatment attempted to simulate drier conditions
with fewer rain events. Drought condition pots were watered with 120 mL fresh water once every 2 weeks, which allowed the plants to experience significant periods with little to no water.

The saltwater treatments simulated storm surge conditions; wherein, soils experience a one-time pulse of saltwater. The pots receiving the saltwater treatment were treated with approximately 120 mL sea water collected in nearby Apalachicola Bay. The saltwater treatments were administered concurrently with inundation watering treatments at the beginning of the experiment.

**Data collection and analysis**

After a period of 6 months, plant survival and growth (maximum height, number of leaves, and biomass) were recorded. Maximum height refers to the maximum green height of each plant, including shoot or leaves. The number of leaves refers to the total number of living, green in each pot at the conclusion of the experiment. Plants were harvested from their treatment pots and the stem and root length were measured. Dry mass was obtained after drying the plants in an oven at 60 °C for a minimum of 72 hours.

Effects of crusts, rain, and salt on plant survival was analyzed in a separate generalized linear model (GLM) for each species, with Chi-squared tests assuming binominal distributions for each of the independent variables, and their interaction terms. Effects of crusts, rain, and salt on final maximum height, number of leaves, and dry mass (root, shoot, and total biomass) were initially analyzed using a factorial ANOVA including the watering, salt, and crust treatments, as well as species identity. Because the effect of species was always significant and several interactions of other treatments with species were significant, separate ANOVA were then conducted for treatment effects within each species. Resulting P-values from the factorial ANOVA were adjusted using the Benjamini-Hochberg method of correction for multiple comparisons, which limits the false discovery rate by controlling the proportion of rejected nulls (Benjamini and Hochberg 1995). Effect sizes were calculated using partial Eta-squared, a measure of the degree to which variation in data is attributed to experimental conditions (Cohen 1973).
Results

Of the original 360 plants, 84% of plants survived. Overall, individuals treated with crust had lower survival (79%) compared to those treated with the water control (90% survival). Survival increased with increased water input, such that drought conditions had the lowest survival (72%), average conditions had more moderate survival (85%) and flood condition treatments had the highest overall survival (95%). Salt treatment individuals had slightly lower survival (82%) compared to the control (87%).

Survival varied across species and treatments (Table 2.1). Negative effects of drought watering conditions were seen in survival of *C. asiatica* (Chi-squared, df = 2, P << 0.001, \( \eta^2 = 0.036 \); Figure 2.3), which had the lowest overall survival rate (39%). Interaction effects were also seen in *C. asiatica* between crust and salt, and crust and rain treatments before correction (Chi-squared, df = 2, P = 0.029, df = 1, P = 0.048, respectively); however, the effect sizes of these were small (\( \eta^2 > 0.03 \); Table 2.2). *I. imperati, F. caroliniana*, and *U. paniculata* had moderate survival (92%, 90%, and 90%, respectively); whereas survival of *P. distichum* and *S. maritimum* was the highest of the six species (95% and 98%, respectively). A negative effect of crust on survival was also seen in *F. caroliniana* (Chi-squared, df = 1, P = 0.007) and *U. paniculata* (Chi-squared, df = 1, P = 0.003); however, these effects were fairly small (\( \eta^2 > 0.03 \); Table 2.2).

<table>
<thead>
<tr>
<th></th>
<th>Centella asiatica</th>
<th>Paspalum distichum</th>
<th>Fimbristylis caroliniana</th>
<th>Ipomoea imperati</th>
<th>Schizachyrium maritimum</th>
<th>Uniola paniculata</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crust</td>
<td>1.061</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rain</td>
<td>2.007*</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salt</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crust*Rain</td>
<td>0.029</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crust*Salt</td>
<td>0.048</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rain*Salt</td>
<td>0.748</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Crust<em>Rain</em>Salt</td>
<td>1.000</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 2.3. Total proportion survival of *Centella asiatica* overall in (A) crust, (B) rain, and (C) salt treatments. In this depiction each treatment is considered when averaged across all other treatments (i.e. (A) illustrates the relationship based on survival of individuals treated with crust regardless of rain or salt treatment). Rain treatments differed significantly, and increase rain had a positive effect. Salt additions had a significant negative effect on survival. Crust effects were marginal, though, tended to be negative.
Table 2.3. Results of the factorial ANOVA for the effects of all independent variables and interaction terms on final maximum height, total leaves, total mass, and shoot biomass. P-values less than 0.05 are in bold, and those significant after Benjamini-Hochberg correction are denoted with an asterisk.

<table>
<thead>
<tr>
<th></th>
<th>Height F</th>
<th>Height P</th>
<th>Leaves F</th>
<th>Leaves P</th>
<th>Total Biomass F</th>
<th>Total Biomass P</th>
<th>Root Biomass F</th>
<th>Root Biomass P</th>
<th>Shoot Biomass F</th>
<th>Shoot Biomass P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>34.705</td>
<td>&gt;0.001*</td>
<td>23.634</td>
<td>&gt;0.001*</td>
<td>38.345</td>
<td>&gt;0.001*</td>
<td>27.624</td>
<td>&gt;0.001*</td>
<td>34.562</td>
<td>&gt;0.001*</td>
</tr>
<tr>
<td>Crust</td>
<td>2.924</td>
<td>0.089</td>
<td>1.530</td>
<td>0.218</td>
<td>0.062</td>
<td>0.803</td>
<td>0.021</td>
<td>0.884</td>
<td>0.574</td>
<td>0.450</td>
</tr>
<tr>
<td>Rain</td>
<td>2.657</td>
<td>0.073</td>
<td>3.726</td>
<td>0.026</td>
<td>12.147</td>
<td>&gt;0.001*</td>
<td>6.305</td>
<td>0.002*</td>
<td>5.576</td>
<td>0.004*</td>
</tr>
<tr>
<td>Salt</td>
<td>0.078</td>
<td>0.781</td>
<td>0.005</td>
<td>0.944</td>
<td>0.054</td>
<td>0.816</td>
<td>0.797</td>
<td>0.373</td>
<td>0.561</td>
<td>0.454</td>
</tr>
<tr>
<td>Species*Crust</td>
<td>0.586</td>
<td>0.673</td>
<td>1.414</td>
<td>0.221</td>
<td>0.443</td>
<td>0.818</td>
<td>1.203</td>
<td>0.308</td>
<td>0.348</td>
<td>0.883</td>
</tr>
<tr>
<td>Species*Rain</td>
<td>0.993</td>
<td>0.444</td>
<td>0.654</td>
<td>0.766</td>
<td>2.623</td>
<td>0.005*</td>
<td>1.692</td>
<td>0.083</td>
<td>1.216</td>
<td>0.282</td>
</tr>
<tr>
<td>Species*Salt</td>
<td>0.777</td>
<td>0.541</td>
<td>4.200</td>
<td>0.001*</td>
<td>3.226</td>
<td>0.008*</td>
<td>3.499</td>
<td>0.004*</td>
<td>1.655</td>
<td>0.146</td>
</tr>
<tr>
<td>Crust*Rain</td>
<td>0.182</td>
<td>0.834</td>
<td>0.661</td>
<td>0.518</td>
<td>0.886</td>
<td>0.414</td>
<td>0.139</td>
<td>0.870</td>
<td>1.192</td>
<td>0.306</td>
</tr>
<tr>
<td>Crust*Salt</td>
<td>0.324</td>
<td>0.570</td>
<td>0.905</td>
<td>0.343</td>
<td>0.153</td>
<td>0.696</td>
<td>0.560</td>
<td>0.455</td>
<td>0.147</td>
<td>0.702</td>
</tr>
<tr>
<td>Rain*Salt</td>
<td>1.331</td>
<td>0.267</td>
<td>2.012</td>
<td>0.136</td>
<td>1.180</td>
<td>0.309</td>
<td>1.158</td>
<td>0.316</td>
<td>0.976</td>
<td>0.378</td>
</tr>
<tr>
<td>Species<em>Crust</em>Rain</td>
<td>0.482</td>
<td>0.847</td>
<td>0.405</td>
<td>0.931</td>
<td>0.858</td>
<td>0.573</td>
<td>1.621</td>
<td>0.101</td>
<td>0.204</td>
<td>0.994</td>
</tr>
<tr>
<td>Species<em>Crust</em>Salt</td>
<td>1.354</td>
<td>0.253</td>
<td>1.093</td>
<td>0.365</td>
<td>0.105</td>
<td>0.991</td>
<td>0.148</td>
<td>0.981</td>
<td>0.254</td>
<td>0.937</td>
</tr>
<tr>
<td>Species<em>Rain</em>Salt</td>
<td>0.862</td>
<td>0.508</td>
<td>1.007</td>
<td>0.427</td>
<td>1.005</td>
<td>0.440</td>
<td>1.628</td>
<td>0.099</td>
<td>0.738</td>
<td>0.658</td>
</tr>
<tr>
<td>Crust<em>Rain</em>Salt</td>
<td>1.138</td>
<td>0.323</td>
<td>0.537</td>
<td>0.585</td>
<td>0.384</td>
<td>0.682</td>
<td>0.096</td>
<td>0.908</td>
<td>0.240</td>
<td>0.787</td>
</tr>
<tr>
<td>Species<em>Crust</em>Rain*Salt</td>
<td>2.932</td>
<td><strong>0.035</strong></td>
<td>0.715</td>
<td>0.613</td>
<td>0.669</td>
<td>0.737</td>
<td>0.801</td>
<td>0.616</td>
<td>0.549</td>
<td>0.796</td>
</tr>
</tbody>
</table>
Table 2.4. Results of the ANOVA for the effects of all independent variables (height, leaves, total biomass, and shoot biomass) and interaction terms on plant growth variables for each species analyzed separately. P-values less than 0.05 are in bold, and those significant after Benjamini-Hochberg correction are denoted with an asterisk.

<table>
<thead>
<tr>
<th></th>
<th>Crust</th>
<th>Crust*Rain</th>
<th>Rain</th>
<th>Rain*Salt</th>
<th>Crust<em>Rain</em>Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>P</td>
<td>F</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Height</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centella asiatica</td>
<td>0.115</td>
<td>0.74</td>
<td>1.95</td>
<td>0.182</td>
<td>0.242</td>
</tr>
<tr>
<td>Fimbristylis caroliniana</td>
<td>0.525</td>
<td>0.475</td>
<td>1.603</td>
<td>0.219</td>
<td>0.531</td>
</tr>
<tr>
<td>Paspalum distichum</td>
<td>0.408</td>
<td>0.527</td>
<td>1.072</td>
<td>0.353</td>
<td>0.859</td>
</tr>
<tr>
<td>Schizachyrium maritimum</td>
<td>0.339</td>
<td>0.564</td>
<td>4.359</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Uniola paniculata</td>
<td>1.296</td>
<td>0.262</td>
<td>1.977</td>
<td>0.153</td>
<td>2.125</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centella asiatica</td>
<td>6.676</td>
<td>0.02</td>
<td>1.746</td>
<td>0.213</td>
<td>0.273</td>
</tr>
<tr>
<td>Ipomoea imperati</td>
<td>1.637</td>
<td>0.124</td>
<td>0.578</td>
<td>0.606</td>
<td>1.465</td>
</tr>
<tr>
<td>Fimbristylis caroliniana</td>
<td>0.807</td>
<td>0.377</td>
<td>0.346</td>
<td>0.71</td>
<td>2.409</td>
</tr>
<tr>
<td>Paspalum distichum</td>
<td>2.614</td>
<td>0.115</td>
<td>1.609</td>
<td>0.214</td>
<td>1.834</td>
</tr>
<tr>
<td>Schizachyrium maritimum</td>
<td>0.001</td>
<td>0.976</td>
<td>0.831</td>
<td>0.444</td>
<td>14.65</td>
</tr>
<tr>
<td>Uniola paniculata</td>
<td>2.844</td>
<td>0.1</td>
<td>1.962</td>
<td>0.155</td>
<td>0.154</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Biomass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centella asiatica</td>
<td>0.488</td>
<td>0.491</td>
<td>5.154</td>
<td>0.01</td>
<td>0.359</td>
</tr>
<tr>
<td>Ipomoea imperati</td>
<td>0.126</td>
<td>0.623</td>
<td>7.875</td>
<td>0.001*</td>
<td>8.844</td>
</tr>
<tr>
<td>Fimbristylis caroliniana</td>
<td>0.774</td>
<td>0.384</td>
<td>5.81</td>
<td>0.006*</td>
<td>0.531</td>
</tr>
<tr>
<td>Paspalum distichum</td>
<td>2.448</td>
<td>0.125</td>
<td>3.313</td>
<td>0.045</td>
<td>0.547</td>
</tr>
<tr>
<td>Schizachyrium maritimum</td>
<td>0.039</td>
<td>0.845</td>
<td>0.516</td>
<td>0.6</td>
<td>12.97</td>
</tr>
<tr>
<td>Uniola paniculata</td>
<td>0.237</td>
<td>0.629</td>
<td>5.365</td>
<td>0.008*</td>
<td>1.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot Biomass</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centella asiatica</td>
<td>0</td>
<td>0.987</td>
<td>0.007</td>
<td>0.993</td>
<td>0.974</td>
</tr>
<tr>
<td>Ipomoea imperati</td>
<td>0.114</td>
<td>0.389</td>
<td>1.638</td>
<td>0.04</td>
<td>0.472</td>
</tr>
<tr>
<td>Fimbristylis caroliniana</td>
<td>0.917</td>
<td>0.344</td>
<td>2.408</td>
<td>0.103</td>
<td>0.507</td>
</tr>
<tr>
<td>Paspalum distichum</td>
<td>7.129</td>
<td>0.011*</td>
<td>3.458</td>
<td>0.04</td>
<td>0.335</td>
</tr>
<tr>
<td>Schizachyrium maritimum</td>
<td>0.013</td>
<td>0.91</td>
<td>0.206</td>
<td>0.815</td>
<td>10.62</td>
</tr>
<tr>
<td>Uniola paniculata</td>
<td>0.56</td>
<td>0.459</td>
<td>2.402</td>
<td>0.103</td>
<td>0.882</td>
</tr>
</tbody>
</table>
Table 2.5. Effect sizes of each treatment for each of the species and variables measured. Effect size was calculated based on each of the response variables (height, number of leaves, total biomass, and shoot biomass) individually using partial Eta-squared. Values greater than 0.06 are bolded (medium effects) and values greater than 0.13 are denoted with asterisks (large effects).

<table>
<thead>
<tr>
<th></th>
<th>Crust</th>
<th>Rain</th>
<th>Salt</th>
<th>Crust*Rain</th>
<th>Crust*Salt</th>
<th>Rain*Salt</th>
<th>Crust<em>Rain</em>Salt</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Height</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Centella asiatica</td>
<td>0.001</td>
<td>0.126</td>
<td>0.030</td>
<td><strong>0.063</strong></td>
<td>0.001</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>Fimbristylis caroliniana</td>
<td>0.047</td>
<td>0.069</td>
<td>0.001</td>
<td>0.002</td>
<td><strong>0.012</strong></td>
<td>0.021</td>
<td></td>
</tr>
<tr>
<td>Paspalum distichum</td>
<td>0.002</td>
<td>0.076</td>
<td>0.019</td>
<td>0.008</td>
<td>0.071</td>
<td>0.031</td>
<td>0.008</td>
</tr>
<tr>
<td>Schizachyrium maritimum</td>
<td>0.024</td>
<td>0.071</td>
<td>0.003</td>
<td>0.023</td>
<td>0.006</td>
<td>0.005</td>
<td>0.000</td>
</tr>
<tr>
<td>Uniola paniculata</td>
<td>0.010</td>
<td>0.065</td>
<td>0.060</td>
<td>0.028</td>
<td>0.005</td>
<td>0.051</td>
<td>0.044</td>
</tr>
</tbody>
</table>

| **Leaves**       |       |      |      |            |            |           |                |
| Centella asiatica| **0.188** | 0.063| 0.048| 0.005      | 0.009      | 0.000     | 0.000          |
| Fimbristylis caroliniana | 0.055 | 0.047| 0.045| 0.045      | 0.036      | 0.019     | 0.031          |
| Paspalum distichum| **0.088** | **0.087**| 0.013| 0.002      | **0.063**  | 0.021     |                |
| Ipomoea imperati  | 0.016 | 0.034| 0.059| 0.023      | 0.009      | 0.030     | 0.024          |
| Schizachyrium maritimum | 0.000 | 0.031| **0.172**| 0.018      | 0.025      | **0.075** | 0.000          |
| Uniola paniculata | 0.016 | 0.072| 0.004| 0.007      | 0.009      | 0.005     | 0.070          |

| **Total Biomass**|       |      |      |            |            |           |                |
| Centella asiatica| 0.022 | **0.184** | 0.009| 0.018      | 0.007      | 0.044     | 0.000          |
| Fimbristylis caroliniana | 0.000 | **0.064** | **0.075**| 0.015      | 0.016      | 0.027     | 0.051          |
| Paspalum distichum| 0.059 | **0.135** | 0.002| 0.013      | 0.021      | 0.031     | 0.054          |
| Ipomoea imperati  | 0.005 | **0.102** | 0.036| **0.100**  | 0.001      | 0.012     | 0.005          |
| Schizachyrium maritimum | 0.007 | 0.002| **0.110**| **0.088**  | 0.000      | 0.043     | 0.038          |
| Uniola paniculata | 0.014 | **0.101** | 0.031| **0.061**  | 0.004      | 0.017     | 0.045          |

| **Shoot Biomass**|       |      |      |            |            |           |                |
| Centella asiatica| 0.000 | 0.018| 0.079| 0.004      | 0.001      | 0.000     | 0.000          |
| Fimbristylis caroliniana | 0.033 | 0.050| 0.060| 0.004      | 0.002      | 0.022     | 0.039          |
| Paspalum distichum| **0.114** | **0.128**| 0.006| 0.013      | 0.016      | **0.075** |                |
| Ipomoea imperati  | 0.013 | 0.019| 0.017| **0.077**  | 0.002      | 0.054     | 0.021          |
| Schizachyrium maritimum | 0.011 | 0.002| **0.073**| **0.065**  | 0.000      | 0.026     | **0.070**      |
| Uniola paniculata | 0.010 | **0.061** | 0.044| 0.014      | 0.004      | 0.014     | 0.049          |
Using the full model of growth responses, there were main effects of species for all or most of the growth measures (height, leaves, and biomass; see Table 2.3). However, because there were significant interactions between species and other treatments, treatments were then analyzed separately for each species (Table 2.4). This revealed several significant effects that were not apparent in the full model.

Each of the manipulated variables exhibited main effects on at least one species. The presence of crust had very little effect on most species. However, there was a significant effect of crusts increasing *P. distichum* shoot biomass (df = 1, F = 7.129, P = 0.011; $\eta^2 = 0.114$; Figure 2.4), and decreasing *C. asiatica* leaf number (ANOVA, df = 1, F = 6.67, P = 0.023; $\eta^2 = 0.188$; Figure 2.5), which was not significant after correction for multiple comparisons.

The simulated rain had the strongest effects on plant growth, overall. There were positive significant effects of rain on total biomass of both *I. imperati* (df = 2, F = 7.875, P < 0.001, Figure 2.6), *F. caroliniana* (df = 2, F = 5.810, P = 0.006), and *U. paniculata* (df = 2, F = 5.365, P = 0.008; Table 2.4; Table 2.5). Rain also increased plant height in *S. maritimum* and shoot biomass in *I. imperati* and *P. distichum*. As expected, the salt treatments were generally negative, but mostly on one species. Salt treatments had a large negative effect on *S. maritimum* leaf number (df = 1, F= 14.648, P << 0.001; $\eta^2 = 0.172$), total biomass (df = 1, F= 12.970, P = 0.001; $\eta^2 = 0.110$; Figure 2.7), and shoot biomass (df = 1, F= 1.620, P = 0.002; $\eta^2 = 0.073$).

In general, across the 6 species, there were few interactions between main treatment effects; however, these were not significant after correction (Table 2.4). Crust and rain interaction effects were seen in *I. imperati*, wherein crust had a positive effect on total biomass in drought conditions, in both salt treatments (df = 2, F = 3.392, P = 0.036; $\eta^2 = 0.100$). Smaller effects of crust and rain interactions are also seen in *C. asiatica, S. maritimum*, and *U. paniculata* (Table 2.5). There was also a positive effect of crust by salt interactions on which moderated the negative effects of salt stress on height in *P. distichum*, which was not significant after correction for multiple comparisons (df = 1, F = 4.129, P = 0.050), as well as medium-sized interaction effects of crust, rain, and salt on *P. distichum* and *S. maritimum* (Table 2.5). Similar trends were seen in *P. distichum* shoot biomass (Figure 2.4).
Figure 2.4. Effects of watering (drought, average, and flood), salt addition, and presence of biocrust on the shoot biomass of *Paspalum distichum*. Treatments containing biocrust additions are green and crust control treatments are denoted in by the tan bars. The results are ordered by increasing moisture: drought, average, and flood. *Paspalum distichum* demonstrated significant effects of crust on shoot biomass (df = 1, F = 7.129, P = 0.011), and a salt by crust interaction, which was not significant after correction for multiple comparisons (ANOVA; df = 1, F = 4.129, P = 0.050).

Figure 2.5. Results of the full factorial design for *Centella asiatica* number of leaves. Treatments containing biocrust additions are green and crust control (water) treatments are denoted in by the tan bars. The results displayed here are organized by increasing moisture conditions: drought, average, and flood. There was an effect of crusts detected in *Centella asiatica* leaves, which was not significant after correction (ANOVA; df = 1, F = 6.67, P = 0.023).
Figure 2.6. Results of the full factorial design for *Ipomoea imperati* total biomass. Treatments containing biocrust additions are green and crust control (water) treatments are denoted in by the tan bars. The results displayed here are organized by increasing moisture conditions: drought, average, and flood. *Ipomoea imperati* displayed significant effects of rain (df = 2, F = 7.875, P = 0.001), and salt (df = 1, F = 8.844, P = 0.02), and a statistical interaction effect of rain and crust (df = 2, F = 3.392, P = 0.04).

Figure 2.7. Results of the full factorial design for *Schizachyrium maritimum* number of leaves. Treatments containing biocrust additions are green and crust control (water) treatments are denoted in by the tan bars. The results displayed here are organized by increasing moisture conditions: drought, average, and flood. There were significant effects of salt detected on *Schizachyrium maritimum* total biomass (ANOVA; df = 1, F= 12.970, P = 0.001).
**Discussion**

The main focus of this chapter was to understand the effects of crusts on coastal dune plants, and how those effects might change under different environmental stressors. Crusts had surprisingly few main effects on these dune species in the greenhouse (Table 2.4). As predicted, these effects did differ among the dune species, which provides insight as to the potential effects of crusts across the dunes.

A positive effect of crust was observed in *P. distichum* (shoot biomass) and a negative effect was observed in *C. asiatica* (number of leaves in flood conditions). One potential explanation for the different effects of crusts on these two species is the growth form of their roots. *Ce. asiatica* is a delicate, herbaceous forb with fairly shallow root structures near the soil surface, whereas the grass *P. distichum* has deeper roots and aggressive growth (Pompeiano et al. 2014, Lonard et al. 2015). Thiet et al (2013) found that biocrusts tend to inhibit the growth of seedlings; however, little is known as to whether biocrusts may similarly inhibit vegetative growth. As with seedlings, the crusts may have acted as a barrier to the growth of new emergent nodes in *C. asiatica*, such that, when old leaves on *C. asiatica* senesced, new leaf structures were unable to emerge and replace them. *P. distichum*, on the other hand, often grow vegetative stolons at the soil surface (Lonard et al. 2015), which may bypass the barrier to new vegetative growth and allow *P. distichum* to take advantage of the potential beneficial effects of the crusts, such as increased soil moisture and nutrients (Smith et al. 2004, Thiet et al. 2014).

As expected, there were effects of water on growth; however they differed between species. Water tended to have a positive effect on most species, particularly in total biomass. I did not observe any negative effects of flooding on species growth or survival, which suggests that these species are tolerant to temporary flood conditions. *C. asiatica* had low overall survival (39%), particularly in the drought and average rain conditions. *C. asiatica* is known to be sensitive to moisture conditions, and to prefer soils with very high soil moisture (Devkota and Jha 2011). Likewise, a negative effect of drought conditions was observed on *F. caroliniana* survival. Both *C. asiatica* and *F. caroliniana* are associated with lower elevation areas on the dunes (Miller 2015a) where moisture is highest. Rain and salt conditions were designed to simulate conditions in the natural dunes, but this may have resulted in drier conditions than *C. asiatica* and *F. caroliniana* generally experience in the field.
Additionally, effects of salt were only seen in a few species and traits; particularly *C. asiatica* survival and *S. maritimum* growth were significantly reduced by salt. This is unsurprising for *C. asiatica*, as salt has been shown to negatively impact *C. asiatica*’s growth and germination (Devkota and Jha 2010). The relationship between *S. maritimum* and salt is more surprising. *S. maritimum* tends to dominate the leeward sides of dunes and further inland in the backdunes where it is sheltered from salt spray; however, this species is also prevalent on the margins of tidal flats and overwash areas characterized by salt tolerant species (Lonard and Judd 2010). This experiment suggests, at least for the population of *S. maritimum* on St George Island, salt may have surprisingly important effects in limiting this species.

In addition to main effects of crust, rain, and salt, a couple of statistical interactions were observed between crust and the abiotic variables; though these interactions were not significant after correction for multiple comparisons. For instance, an interaction effect between crust and salt was observed in *P. distichum*; salt conditions coupled with crust treatments exhibited greater average growth compared to no crust controls across all rain conditions (Figure 2.4). *P. distichum* is generally associated with wetter soils and salt tolerance, and has been shown to respond positively to nitrogen inputs despite salt stress, whereas other turf grasses’ response to nitrogen has been shown to be minimized by salt stress (Pompeiano et al. 2014). However, this does not explain why *P. distichum*’s positive response to crust is observed mainly in salt treatments, or why salt-crust combinations tended to have higher growth values with salt compared to no salt treatments. No other species illustrated clear trends of a crust by salt interaction.

The effect of crust on total biomass of *I. imperati* differed among rain treatments; though, the interaction between crusts and rain on *I. imperati* biomass was not significant after correction for multiple comparisons (Figure 2.6). In average and flood conditions, the effects of crust were negligible; however, in drought conditions, *I. imperati*, a generally dry species, exhibited greater total biomass compared to no crust controls. Other studies, particularly in arid environments, suggest that biocrusts may retain moisture (Kutiel 1998, Smith et al. 2004, Thiet et al. 2014). Given the effect of crusts on *I. imperati* is only seen in drought rain conditions, and across salt treatments, a similar mechanism could be operating here. Although these effects were not significant after correction for multiple comparisons, effect sizes (Table 2.5) suggest a moderate facultative interaction between crusts and coastal dune plant species with increasing
environmental stress. There were no additional significant interactions detected among crust, rain, and salt treatments; however, effect sizes suggest moderate interaction between these treatments in *P. distichum* and *S. maritimum*. These statistical interaction effects suggest crust effects vary slightly among rain and salt conditions in some species.

Previous research has identified several mechanisms that might underlie positive effects of algae-dominated crusts on plant growth in coastal dunes such as St. George Island. First, crusts have been shown to increase soil moisture (Belnap and Lange 2003, Smith et al. 2004), and soil moisture has positive effects on plant growth (Thiet et al. 2014). Increased moisture retention is a potential explanation for the positive effect of crusts in drought, as was seen in *I. imperati*. However, in *P. distichum*, the effect of crust is not greater in low moisture rain conditions; which would suggest that either moisture is not driving this positive reaction, or the relationship between the effect of crust and water stress is not monotonic (Kawai and Tokeshi 2007). Second, crusts have been proposed to increase or modulate nutrient availability, particularly nitrogen (Belnap 2002, Smith et al. 2004, Delgado-Baquerizo et al. 2013). Increased soil nutrients could account for the positive effect of crusts on *P. distichum*, but these effects are not significant after correcting for multiple comparisons. Third, crusts have been shown to stabilize soils and reduce erosion (Pluis and de Winder 1989, Kutiel 1998, Smith et al. 2004). Sand movement, bombardment, and changes in elevation have been shown to affect plants (Wilson and Sykes 1999, Maun and Perumal 1999, Miller 2015b). This third point presents the potential for additional positive effects of crusts on plants not examined in this study.

Some interaction results, although not significant, are in line with the stress gradient hypothesis, which postulates that interactions will shift from negative (competitive) to less negative or even positive (facilitative) across gradients of environmental stress (Bertness and Callaway 1994). Although not significant after correction for multiple comparisons, the data suggest that crust can ameliorate effects of low moisture and salt stress in *I. imperati* and *P. distichum*, respectively, implying facilitative effects of crusts on plants. However, these patterns are not consistent across the levels of watering used in this experiment. For instance, the interaction between *I. imperati* and crusts based on growth data changed from positive to neutral, or slightly negative. This shifting of interaction effects along these simulated stress gradients implies a range of positive to negative plant-crust interactions along the gradient of low to high moisture, as might be expected by Bertness and Callaway (1994). However, such effects were
only observed in growth data. The overall plant-crust relationship on the population level may be complicated by other factors, such as the negative effects of crusts on survival seen in several species (e.g. *C. asiatica*, *F. caroliniana*, and *U. paniculata*). To understand the effect of crusts on populations, it is also likely important to consider potential tradeoffs between factors like growth and survival over time. These effects may be investigated by monitoring plant’s response to field manipulations of crust presence or absence over time.

Overall, I did not find strong evidence for context-dependent plant-crust interactions that change the way species interact, and communities function, in various abiotic contexts. This is interesting given the strong potential for treatment interactions. It is important to note that crusts vary in structure in the field. In the wettest areas, such as interdunes which flood periodically, thicker, more mat like structures form that blanket the soil surface; in drier areas, crusts form within the top layer of sand and bind sand particles together. These two crust types may vary in their function or effect. Particularly, thicker, more mat-like crusts may have stronger effects on factors like moisture retention or seedling inhibition. Crusts in this experiment resembled drier crusts (see Figure 2.2), found more sparsely at higher elevations, and thus may not reflect the effects of more mat like crusts found in interdunes.

Another study investigating the mechanisms of positive species interactions on St. George Island alludes to potential indirect facilitation affecting patterns in community composition, particularly in interdune habitats where crusts are most common (Monge and Gornish 2015). Effect size data in this study suggests moderate positive interactions among crust, rain, and salt conditions. It is possible that crusts may be circuitously influencing plant species through the amelioration of stressors, such as low moisture and salt inputs, resulting in a facilitative effect; if so, these interactions would likely affect community composition as a function of storm conditions. Time series data, including precipitation, surge, crust abundance, and the plant community data, may be necessary to better understand the role of crusts in plant communities as a function of storm disturbance.

This experiment is focused on the effects of crusts on individual species in isolation. However, in the field, plants also interact with abiotic stressors, and combined effect of interactions among plants, crusts, and abiotic factors could in turn affect plant community dynamics. If species have differential responses to crust, crusts may affect competitive interactions between species. For instance, a positive effect of crust was observed in *P.*
distichum (shoot biomass) and a negative effect was observed in C. asiatica (number of leaves). P. distichum and C. asiatica co-occur fairly frequently in the field (Miller et al. 2009). This suggests the potential for crusts to give P. distichum a competitive advantage over C. asiatica in plots where biocrusts are abundant. Such changes in pairwise interactions between species have the potential to affect community composition and dynamics. Additional studies are needed to investigate the potential effects of crusts on plant community interactions in the field in order to best understand the implications of these effects in plant communities.
CHAPTER 3
COMPETITION EXPERIMENT

Introduction

In plant communities, individuals may interact through resource competition, or indirectly by affecting interactions with a third species (Miller 1994). Likewise, plants may circuitously affect other individuals in the system by altering environmental conditions in ways other than resource uptake. Examples include allelopathy, and nitrogen-fixation by legumes. Plants in a community will necessarily in their response to these abiotic changes. Variation in responses to changes in abiotic conditions presents the potential for differential plant-plant interactions in a third species. For example, if one plant is well equipped to use nitrates supplied by neighboring legumes, it may increase its competitive ability relative to other neighbors less efficient at utilizing these resources.

Biocrusts, particularly algae-dominated biocrusts, have been shown to affect a number of environmental variables, especially in disturbed or high stress environments. In arid desert systems, algae and cyanobacteria-dominated biocrusts have been shown to increase moisture (Belnap and Lange 2003, Pointing and Belnap 2012, Zellman 2014), contribute to nutrient inputs and regulation (Belnap 2002, Castillo-Monroy et al. 2010, Delgado-Baquerizo et al. 2013), and reduce erosion (Williams et al. 1995). In wetlands, algae and cyanobacteria-dominated periphyton mats have been shown to affect nutrients, stabilize soils, and contribute significantly to the food web (Gaiser et al. 2011). Crusts have also been shown to contribute to successional transitions in desert dunes (Kutiel 1998). Each of these abiotic effects of crusts has the potential to affect plants that co-occur with crusts, either directly or indirectly by affecting other vegetation.

Coastal dune systems can be quite arid and, like desert systems, tend to support biocrusts dominated by algae and cyanobacteria (Schulz et al. 2015). The few studies of these systems suggest that some of the abiotic effects of crust may be similar to desert crusts. Coastal dune crusts occur primarily in the wet, lower elevations, especially in the interdunes that are prone to flooding after storms (Álvarez-Rogel et al. 2007). These systems experience a variety of abiotic stressors similar to desert systems, such as low resource availability, and sand bombardment, in addition to periodic storm disturbances, all of which are known to affect the local plant
community (Gornish and Miller 2010, Mallinger 2014b). Stressful environments, such as desert and coastal dune systems, can promote facilitative relationships or reduce competitive relationships between heterospecific and conspecific neighbors (Maestre et al. 2005, 2009). While some studies have described positive interactions between plants in coastal dunes (Franks 2003a, Martínez 2003, Monge and Gornish 2015), the potential facilitative contributions of crusts in coastal dunes have not been studied.

There are a few studies describing abiotic effects of crusts in coastal dunes. For instance, coastal dune crusts have been shown to decrease erosion, increase water retention, and increase nutrients in the soil (Smith et al. 2004), as well as promote increases in silt and clay (Zellman 2014). Each of these environmental variables is known to affect plant species growth and survival. Hence, it is probable that the abiotic effects of coastal dune crusts affect plant species with which they co-occur.

Only a single study has investigated the effect of crusts on vegetation in coastal dune plant species. Thiet et al. (2013) found that algae-dominated crusts on coastal dunes on Cape Cod tended to increase soil moisture and seedling survival of two dune plant species, while decreasing growth relative to no crust controls. In these ways, crusts may indirectly confer a facilitative effect on individuals in these stressful systems by changing local abiotic conditions. However, crusts may also negatively affect plant species through competition for resources, such as water; hence, the direction of crust effects on dune vegetation may vary from positive in average, dry conditions (Thiet et al. 2014) to negative in the context of extreme drought (Kidron 2014).

Effects of crusts on individual plants have the potential to incite changes in pairwise interactions between plants, particularly if the effect of crust varies among species. For instance, if one competitor gains a greater benefit from crust-generated abiotic changes, such as moisture retention or nutrient availability, that competitor species may then exert a stronger competitive pressure on surrounding neighbors in crust conditions relative to crustless conditions. Alternatively, plant species that compete more strongly with crusts will exert less competitive pressure on neighbors in the presence of crust.

While several studies have investigated the effects of crusts on environmental variables and individual plant species, no previous studies have investigated the effects of biocrusts on species interactions in coastal dune communities. In this greenhouse experiment, I tested the
effect of coastal dune crusts on competition among four abundant coastal dune plant species using a target-neighbor design. Explicitly, I used a target-neighbor design to test (1) competitive interactions between plant species, (2) direct effects of crusts on these same species, and (3) interactions between the competitive effects of neighbors and the presence of soil crusts.

Methods

Experimental Design

To quantify competitive interactions among 4 species of coastal dune plants, I conducted a competition experiment using a 4x5 factorial target-neighbor design (Goldberg and Werner 1983). This included 3 heterospecific neighbor treatments, a conspecific neighbor treatment, and a “target only” treatment for each of the 4 species (Figure 3.1). A target individual was planted in the center of round 15 cm x 15cm pots, either alone, or with 3 neighbors of the same or a different species equally spaced around the target. Each target-neighbor combination was grown with and without crust, adding an additional level to the factorial design. These 40 experiment units (4 species, 5 neighbor treatments including “target only”, and 2 crust treatments - crust or no crust control) were replicated 5 times each for a total of 200 pots. After 6 months of growth, the biomass of target individuals in different treatments were compared to quantify intra- and interspecific interactions. Each of the neighbor treatments was also compared between corresponding crust and no crust treatments (i.e. conspecific no crust and conspecific crust treatments).

Plant selection, collection and rearing

I selected four plant species for this experiment that are common across the coastal dunes of St George Island for this experiment. Approximately 20 individuals of each species were collected from St George Island State Park in the winter of 2014 at a variety of locations on the easternmost tip of the island (under Florida DEP permit 10211410 to T. E. Miller). Two graminoid species (S. maritimum, and Paspalum distichum) and 2 forbs (Centella asiatica and I. imperati) were chosen for these experiments, all of which are perennial, and reproduce vegetatively. While each of the selected species occurs broadly across the dunes and co-occurs
with crust, they have been described by Miller et al. (2009) as being either "wet species" (*P. distichum*, and *C. asiatica*) or "dry species" (*S. maritimum*, and *I. imperati*).

Wet species tend to occur in lower elevation areas where they are more likely to co-occur with biocrusts, such as interdune habitats or troughs between larger dunes (see Chapter 1), whereas dry species tend to occur on the tops or sides of dunes, and predominantly in foredune and backdune habitats where crusts are more rare.

![Figure 3.1. Target-neighbor experimental design, including an “target only” treatment (NA) with no neighbors and 4 species of coastal dune plant: *Ipomoea imperati* (I), *Schizachyrium maritimum* (S), *Paspalum distichum* (P), and *Centella asiatica* (C), each grown with and without crust treatment.](image)

Individuals from each species were allowed to reproduce vegetatively in the greenhouse between the winter of 2014 and summer 2015 until 170 or more individuals of each species were produced, for a total of 680 plants used in the experiment. During this period, plants were grown in a 1:1 ratio of fine sand and potting soil in the greenhouse. A small amount of fertilizer was added at the beginning of the rearing process to facilitate vegetative growth during the rearing period. As the plants outgrew their pots they were separated into individual clumps or ramets and transplanted into separate pots.

To initiate the experiments, single ramets (forbs) or clumps of grasses were separated, cleaned, and weighed, and then transplanted to individual pots, consisting of a 15 x 15 cm round pot filled with sand. Treatment pots were transferred to the FSU Coastal and Marine Laboratory.
Biocrusts: collection, cultivation, and treatment

Natural biocrusts on St. George Island are often initiated in standing freshwater in low-lying areas such as interdunes. To create similar conditions in the greenhouse, biocrusts were collected from multiple sites across the long term research site at the eastern tip of St George in the winter of 2014. Collected crusts were dry due to a lack of rain and storms on the island during this period. Crust samples from across the dunescape were combined and homogenized, and then added to a glass 5 gal tank of fresh water with approximately 1 tablespoon of fertilizer. Given the low concentration of fertilizer, and the long growth period, the initial fertilizer addition was unlikely to be directly available for the plants used in the experiments. As the slurry grew, the mixture was divided into multiple tanks diluted with fresh water. Algae in tanks were combined into a larger tank, and thoroughly mixed to create a homogeneous slurry prior to biocrust treatment application.

After recovering from transplant stress, plants were flooded until up to approximately 2 cm above the sand surface. Half of all the plants in the experiment were then treated with approximately 120 mL of biocrust slurry, while half received an equal amount of fresh water as a control. After being treated with either the biocrust slurry or the water control, biocrusts were given two weeks in inundated conditions to establish. After this period, all pots were watered daily via an automated sprinkler system.

Data collection and analysis

Plant survival and growth (maximum height and number of leaves or blades) for the target plants were recorded throughout the duration of the experiment. After a period of 6 months, both the target and neighbor plants were harvested from their treatment pots and the stem and root length of the targets recorded. Target plants and the neighbors for each pot were then dried at 60 °C for a minimum of 48 hours and weighed.
Survival was analyzed as a function of crust and neighbor treatments using a Chi-squared tests with a binomial distribution for each target species. The effects of neighbor species on target growth were calculated as the difference between the target biomass for each neighbor treatment and the average biomass of the target alone, standardized by the average biomass of the target alone. This calculation is illustrated in Equation 1 below; \( W \) describes the target biomass for each neighbor (N) and crust (C) treatment combination or “target only,” or alone, individuals (I). In order to retain variance in target-neighbor treatment data, “target only” treatments were averaged to use as a constant in relative difference calculations. The relative difference values generated for each target were adjusted to positive values by adding the absolute value of the largest negative value and square root transformed in order to meet the normality assumptions of ANOVA. Values were analyzed using a factorial ANOVA for each target species with effects of neighbor identity, crust treatment, and their interaction. Effect sizes for relative competitive effects were calculated using partial Eta-squared in the “lsr” package in R (Navarro 2015).

\[
\text{(1)} \quad \frac{W_{NC} - \overline{W}_I}{\overline{W}_I}
\]

Results

Overall survival of target individuals was high (approximately 93%). Most of the mortality occurred in \( C. asiatica \) (7/50 target individuals) and \( P. distichum \) treatments (6/50 target individuals). Only \( C. asiatica \) survival was significantly affected by neighbor species (Table 3.1; \( df = 4, P = 0.048 \)). Survival was not significantly affected by crusts or crust-neighbor interactions in any of the other species.

The effects of treatments (neighbor identity and crust) were more apparent on growth for all four species. The full factorial ANOVA of competitive interaction effects show strong effects of target (\( F = 6.804, P << 0.001 \)), neighbor (\( F = 4.432, P = 0.005 \)), and crust (\( F = 21.275, P << 0.001 \); Table 3.2). The strongest effects were seen in crust treatments. No significant interaction effects were observed among target, neighbor, and crust treatments.
Table 3.1. Results of GLM for survival of each species as a function of both crust and neighbor treatments (including no neighbor control). P-values less than 0.05 are bolded. None of the effect sizes were greater than 0.06.

<table>
<thead>
<tr>
<th></th>
<th>Centella asiatica</th>
<th>Ipomoea imperati</th>
<th>Paspalum distichum</th>
<th>Schizachyrium maritimum</th>
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<tbody>
<tr>
<td>Crust</td>
<td>df 1 P 0.683 η² 0.004</td>
<td>df 3 P 0.048 η² &lt;0.001</td>
<td>df 3 P 0.687 η² &lt;0.184</td>
<td>df 1 P 1.00 η² &lt;0.001</td>
</tr>
<tr>
<td>Neighbor</td>
<td>df 3 P 0.048 η² &lt;0.001</td>
<td>df 3 P 0.494 η² &lt;0.001</td>
<td>df 1 P 0.702 η² -0.139</td>
<td>df 3 P 1.00 η² &lt;0.001</td>
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<tr>
<td>Crust*Neighbor</td>
<td>df 3 P 0.687 η² &lt;0.001</td>
<td>df 3 P 0.494 η² &lt;0.001</td>
<td>df 1 P 0.702 η² -0.139</td>
<td>df 3 P 1.00 η² &lt;0.001</td>
</tr>
</tbody>
</table>

Table 3.2. Results of factorial ANOVA of competitive interaction effects (based on the relative difference between each individual in competition and the average “target only” biomass) as a function of target, neighbor, and crust treatments. P-values less than 0.05 are bolded. Effect sizes greater than 0.06 are bolded.

<table>
<thead>
<tr>
<th></th>
<th>df 3 F 6.804 P &lt;0.001 η² 0.109</th>
<th>df 3 F 4.432 P 0.005 η² 0.227</th>
<th>df 1 F 21.275 P &gt;0.001 η² 0.079</th>
<th>df 3 F 1.597 P 0.124 η² 0.104</th>
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<td>Target</td>
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<td>Neighbor</td>
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<td>Crust</td>
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<td>Target*Neighbor</td>
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<td>Target*Crust</td>
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<td>Neighbor*Crust</td>
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<td>Target<em>Neighbor</em>Crust</td>
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Table 3.3. The ANOVA results for the effect of crust on “target only” treatments. P-values less than 0.05 are bolded. Effect sizes greater than 0.06 are bolded.

<table>
<thead>
<tr>
<th></th>
<th>df 1 F 10.863 P 0.011 η² 0.576</th>
<th>df 1 F 3.108 P 0.116 η² 0.280</th>
<th>df 1 F 2.241 P 0.178 η² 0.242</th>
<th>df 1 F 6.505 P 0.034 η² 0.448</th>
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<tbody>
<tr>
<td>Centella asiatica</td>
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<td>Ipomoea imperati</td>
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<td>Paspalum distichum</td>
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<tr>
<td>Schizachyrium maritimum</td>
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</table>

“Target only” treatments tended to have higher biomass when treated with crust, although this difference was only significant for C. asiatica (Table 3.3; Figure 2; df = 1, F = 17.41, P = 0.003; η² = 0.576) and S. maritimum (Table 3.3; Figure 2; df = 1, F = 6.505, P = 0.034; η² = 0.448). Marginal effects were seen in I. imperati and P. distichum. The effects of crusts were more variable when neighbors were present, though the interaction effect was marginal (Table 3.2; df = 3, F = 1.736, P = 0.089; η² = 0.081). There was a trend of greater target biomass in crust treatments compared to no crust controls for both P. distichum and S. maritimum targets paired with C. asiatica, and all
targets paired with *S. maritimum* neighbors, except *S. maritimum* monocultures, which did poorly in both crust and no crust treatments. Among neighbor treatments, the greatest effects of crust were seen in *C. asiatica* and *P. distichum* biomass; specifically, crust had a positive effect in combination with *C. asiatica* and *S. maritimum* neighbors.

Table 3.4. ANOVA results and effect sizes of relative competitive interaction effects for each of the four species used as targets in competition experiments. P-values less than 0.05 are bolded. Effect sizes were calculated using Eta-squared. All effect sizes greater than 0.06 are bolded, and those greater than 0.13 are denoted with asterisks.

<table>
<thead>
<tr>
<th></th>
<th>Centella asiatica</th>
<th>Ipomoea imperati</th>
<th>Paspalum distichum</th>
<th>Schizachyrium maritimum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Df</td>
<td>F</td>
<td>P</td>
<td>η²</td>
</tr>
<tr>
<td>Neighbor</td>
<td>3</td>
<td>0.176</td>
<td>0.911</td>
<td>0.016</td>
</tr>
<tr>
<td>Crust</td>
<td>1</td>
<td>4.954</td>
<td><strong>0.035</strong></td>
<td><strong>0.165</strong></td>
</tr>
<tr>
<td>Neighbor*Crust</td>
<td>3</td>
<td>1.447</td>
<td>0.253</td>
<td><strong>0.147</strong>*</td>
</tr>
</tbody>
</table>

This design allows the separation of competitive effects and responses, which varied among target and neighbor species combinations. Conspecific neighbor treatment and in combination with *P. distichum*; for *I. imperati* targets, species competitive effects tended to be less negative in combination with *C. asiatica* and in with conspecific neighbors; little effect was seen across neighbor treatments in no crust control for *P. distichum*; competition effects in in *S. maritimum* tended to be less negative, or even slightly positive, in combination with *I. imperati* and *P. distichum* (Figure 3.3). In general, these relationships suggest some species are fairly strong competitors in general, such as *P. distichum*; some species are relatively weaker competitors, such as *C. asiatica*; and other species responses and competitive effects are variable. These relationships varied across crust treatments, which tended to have a negative competitive effect among neighbor treatments.

**Discussion**

In this experiment, the effects of coastal dune crusts on competition between four abundant coastal dune plant species were quantified using a target-neighbor design. Plants grown without competitors usually had a greater biomass than those grown with neighbors, demonstrating
Figure 3.2 Final biomass of each of the target species as a function of their neighbor treatment (X axis), including “target only” controls (alone). Crust treatments are denoted by green bars, and no crust treatments are denoted by tan bars. Note that the Y axes vary by target species, as some of these species tend to differ in size, especially between grasses and forbs.
Figure 3.3 The relative effect competitive effect of each target species in neighbor treatments compared to no competition ("target only") treatment averages for each target species. Crust treatments are denoted by green bars, and no crust treatments are denoted by tan bars. The dashed line denotes no difference between "target only" treatment and the relative competitive interaction effect. Note that the Y axes differ in scale between target species.
a significant and sometimes quite strong effect of competition. There was a significant effect of neighbor treatment on survival in *C. asiatica*, as well as significant main effects of target, neighbor, and crust treatments on the growth of all four species when grown in competition. These results show that crusts do promote the growth of these dune species and that species vary in both their response to competition and their competitive effects on other species.

The effects of crusts in this experiment were complex. Crust had a positive effect on biomass in “target only” treatments, particularly in *C. asiatica* (Figure 3.2). However, the effect of crusts on biomass in competition varied among species, but was usually negative overall. Likewise, when the relative competitive effects of crusts were examined, crusts tend to have a negative effect overall, and the variation seen was not statistically significant. Yet, for some pairwise combinations there was a positive effect of crust on biomass relative to no crust controls which rivaled the “target only” biomass for that species, particularly *C. asiatica* and *P. distichum* (Figure 3.2). The different effects of crust for “target only” treatments and in competition treatments suggest other forces may be at play in competition. For instance, though not significant, “target only” *P. distichum* and *I. imperati* biomass tended to be greater in crust treatments compared to no crust controls (P = 0.090 & 0.085, respectively); the effect of crust on *P. distichum* in competition with *I. imperati* was negative; and there was seemingly no effect of crust on *I. imperati* in competition with *P. distichum*. This trend indicates the potential for interactions between *I. imperati* and crust that exaggerate the effect of competition on *P. distichum*.

Trends in the data suggest differential responses to crusts and neighbors among species (Figure 3.3). For instance, *P. distichum* targets paired with *C. asiatica* neighbors on average had higher biomass in crust treatments compared to no crust controls; as did *P. distichum* paired with *S. maritimum* neighbors, and *C. asiatica* with *I. imperati* or *S. maritimum* neighbors. Alternatively, some target species demonstrated little to no effect between crust and no crust treatments across neighbors. For instance, *I. imperati* and *S. maritimum* targets saw little variation between most neighbor treatments and between crust treatments. Likewise, some species, such as *P. distichum*, exhibited stronger effects of crusts on the relative competitive interaction effects compared to other neighbor species. *P. distichum* often occurs with crust, and may have adapted to crust conditions. Likewise, these species differ in growth and size. *P. distichum* and *S. maritimum* tend to be larger than *C. asiatica* and *I. imperati*. In the field,
differential growth responses in the presence of crusts presents the potential for variation in competitive outcomes in the context of crusts, which were not detected here.

Figure 3.4. Overall effect of crust on biomass of targets in competition treatments. Values depicted group all crust and no crust treatments per species regardless of neighbor treatment.

The effects of crusts observed in this experiment differ slightly from the research presented in Chapter 2. In this earlier experiment using only single plants per pot, only *P. distichum* illustrated a significant positive growth response to crust, and *C. asiatica* illustrated a significant negative response. However, Chapter 2 focused on the effect of crusts in various abiotic conditions, and was conducted in a different greenhouse with different growth conditions. One potential explanation for the difference in crust effects between the two experiments is the alteration of watering schemes. In Chapter 2, watering was designed to mimic different rain or storm conditions in the field, including storm rain, average drought conditions, and average conditions, which were still fairly dry. In the competition experiment, pots were watered daily via a sprinkler system, which maintained a moister environment compared to the previous
experiment. Higher algae and cyanobacteria abundance is thought to be promoted in more moist conditions (Álvarez-Rogel et al. 2007), and hence, the effects of crusts may have been stronger in these more moist conditions. Likewise, the experiment described in Chapter 2 found a positive crust by salt interaction effect in *P. distichum*. The positive effects of crusts seen in Chapter 2 may have been largely influenced by interactions between crusts and abiotic variables in various stress conditions; whereas, plants in this experiment did not experience water or salt stress. Given the variation in response to crust and abiotic stressor combinations among plants, competitive interaction effects between these species may also change in the context of crust and abiotic stressors.

Figure 3.5. Relative effects of competition, compared to no competition treatments, with and without crust. Values depicted group all crust and no crust treatments per species regardless of neighbor treatment.

The stress gradient hypothesis suggests that species in harsh or stressful environments, such as coastal dune systems, have a higher tendency for facilitative or less competitive
interactions (Bertness and Callaway 1994). In some cases in my experiment, targets in competition treatments with crust had fairly similar biomass to “target only” treatments (e.g. *P. distichum* targets with *C. asiatica* and *S. maritimum* neighbors, and *S. maritimum* targets with *C. asiatica* neighbors). This suggests crusts may have a positive effect on these target species, or a negative effect on neighbor species, mitigating the negative effects of competition. While I did find main effects of crusts, abiotic factors, and competition on biomass, and patterns in the data suggest differences in plant growth among these variables and among species, interaction effects between these variables per species were not significant. In general, these data do not support the stress gradient hypothesis, since the positive effects of crust occur under low, rather than high, competitive stress.

Previous research suggests biocrusts act as ecosystem engineers by enhancing moisture retention, reducing erosion, and contributing to soil nutrients (Williams et al. 1995, Belnap 2002, Smith et al. 2004, Thiet et al. 2014). My results do not support the idea that crusts operate as ecosystem engineers through modulation of soil nutrients for established plants. While they do seem to have largely positive effects on individuals, crusts do not appear to affect the largely negative effects of competition among dune species, except possibly for *P. distichum* targets with *C. asiatica* and *S. maritimum* neighbors, and *S. maritimum* targets with *C. asiatica* neighbors. However, coastal dune plants are known to be affected by a number of stressors not explicitly investigated here, that could also affect plant survival, growth, or competition, such as sand bombardment and erosion, which have been shown to be reduced by crusts (Smith et al. 2004, Levin et al. 2007). Further, my study was initiated from clonal material; other results might have been found using seeds and seedlings. A simultaneous examination of species interactions in various environmental conditions and life-history stages would be useful to examine changes in plant-plant interaction effects as a function of crust and environmental variables.

This data suggests that the interactions between coastal dune crusts and plant communities may be very important, but that these interactions vary in both magnitude and direction among species and do not interact with competitive effects. While some studies have examined the abiotic effects of coastal dune crusts on abiotic factors (Smith et al. 2004, Kidron and Büdel 2014), and their interaction with individual plant species (Thiet et al. 2014) or overall community characteristics (Kidron 2014), this is the first study to explicitly examine the effects
of crusts on plant-plant interactions between four dominant coastal dune plant species. However, these systems are complex, and plant responses and interactions vary as a function of space, time, and abiotic stress. In order to better understand the role of biocrusts in coastal dune communities, future work will need to incorporate variables, like stress, in tests of competitive interaction effects to determine if crust presence affect plant-plant interactions differently under stress. Understanding these interactions, and how they change as a function of community composition, will allow us to better understand community dynamics, and optimize management regimes to increase ecosystem services and function in the face of changing landscapes and increased stressors.
CONCLUSIONS

Previous studies have investigated the effects of biocrusts on abiotic factors in coastal dunes (Williams et al. 1995, Smith et al. 2004), but few have investigated the effects of biocrusts on vegetation. Those studies that do examine the effects of crusts on plant species studied one or two species in static conditions, and the effects observed were complex. For instance, Thiet et al. (2005) found that algae-dominated crusts tend to have a negative effect on plant growth, and a positive effect on seedling survivorship. Likewise, in desert systems, while crusts tend to be associated with positive environmental effects, like increased soil moisture, some studies have found overall negative effects on plant species growth and survival (Kidron 2014). In order to understand the role of biocrusts in coastal dune communities, a more comprehensive examination of crust effects, including many species, and ideally whole communities, are necessary.

I analyzed data on the spatial distributions of plants and crust from a coastal dune community to examine the relationships among the occurrence of crusts, individual plant species, and the plant community as a whole. I identified five species with positive correlations with crust and six species with negative correlations, as well as abiotic and community characteristics associated with crusts. I found that crust cover was not correlated with soil moisture or organic content. These associations suggest species specific interactions with crust that might moderate plant-plant interactions.

Coastal dunes are dynamic environments that experience regular stressors, such as sand bombardment, erosion, and salt spray and the stressors and disturbances that affect these systems are forecasted to change with climate change (Feagin et al. 2005). In the storm simulation experiment, I found differential responses of plant growth and survival to crusts treatments among species ranging from positive to negative. I did not detect significant statistical interaction effects of crusts and abiotic factors, but patterns suggested interactions between effects of crusts and abiotic conditions on plant growth and survival. Differential responses, such as those observed here, crust could drive variation in the strength and direction of plant species interactions.

Interactions between individuals in coastal dune communities are complex. While competition between plant species is evident (Martinez and Garcia-Franco 2003), the stress
gradient hypothesis would suggest facilitative interactions between otherwise competitive species at stress gradient extremes (Bertness and Callaway 1994). Other studies of coastal dunes have documented positive interactions between coastal dune species, supporting the stress gradient hypothesis (Franks 2003b, Monge and Gornish 2015). While I did not detect significant interactions between crusts and neighbor species on target growth and survival, patterns in the data suggest the potential for interactive effects between these variables, which might be more pronounced across a larger gradient of conditions, including varieties of biocrusts found in the field, or with larger sample sizes. These sorts of changes in the direction of pairwise interactions between species have the potential to affect community composition and dynamics.

I postulate that the observed effects of crusts in these analyses may be driven by changes in the environmental context caused by crust, and create potential positive feedbacks between crusts and abiotic factors. For instance, crusts are promoted by ephemeral inundation, or high soil moisture, and are also associated with retaining moisture, creating a locally moister environment, which may promote the growth of more crust. Investigating species turnover as a function of crust over time may elucidate positive feedback effects between crusts and plant species. Understanding the causal relationship between crusts, environmental factors, and plant community composition will allow us to better understand community dynamics, and optimize management regimes to increase ecosystem services and function in these socially and economically important coastal dune systems.
REFERENCES


Miller, T. E. 2015b. Effects of disturbance on vegetation by sand accretion and erosion across coastal dune habitats on a barrier island. AoB plants 7:plv003–.


**BIOGRAPHICAL SKETCH**

**Education**

<table>
<thead>
<tr>
<th>Degree</th>
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<tr>
<td>MS</td>
<td>2016</td>
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<td>BS</td>
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**Research**

- The role of biocrusts in coastal dune plant communities; Advisor: Thomas E. Miller; Florida State University 2014-2016

- Pollination of *Manzanita* by thrips; Principle Investigator: Dr. Dorit Eliyahu; University of Arizona 2013-2014

- Adapting the pin-intercept method for estimation of biomass in sawgrass communities of the Florida Everglades; Advisor: Dr. Brian Benscoter; Florida Atlantic University 2012-2014

- Vegetation analysis for management of *Gopherus polyphemus* habitat; Advisor: Dr. E. Frazier, Florida Atlantic University 2011-2012

- Habitat suitability assessment for wading birds in the Florida Everglades; Advisor: Dr. Dale Gawlik, Florida Atlantic University 2010-2013

**Publications**


**Presentations**

- Ecological Society of America Conference, Poster Presentation 2015
- Southeastern Ecology and Evolution Conference, Oral Presentation 2015
- Florida State University Natural Sciences Symposium, Poster Presentation 2014
Florida Atlantic University Undergraduate Research Symposium, Poster Presentation 2013
Florida Atlantic University College of Science Symposium, Poster Presentation 2013
South Florida STEM Research Symposium, Poster Presentation 2013
Women’s Leadership Forum Meeting, Poster Presentation 2013
Southeastern Ecology and Evolution Conference, Poster Presentation 2013
Ecological Society of America Conference, Poster Presentation 2012
Florida Atlantic University Undergraduate Research Symposium, Poster Presentation 2012
Stetson Undergraduate Research Conference, Poster Presentation 2012

Research Grants

Florida State University Coastal and Marine Laboratory Fellowship 2014
Robert K. Godfrey Endowment for Botany 2014
Broward Undergraduate Research Grant 2013
Florida Atlantic University Undergraduate Research Grant 2011

Academic Awards

ESA-SEEDS Travel Award, ESA Annual Meeting 2014
Best Undergraduate Poster, Southeastern Ecology and Evolution Conference 2013
Society of Wetland Scientists Travel Grant 2013
Florida Atlantic University Undergraduate Grant 2013
Edward Shoaf Scholarship 2013
ESA-SEEDS Travel Award, SEEDS Annual Leadership Meeting 2013
INTECOL International Wetlands Conference Travel Award 2012
National Science Foundation Travel Grant 2012
National Science Foundation Undergraduate Research and Mentoring Fellowship 2011
Florida Academic Scholarship 2008
Academic Competitiveness Grant 2008

Teaching Experience

Lead Teaching Assistant - Introductory Biology II 2016
Graduate Teaching Assistant - Introductory Biology II; Principles of Ecology; Evolution, Medicine and Evidence 2014-2016
Undergraduate Teaching Assistant – Life of a Scientist 2012-2013
Workshop Leader – Environmental Education 2012-2013

Service

Southeastern Ecology and Evolution Conference Chair 2015-2016
Associate Editor, Florida Atlantic University Undergraduate Research Journal 2012-2013
ESA - Strategies for Ecology Education, Diversity and Sustainability (SEEDS) 2011-2013

Societies

Ecological Society of America
American Society of Naturalists