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Emergent interactions influence functional traits and success of dune building ecosystem engineers

Joseph K. Brown
Virginia Commonwealth University

EMERGENT INTERACTIONS INFLUENCE FUNCTIONAL TRAITS AND SUCCESS OF
DUNE BUILDING ECOSYSTEM ENGINEERS

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
at Virginia Commonwealth University.

By

Joseph Karl Brown,
B.S. Biology Virginia Commonwealth University 2013

Directors: Julie Zinnert, Phd and Donald Young, PhD, Department of Biology

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Acknowledgement

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Abstract

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Stability of coastal systems are threatened by oceanic and atmospheric drivers of climate change. Sea-level rise compounded with increased frequency and intensity of storms emphasizes need for protection of inner island systems by dune formations. Dune building processes are affected by interactions between growth of ecosystem engineering dune grasses and environmental factors associated with disturbance such as sand burial and salt spray. Climate change may also cause latitudinal expansion of some species, resulting in emergence of competitive interactions that were previously absent. Topographic structure of coastlines, traditionally influenced by sand burial, could change as a result of competition emergence. My goal was to determine if species functional trait responses to common abiotic factors are altered by novel and current biotic interactions. I performed a multi-factorial greenhouse experiment by planting three common dune grasses (*Ammophila breviligulata, Uniola paniculata, and Spartina patens*) in different biotic combinations, using sand burial and salt spray as abiotic stressors. I hypothesized that biotic interactions will cause these dune grasses to shift functional trait responses to abiotic factors that are associated with dune building. I found that plants consistently decreased in biomass when buried. I also found that competition between *A.*

breviligulata and *U. paniculata* negatively affected dune building function traits of *A. breviligulata*. This indicates that competition with *U. paniculata* could alter dune structure. In comparison *A. breviligulata* had a positive interaction with *S. patens,* which increased functional trait responses to abiotic stress. Last, we found that competitive intransitivity could occur between these species. My results can be used to make predictions on cross-scale consequences of novel competitive events. This experiment also provides evidence that consideration of local biotic interactions is important in understanding connections between plant level dynamics and large-scale landscape patterns in high stress environment.

Introduction

Barrier islands are vital ecosystems that provide the first line of defense against increasing disturbances (Feagin et al. 2015). These ecosystems are significantly affected by sea level rise (SLR) and high intensity storms, both increasing with current climate change (Duran and Moore 2013, Prisco et al. 2013, Brantley et al. 2014). Dunes are important geomorphological formations of barrier islands that serve in protecting inner island processes as well as associated wetlands (Stallins 2003, 2005, Duran and Moore 2013). Coastal dune formations rely on interactions between sediment movement, plant presence, and plant physiological response to sand burial (Stallins 2003, 2006, Duran and Moore 2013, Brantley et al. 2014).

While dune morphology is a function of what grass is species present, dunes themselves influence abiotic stressors that affect plants such as water limitation, nutrient limitation, sea-spray, and burial (Seliskar 1993, Maun 1998, Stallins 2006, Gornish and Miller 2010, Emery and Rudgers 2014). These constant plant-environment interactions influence dune grass community establishment (Qu et al. 2014, Stallins 2006, Miller 2015). Many studies have focused on effects of sediment-plant interactions on dune formation (Stallins 2006, Duran and Moore 2013, Brantley et al. 2014, Emery and Rudgers 2014); however, the influence of biotic interactions on functional trait responses of dune grasses to abiotic stressors has had little focus (Zarnetske et al. 2012). Here I define functional traits as any morphological or physiological characteristic of an organism that indicates ecological strategies of growth relative to environmental conditions across spatial and temporal scales (Westoby and Wright 2006, Kraft and Ackerly 2010). In this study, functional traits consist of morphological measurements reflective of both stability within a community and success in response to abiotic stress; such as aboveground biomass,

belowground biomass, elongation, and maximum root length (Seliskar 1993, Brewer et al. 1998, Maun 1998, Qu et al. 2014). **The objective of my research was to investigate the net effect of species interactions on morphological functional trait responses of three common dune grasses to abiotic stressors (burial and sea-spray).**

I tested the hypothesis that current and novel species interactions cause functional trait modifications that either promote or hamper plant response to abiotic factors. Dune grasses are subjected to high stress environments due to high sediment deposition via aeolian sand transport, overwash events, high wind events, and high sea-spray exposure (Seliskar 1993, Maun 1998, Stallins 2002, Brantley et al. 2014, Emery and Rudgers 2014). These harsh environmental factors could possibly be mitigated with positive species interactions (Bertness and Callaway 1994, Callaway et al. 2002, Zarnetske et al. 2012), while emergent competitive interactions could increase plant vulnerability to abiotic stress (Esquivias et al. 2015). While facilitation and competition differ in result, they are similar in mechanism and can be drivers of cross-scale ecological change and biodiversity (Zarnetske et al. 2012, Bertness et al. 2015, Esquivias et al. 2015). It is important to consider plant functional traits as both the origin and result of biotic interactions in high stress environments (He et al. 2013, Kraft et al. 2015). Plant-to-plant interactions can cause small scale changes in individual plant physiology by altering functional trait response to abiotic factors and provide either a facilitative interaction or gives rise to negative competition (Hacker and Bertness 1995, Zarnetske et al. 2012 Esquivias et al. 2015). Significant interactions can also cause large-scale consequences dependent on resulting dune grass establishment (Stallins 2006, Zarnetske et al. 2012, Duran and Moore 2013, Brantley et al. 2014, Monge and Gornish 2015). For example, if novel species interactions were to arise between dune grasses and functional traits begin to shift we may see consequential shifts in dune

engineering (hummock vs. ridge formation), recovery capacities from disturbances, and overall habitat complexity (Stallins 2006, Zarnetske et al. 2012, Brantley et al. 2014, Duran and Moore 2015).

As dune formation is shifted in response to dune grass functional trait modifications, changes in vegetation distribution and biodiversity at a local scale as well as in connected dune-swale complexes can be expected (Young et al. 2007, Monge and Gornish 2015, Miller 2015). A decrease in functional trait abilities to build large dunes can result in self-perpetuating breaching events during storms and high water events (Duran and Moore 2015). If dune protection is compromised, swale communities could experience biodiversity collapse resulting in decreased habitat space and overall species richness (Young et al. 2007). This is critical in a system that is already characterized by low diversity (Stalter and Odum 1993).

Ammophila breviligulata Fern., (Poaceae) is a common dune grass found in northern latitudes from North Carolina to Canada (Emery and Rudgers 2014). *Ammophila breviligulata* builds large continuous ridges and has functional traits that allow for success in dune habitats such as deep roots, increased growth response to burial, and adventitious root production (Seliskar 1993, Emery and Rudgers 2014, Qu et al. 2014). On the Virginia barrier islands *A. breviligulata* coexists with *S. patens* (Day et al. 2001), however interactions between these species is relatively unknown. *Spartina patens* Muhl., (Poaceae) is a generalist species found across moisture gradients of barrier islands and exists along the Atlantic coast from Canada down to Florida (Stalter 1994). *Spartina patens* has been suggested to maintain flatter areas on islands allowing for more frequent overwash and salt water inundation to occur (Brantley et al. 2014). In its southern distribution *S. paten* coexists with *U. paniculata* (Stallins 2002), interactions between these species is also relatively unknown. *Uniola paniculata* L., (Poaceae) is

a semi-tropical dune grass that grows on the southern Atlantic coast from southern Virginia into the Gulf Coast (Wagner 1964, Hodel and Gonzalez 2013). This species exhibits a phalanx growth strategy resulting in hummock dunes and recolonizes newly disturbed areas slowly (Wagner 1964, Mendelssohn et al. 1991, Stallins 2006). Since the last glacial maximum *U. paniculata* has continued to migrate north, a pattern that is expected to continue as climate change persists and global temperatures rise (Hodel and Gonzalez 2013).

Native species can be considered invasive when their ranges are expanded in response to rapid climatic changes (Alpert et al. 2000). This suggests that emergent competition with *U. paniculata*, acting as an invasive, could have significant effects on *A. breviligulata* or *S. patens* functional trait responses to abiotic stressors. It is important to understand how interactions cause functional trait shifts because resulting plant communities will influence dune topography, dune building processes, and ultimate island formation (Stallins 2006).

Functional trait outcomes resulting from species interactions can cause variable feedbacks with physical processes present, thereby changing barrier island morphology (Hayden et al. 1995, Stallins 2006, Zinnert et al. 2016). Coastal ecosystems are ideal for demonstrating the proposed theories of multiple causality of land formation processes because of these variable feedbacks (Stallins 2006). The consideration of cross-scale interactions in barrier island formation is critical in understanding how physical process at one temporal scale can lead to changes in plant distribution which will have subsequent effects on a second physical process at a second temporal scale (Stallins 2006, Young et al. 2007, Miller 2015, Zinnert et al. 2016). Brantley et al. (2014) described how islands differing in topography displayed different plant distributions. Islands with large dunes and high *A. breviligulata* abundance are coupled with less disturbed swales (Brantley et al. 2014). Conversely, islands with low elevation and active

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overwash areas are completely dominated by *S. patens* (Brantley et al. 2014), and as

aforementioned, *U. paniculata* is associated with hummocky dunes that experience intermediate

overwash disturbance due to phalanx growth strategy that results in large spaces between dune

engineering ramets (Wagner 1964, Mendelssohn et al. 1991, Stallins 2006). In order to

understand how plants will affect barrier island formation and protection, we must understand

how plant functional traits respond to abiotic factors when compounded with complex biotic

interactions.

It is clear that plant-to-plant interactions have significant effects on individual plant

morphological or physiological responses (Hacker and Bertness 1995, Zarnetske et al. 2012,

Esquivias et al. 2015). However, this type of research is lacking in dune habitats and can

potentially explain how emergent competition, driven by species range expansion from climate

change, could alter dune morphology, barrier island stability, and island biodiversity. Here I

performed a multi-factorial greenhouse experiment to examine how plant functional trait

responses to abiotic factors change under different biotic mixtures. If my hypothesis is

supported and functional trait responses to abiotic stress are modified by biotic interactions it

could begin to explain how dune community structure could influence dune structure.

Methods

A multi-factorial greenhouse experiment was designed to measure how *A. breviligulata*

(A*), U. paniculata* (U), and *S. patens* (S) respond to varying biotic mixtures and a combination

of abiotic treatments (Figure 1). Four different plant biotic mixture levels (AU, AS, US, USA),

as well as monoculture levels (A, S, U), were planted in 14.5 x 15 cm plastic pots to induce

competition. Each pot was planted with an even density of 6 stems and even species density in

each mixture (i.e. when 2 species were present there were 3 individuals of each species, when 3

species were present there were 2 individuals of each species; Figure 1). Each biotic mixture was treated with abiotic treatments common to barrier islands: control (no treatment), sea-spray with no burial, burial with no sea-spray, and burial + salt spray (Figure 1). Sample size was even across all species × biotic mixture × treatment combinations (n = 5).

All plants were purchased in plugs from nurseries (*A. breviligulata* from Cape Coastal Nursery in South Dennis, MA, *S. patens* from Green Seasons Nursery in Parrish, FL, and *U. paniculata* from Coastal Transplants in Bolivia, NC). Upon arrival, plants were planted and a two week growth period was permitted for plant establishment. All plants were trimmed to 35 cm height. Burial collars made from PVC piping, 10.3 cm in diameter, were placed around each group of plants and pushed 1 cm into the soil. Plants being buried received collars 25 cm in length, while unburied groups received collars of 5 cm in length. Burial and burial + sea-spray pots were treated with 25 cm of sand burial once at the beginning of the study to simulate large sand deposition events found in natural systems.

A mixture of water and Instant Ocean (Aquarium Systems) was used to create a 20 ppt sea-spray solution to be applied to designated groups. In order to retain consistency during the experiment a spray bottle was calibrated before each sea-spray treatment to administer as close to a fixed amount of salt solution as possible. Five sprays per pot were applied twice per week, two sprays on the front and back of each pot and one spray from the top. When watering plants, salt crystal buildup on leaves was rinsed into the sand of each pot. Plants were watered as need with fresh water. All pots were treated once during the study with a 25 mL Hoagland's solution (Hoagland and Arnon 1950) diluted to 50 mL of tap water. The experiment continued from May of 2015 to August of 2015.

Functional trait measurements

I measured morphological trait responses of all species in each treatment group and biotic mixture level. Morphological measurements were collected to provide insight in plant success on a longer temporal scale, there by simultaneously predicting individual and community consequences of functional trait shifts (Lavorel and Garnier 2002).   Elongation was calculated by taking a final height measurement (cm) of each plant and subtracting the original height of each plant (35 cm). This provides a metric that quantifies amount of vertical growth of each species in each treatment group and across all biotic mixture levels. Due to the destructive nature of aboveground biomass, belowground biomass, and maximum root length (MRL) measurements, they were collected at the conclusion of the study during harvesting. Aboveground biomass was defined as any biomass not buried by sand addition treatments. Aboveground biomass was clipped away from belowground biomass during deconstruction of pots and MRL was gathered by extending the longest root of each species and using a ruler to measure length (cm) from the plant base to the root tip. MRL provides evidence of belowground functional trait strategies of water and nutrient capture as well as sediment stabilization (Lou et al. 2014, Qu et al 2014). Belowground biomass and aboveground biomass samples were put in paper bags and dried at 65° C for 72 h and weighed. A relative interaction intensity (RII) index (Armas et al. 2004, Noumi et al. 2016) was calculated with total biomass (aboveground biomass + belowground biomass) to quantify species interactions affecting functional trait responses when plants were grown in a biotic mixture:

$$RII = (X_{ab} - X_{aa})/(X_{ab} + X_{aa})$$

where X_{aa} is total biomass/stem in monoculture and X_{ab} is total biomass/stem of species *a* when grown with species *b*. Quantitative outcomes of RII index are proportional to 0 (i.e. an index of

0 indicates no significant interaction). Indices between 0 and +1 indicated a positive species interaction, and indices between 0 and -1 were indicative of a negative species interaction (Noumi et al. 2016). The nature of this formula is such that monoculture treatments (RII index = 0) was a baseline for all species RII index comparisons.

Statistical analyses

There were no species × treatment × biotic mixture interactions for all measurements (Table S1 - S4). There was a significant effect of species on elongation, MRL, above-, and belowground biomass (Table S1 - S4). After segregating data by species, two-way ANOVA analysis was performed to test treatment × competition interaction on individual species. When two-way interactions were present, one-way ANOVA ($\alpha = 0.05$) testing was used to detect whether competition levels caused functional trait differences within each treatment group. Tukey HSD ($\alpha = 0.05$) test was performed to determine which competition levels caused functional trait changes.

To compare biotic mixture RII index scores to control RII index scores a Dunnett's ($\alpha = 0.05$) test was performed. Using this test confirmed which biotic mixture levels were significantly different from monoculture (control).

Results

Biomass

Ammophila breviligulata

There was a significant treatment × biotic mixture interaction on *A. breviligulata* aboveground biomass ($F_{9, 64} = 2.39$, $P < 0.05$). Aboveground biomass decreased in burial and burial + sea-spray treatments ($F_{3, 64} = 177.68$, $P < 0.0001$, Fig. 2).

In control treatments *Ammophila breviligulata* aboveground biomass was 32% higher when grown with *S. patens* (1.45 ± 0.12 g) than when grown with *U. paniculata* (0.99 ± 0.12 g), but was not different from any other biotic mixture (Fig. 2a). In sea-spray treatments *A. breviligulata* aboveground biomass was hindered by presence of *U. paniculata* (0.89 ± 0.07 g) causing a decrease in aboveground growth by over 34% compared to USA (1.36 ± 0.17 g) mixture, 38% compared to *A. breviligulata* monoculture (1.44 ± 0.09 g), and 49% when compared to AS (1.74 ± 0.07 g) mixture (Fig. 2b). Burial treatment showed no difference between *A. breviligulata* grown in AS mixture (0.53 ± 0.08 g; Fig. 2c) and when grown as a monoculture (0.37 ± 0.03 g; Fig. 2c). However, when burial was present *A. breviligulata* aboveground biomass was higher when grown with *S. patens* (0.53 ± 0.08 g) compared to when grown with *U. paniculata* (0.20 ± 0.06 g; Fig. 2c) or when grown in USA mixture (0.25 ± 0.05 g; Fig. 2c). Biotic mixture within burial + sea-spray treatment showed that aboveground biomass increased by 38% when *A. breviligulata* was grown with *S. patens* (0.46 ± 0.06 g) compared to *A. breviligulata* monoculture (0.28 ± 0.02 g; Fig. 2d) and 46% compared to when grown with *U. paniculata* (0.25 ± 0.02 g; Fig 2d).

There was no treatment $\times$ biotic mixture interaction on belowground biomass of *A. breviligulata* ($F_{9, 64} = 0.32$, $P = 0.9557$). Treatment and biotic mixture were both significant main effects on belowground biomass of *A. breviligulata* ($F_{3, 64} = 32.76$, $P < 0.001$; $F_{3, 64} = 3.75$, $P < 0.05$, respectively), as detailed below.

Belowground growth was 29% higher in *A. breviligulata* when it was grown with *S. patens* (0.52 ± 0.06 g) than when competing with *U. paniculata* (0.37 ± 0.04 g). Belowground biomass also significantly decreased when *A. breviligulata* was grown in USA mixture (0.37 ± 0.05 g) compared to AS mixture (0.52 ± 0.06 g). Plants experiencing burial (Fig. 2c and 2d) had a significantly higher belowground biomass than non-buried plants (Fig. 2a and 2b).

Uniola paniculata

There was no treatment $\times$ biotic mixture interaction for above- or belowground biomass of *U. paniculata* ($F_{9, 64} = 1.07$, $P = 0.4002$; $F_{9, 64} = 2.01$, $P = 0.0530$, respectively).

Biotic mixture and treatment had an effect on aboveground biomass of *U. paniculata* ($F_{3, 64} = 6.62$, $P < 0.001$; $F_{3, 64} = 9.81$, $P < 0.0001$, respectively). *Uniola paniculata* aboveground biomass was over 26% higher when competing with *A. breviligulata* (0.76 ± 0.05 g; Fig. 3a) compared to any other biotic mixture (monoculture = 0.53 ± 0.04 g; US = 0.50 ± 0.06 g; USA = 0.55 ± 0.06 g; Fig. 3a). Burial and sea-spray + burial treatments caused a significant decrease in aboveground biomass compared to non-buried plants (Fig. S1).

In comparison, treatment had an effect on belowground biomass of *U. paniculata* ($F_{3, 64} = 7.41$, $P < 0.0005$). Burial and burial + sea-spray treatments caused a significant increase in belowground biomass compared to belowground biomass production of non-buried plants (Fig. S1).

Spartina patens

No significant treatment × biotic mixture interaction was found for above- or belowground biomass of *S. patens* ($F_{9, 64}$ = 1.02 P = 0.4334).

Aboveground biomass was affected by biotic mixture ($F_{3, 64}$ = 6.78 P < 0.001). When *S. patens* was grown with *A. breviligulata* and *U. paniculata* together (0.70 ± 0.07 g; Fig. 3b) it produced aboveground biomass over 30% higher than when grown with *A. breviligulata* (0.47 ± 0.06 g; Fig. 3b) or when grown in a monoculture (0.49 ± 0.04 g; Fig. 3b). Aboveground biomass was also affected by treatment ($F_{3, 64}$ = 26.3 P < 0.0001), buried plants produced less aboveground biomass when compared to unburied plants (Fig. S2).

Spartina patens belowground biomass was also affected by biotic mixture ($F_{3, 64}$ = 6.26, P < 0.001). Belowground growth decreased by 33% when *S. patens* was grown with *A. breviligulata* (0.41 ± 0.05 g; Fig. 3b) than when grown with *U. paniculata* (0.61 ± 0.05 g; Fig. 3b). Belowground biomass was also 35% higher when all three species were present (0.63 ± 0.06 g; Fig. 3b), compared to when grown with *A. breviligulata* (0.41 ± 0.05 g; Fig. 3b). Treatment also affected belowground biomass ($F_{3, 64}$ = 11.06 P < 0.0001). There was an increase in belowground biomass when *S. patens* was buried compared to non-burial treatments (Fig. S2).

Elongation and Maximum Root Length

Ammophila breviligulata

There was no interaction between biotic mixture and treatment for elongation or MRL of *A. breviligulata* ($F_{9, 64}$ = 1.41 P = 0.2012; $F_{9, 64}$ = 1.37 P = 0.2193, respectively). Treatment had no effect on elongation of *A. breviligulata* ($F_{3, 64}$ = 1.19, P = 0.3191), but did affect MRL ($F_{3, 64}$ = 4.20, P < 0.01).

Biotic mixture also affected elongation of *A. breviligulata* ($F_{3, 64} = 15.85$, $P < 0.0001$). When grown with *U. paniculata*, elongation of *A. breviligulata* (21.9 ± 3.51 cm; Fig. 4a) by decreased by as much as 52% compared to all other biotic mixtures (monoculture: 45.3 ± 2.30 cm, AS: 44.8 ± 2.29 cm, USA: 33.5 ± 3.14 cm; Fig. 4a). Elongation decreased by more than 25% when *A. breviligulata* was competing with all three species (33.5 ± 3.14 cm; Fig. 4a) than when grown in a monoculture (45.3 ± 2.30 cm; Fig. 4a) or with *S. patens* (44.8 ± 2.29 cm; Fig. 4a).

Biotic mixture had a significant effect on MRL of *A. breviligulata* ($F_{3, 64} = 10.95$, $P < 0.0001$), competition with *U. paniculata* inhibited MRL of *A. breviligulata* (9.0 ± 1.32 cm; Fig. 4a) compared to any other biotic mixture by over 42% (monoculture: 16.6 ± 1.07 cm, AS: 16.4 ± 0.91 cm, USA: 15.65 ± 1.37; Fig. 4a). *Ammophila breviligulata* MRL was more elongated in burial treatments compared to sea-spray treatments (Fig. S3).

Uniola paniculata

No treatment × biotic mixture interaction was found for elongation ($F_{9, 64} = 2.03$, $P = 0.05$) or MRL ($F_{9, 64} = 1.05$, $P = 0.4139$). Maximum root length was not significantly affected by treatment or biotic mixture ($F_{3, 64} = 0.44$ $P = 0.6947$; $F_{3, 64} = 0.71$ $P = 0.5500$, respectively; Fig. 4b). However, as described below, treatment and biotic mixture were both main effects on elongation of *U. paniculata* ($F_{3, 64} = 6.53$, $P < 0.001$; $F_{3, 64} = 9.06$, $P < 0.0001$, respectively).

Uniola paniculata elongation was affected by biotic mixture ($F_{3, 64} = 9.06$, $P < 0.0001$). When *U. paniculata* was grown with *A. breviligulata* (70.8 ± 4.17 cm; Fig. 4b), elongation was more than 33% higher than when grown in any other biotic mixture besides monoculture (US: 43.6 ± 4.52 cm, USA: 47.3 ± 5.44 cm; Fig. 4b). *Uniola paniculata* elongation was lower in control groups compared to when burial treatment was present (burial and burial + sea-spray; $F_{3,}$

$_{64}$ = 6.53, $P < 0.001$; Fig. S4). Sea-spray treatment only caused lower elongation when compared to burial + sea-spray (Fig. S4).

Spartina patens

A significant treatment × biotic mixture interaction was found for *S. patens* elongation and MRL ($F_{9, 64}$ = 2.05, $P < 0.05$; $F_{9, 64}$ = 3.95, $P < 0.001$, respectively).

Elongation of *S. patens* increased by over 45% when burial was present (burial = 42.85 ± 1.93 cm; sea-spray + burial = 39.13 ± 3.16 cm; Fig. 5c and 5d) compared to when burial was absent (control = 24.65 ± 1.94 cm; sea-spray = 26.90 ± 2.07 cm; Fig. 5a and 5b).

A significant treatment × biotic mixture interaction in *S. patens* MRL indicates that while there was no difference of MRL in the control group across biotic mixture levels (Fig. 5a), a difference did exist when sea-spray was present. Competition with *U. paniculata* when sea-spray was present decreased MRL of *S. patens* (18.3 ± 1.36 cm, Fig. 5b) by 44% compared to when it was grown in monoculture (32.4 ± 3.14 cm; Fig. 5b). In burial treatments there was no difference in MRL across biotic mixture levels (Fig. 5c). However, when burial was added to sea-spray treatments (sea-spray + burial) *S. patens* MRL was 73% longer when grown with *U. paniculata* (35.0 ± 7.25 cm; Fig. 5d), than when grown with *A. breviligulata* (9.4 ± 2.50 cm; Fig. 5d).

Relative Interaction Intensity Index

Ammophila breviligulata

When grown with *U. paniculata*, *A. breviligulata* RII index (-0.19 ± 0.04; Fig. 6a) was significantly lower than monoculture (Fig 6a). This indicates a negative interaction with *U. paniculata* compared to growth in monoculture.

Relative interaction intensity index of *A. breviligulata* was greater when grown with *S. patens* (0.13 ± 0.03; Fig. 6a) compared to growth in monoculture (Fig. 6a), indicating a positive interaction between *S. patens* and *A. breviligulata*. When *A. breviligulata* was grown in USA mixture (-0.03 ± 0.03; Fig 6a) RII index was not different from monoculture.

Uniola paniculata

There was no difference in RII index of *U. paniculata* at any biotic mixture level (AU: 0.09 ± 0.05; US: -0.10 ± 0.06; USA: -0.09 ± 0.06) compared to *U. paniculata* monoculture (Fig. 6b). This shows that *U. paniculata* performance is not significantly hindered or facilitated by any biotic mixture compared to growth in monoculture.

Spartina patens

Performance of *S. patens* was higher when grown in USA mixture (0.14 ± 0.05; Fig. 6c) compared to grown in monoculture (Fig. 6c). This result indicates that *S. patens* performs better when all three species are present than it does in a monoculture.

Discussion

My results suggest that dune grass functional trait response to abiotic factors common to coastal ecosystems are altered by positive and negative biotic interactions. Interestingly, I found three different biotic relationships occurring as biotic mixture and abiotic stress varied; competition interactions, positive species interactions, and intransitive interactions between species.

When *A. breviligulata* was grown with *U. paniculata*, a negative species interaction (significantly negative RII index) ensued that caused functional trait degradation of *A. breviligulat*a and affected traditional plant response to common abiotic factors (e.g. salt, burial) of dune habitats (Fig. 7). Emergent competition between *A. breviligulata* and *U. paniculata*

consistently inhibited aboveground biomass, belowground biomass, elongation, and maximum

root length (MRL) of *A. breviligulata*. *Uniola paniculata* did not reduce belowground root

length to increase aboveground elongation, but was able to maintain high belowground biomass

when in competition with *A. breviligulata*. Amount of nutrients acquired by a plant is related to

spatial distribution of roots as well as belowground functional traits such as MRL, biomass, and

surface area of root mass (Lambers et al. 2008, Lou et al. 2014). By having a large belowground

biomass and MRL in burial treatments *U. paniculata* was likely able to acquire nutrients faster

and more efficiently, perpetuating fast elongation and thus, theoretically, increasing

photosynthetic efficiency (Lou et al. 2014). Not only could this strategy have increased growth

quickly after burial in *U. paniculata* but it may have also caused decreased growth of competing

species, in this case *A. breviligulata*, by impeding on rooting space and draining available

nutrients and water (Luo et al. 2014). It is possible that increased belowground biomass and

MRL increased water uptake by *U. paniculata*, which could explain decreases in aboveground

growth of *A. breviligulata*, as it utilizes C3 photosynthetic pathway and is less water and nutrient

efficient (Pau et al. 2012).

While not significant, trends suggest that *S. patens* performed well with *U. paniculata* as

evidenced by increased aboveground biomass. Increased MRL was also seen when *S. patens*

was grown with *U. paniculata* in high abiotic stress treatments, which was of interest because *S.

patens* is not known as a dune builder, but may contribute to maintaining a flat topography

(Stallins 2005, Wolner et al. 2013, Brantley et al. 2014). MRL of *S. patens* decreased when

treated with sea-spray (non-burial) and increased in sea-spray and burial treatment, this suggests

facilitation of *S. patens* by *U. paniculata* when abiotic stress is highest (Fig. 5d). This facilitative

relationship caused a functional trait shift in *S. patens* that could potentially enable small dune

engineering (Fig. 7). Responses such as these provide evidence that complex species interactions (competition, facilitation, intransitive interaction) are important factors in functional trait responses to abiotic factors, and that species function can change based on these interactions (Hacker and Bertness 1995, Zarnetske et al. 2012, Bertness et al. 2015, Esquivias 2015).

Simultaneous with facilitation of *S. patens* by *U. paniculata*, trends of decreased success in *U. paniculata* were seen when grown with *S. patens* as evidenced by lower elongation (Fig. 7). A positive interaction was also detected between *A. breviligulata* and *S. patens* explained by increased aboveground biomass and a significantly positive RII index of *A. breviligulata* when treated with abiotic stress (Fig 7.). This explains current coexistence of these two species in natural habitats (Dilustro and Day 1997, Day et al. 2001, Wolner et al. 2013). When *A. breviligulata* was grown with *S. patens* and abiotic stress was at its highest (salinity and burial), aboveground biomass production of *A. breviligulata* was increased relative to monoculture. Interactions between *A. breviligulata* and *S. patens* increased elongation, belowground biomass, and MRL of *A. breviligulata* when compared to *A. breviligulata* competing with *U. paniculata*. Positive species interactions have been theorized to be driven by harsh abiotic environments, and more recent discussions propose that positive species interactions are also driven by other factors, including plant traits (Bertness and Callaway 1994, Callaway et al. 2002, He et al. 2013). My data supports this inference by showing that facilitation caused by abiotic stress, drives functional trait shifts that enable success of certain species (Bertness and Callaway 1994, Olofsson et al. 1999, Zarnetske et al. 2012). Here, positive interactions only occurred in certain species mixtures, indicating species specific traits also play a role in facilitation (Gomez-Aparicio 2004, He et al. 2013). Using my data to synthesize positive and negative interactions, I

found it is possible to infer presence of intransitivity between these three ecosystem engineering dune grasses (Fig. 7).

My data shows that coexistence between these three species is possible via competitive intransitivity (Laird and Shamp 2008, Laird and Shamp 2015, Soliveres et al. 2015). In intransitive competition varying species-to-species interactions create a rock-paper-scissors scenario in which competitive hierarchy no longer exists (Laird and Shamp 2006, Reichenbach et al. 2007, Allesina and Levine 2011). Current models suggest that within plant communities intransitive interactions are most commonly found between dominant species, and is dependent on short disturbance intervals, along with other coexistence mechanisms such as abiotic stress (Laird et al. 2008, Soliveres et al. 2015). Both short disturbance intervals and high abiotic stress are present in coastal areas including the Virginia barrier island system where these species are dominant (Hayden et al. 1995, Stallins 2005, Brantley et al. 2014), making these ideal environments to investigate intransitivity.

I found that within species mixtures intransitivity resulted in an indirect facilitative effect on *S. patens* rather than creating a balance of all species. When *S. patens* was grown with *A. breviligulata* and *U. paniculata* together, increases in above- and belowground biomass were observed, as well as an RII index significantly greater than when grown in monoculture. This is likely due to different functional trait responses between species pairs that determine intransitive stabilization (Allesina and Levine 2011). Each of these species-to-species interactions not only cause functional trait changes on the plant level, but could also create a cross-scale cascade resulting in large scale changes in dune morphology, barrier island topography, and diversity as diagramed below (Fig. 8). Alteration of functional trait responses to abiotic factors, driven by plant-to-plant interactions could bring about more success for certain species (Hacker and

Bertness 1995). If interactions are causing improvements in physiological and morphological responses to burial they can provide another link in substantiating connections between plant physiological response and landscape scale patterns of dune development (Stallins 2006). It is important to continue to consider plant-to-plant interactions as a driver of functional trait shifts because resulting biotopographic interactions will influence barrier island stability and diversity (Stallins 2005, Miller et al. 2015).

On a larger scale these results suggest that as variable abiotic and biotic conditions emerge, different species will prevail, initiating changes in active synergisms between plant presence, sediment movement, and functional trait response (Zinnert et al. 2016). My data strongly suggests that functional trait modifications caused by any of the complex species interactions discussed above can lead to changes in historical island formation causality (Stallins 2006). Island vulnerability and recovery rate relies on resulting interactions between dune grass ecosystem engineers and physical processes (Stallins 2005, Wolner et al. 2013, Duran and Moore 2013, Brantley et al. 2014, Emery and Rudgers 2014). If negative competition emerges as *U. paniculata* experiences northward expansion (Hodel and Gonzalez 2013), *A. breviligulata* could experience damaging alterations to functional traits that specifically make it a good dune engineer (e.g., high aboveground biomass, high MRL, and high elongation). This could ultimately lead to a shift in dune formation processes (Fig. 8). Increased *U. paniculata* presence may slow dune building processes due to phalanx driven hummock dune formation which allows for increased overwash during high water events (Mendelssohn et al. 1991, Stallins 2006, Duran and Moore 2013, Brantley et al. 2014). However, we also found trends of facilitation of *A. breviligulata* by *S. patens* which could offset some negative effects of *U. paniculata* expansion (Fig. 7).

Intransitivity may seem to be a beneficial coexistence mechanism that increases community diversity and species richness (Soliveres 2015). However, an intransitive outcome between these ecosystem engineers would likely result in a similar cross-scale consequence as if *U. paniculata* were to outcompete *A. breviligulata*. While *A. breviligulata* may not be fully outcompeted in intransitive scenarios, functional trait modifications could decrease the primary dune engineering abilities it currently has, coexisting with *S. patens*. In this scenario a result could be development of less effective dunes that allow overwash, sea-spray, and prolonged salt-water inundation to infiltrate swale plant communities, causing a state change and collapsing any establishment of inner island biodiversity (Godfrey 1977, Stallins 2005, Duran and Moore 2015). While intransitivity benefits a system by increasing diversity at a small scale (Soliveres 2015), resulting functional trait shifts could alter ecosystem engineering capabilities of certain species and be detrimental to diversity and species richness at a larger scale (Fig. 8). Therefore intransitivity, while not completely detrimental, could still result in a loss of a dune ridge building species and may have island level biodiversity ramifications in an ecosystem that already has low biodiversity (Stalter and Odum 1993, Hayden et al. 1995).

These new processes brought on by novel biotic interactions thereby cause increased likelihood of salt-water inundation to occur in swale communities normally protected by a dune ridge (Godfrey 1977, Stallins 2006, Duran and Moore 2013). Dune height and Island bistability thresholds are closely linked to interactions between plants and physical processes (Duran and Moore 2013, Duran and Moore 2015) and regime shifts seem to be increasingly more likely as functional trait responses to abiotic stress are altered by a suite of complex biotic interactions. This generates possible regime shifts from stable islands with relatively high biodiversity to unstable islands with more frequent disturbance and low diversity (Stallins 2005, Duran and

Moore 2015). Due to patterns between disturbance intervals and dune grass colonization, once a

dune is breached, portions of a barrier island remains at a low elevation, low diversity state

(Brantley et al. 2014, Wolner et al. 2013, Duran and Moore 2015). Therefore, understanding

processes that cause barrier islands to be more vulnerable is critical. This concept, along with

my results, validates that restoration efforts should consider planting species combinations that

positively influence dune building functional trait responses to common abiotic stressors.

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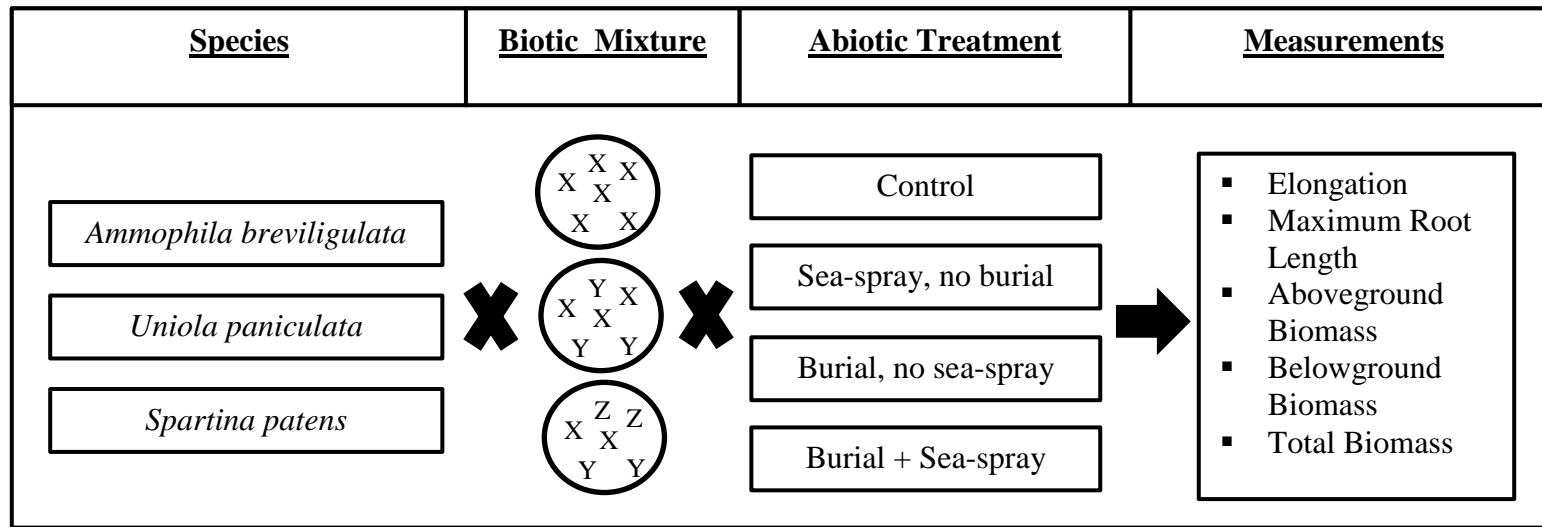
Figure 1. Multi-factorial experiemnental design layout. Three different species (*Ammophila breviligulata* Fern., *Uniola paniculata* L., and *Spartina patens* Muhl.) are placed in three differentcompetition combinations (monouclture (X), 2 species competition (X and Y), 3 species competition (X,Y, and Z)), and given four different treatments (control (no treatment), Sea-spray with no burial, Burial with no sea-spray, and Burial with sea-spray). The final box lists the suite of measurements taken on each species × competition × treatment group.

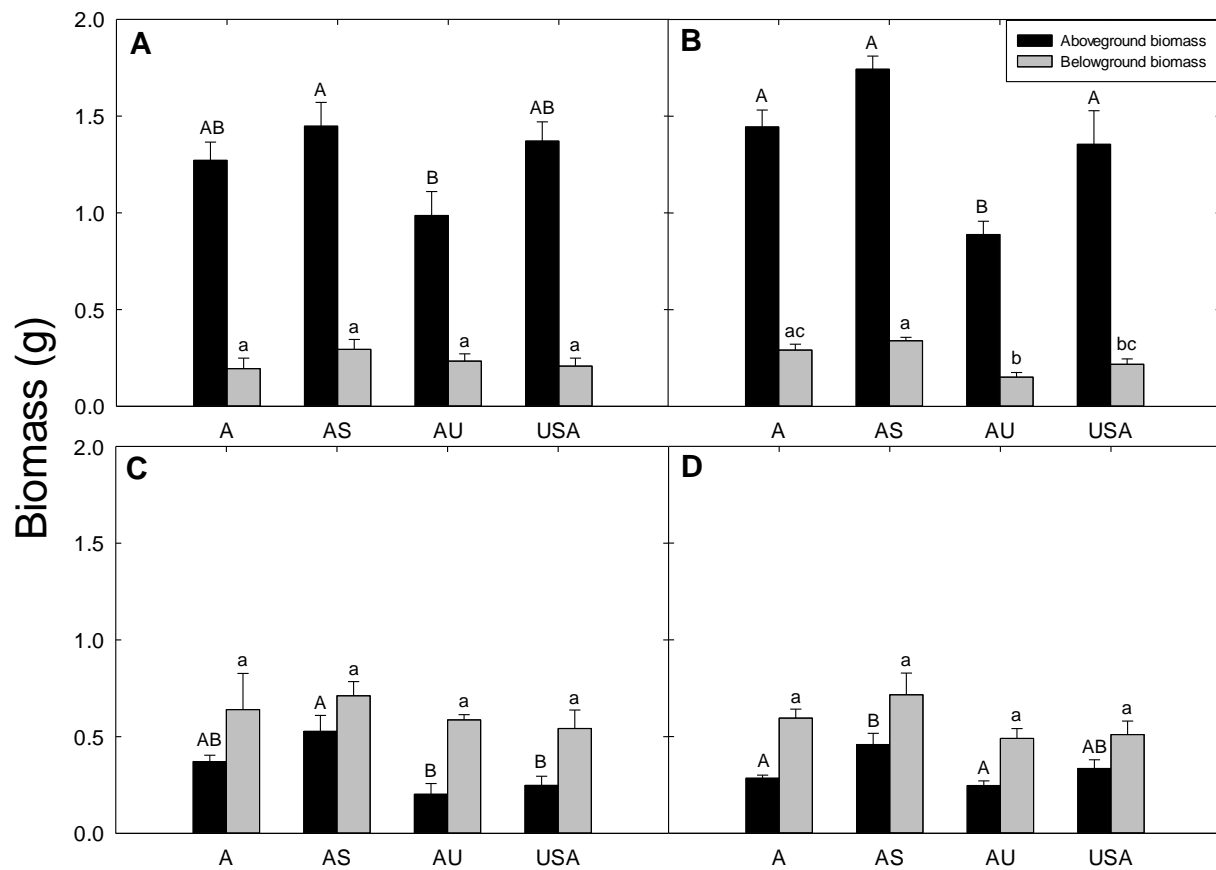**Figure 2.** **A)** Above- and belowground biomass measurements (g) of *A. breviligulata* Fern. under control conditions across all competition levels ± 1 SE. **B)** Above- and belowground biomass measurements (g) of *A. breviligulata* Fern. under sea-spray (no burial) treatments across all competition levels ± 1 SE. **C)** Above- and belowground biomass measurements (g) of *A. breviligulata* Fern. under burial (no sea-spray) conditions across all competition levels ± 1 SE. **D)** Above- and belowground biomass measurements (g) of *A. breviligulata* Fern. under burial + sea-spray conditions across all competition levels ± 1 SE. Where A = *A. breviligulata*, AS = *A. breviligulata + S. patens*, AU = *A. breviligulata + U. paniculata*, and USA = *U. paniculata + A. breviligulata + S. patens*. Bars not connect be letters are significantly different (*P* < 0.05).
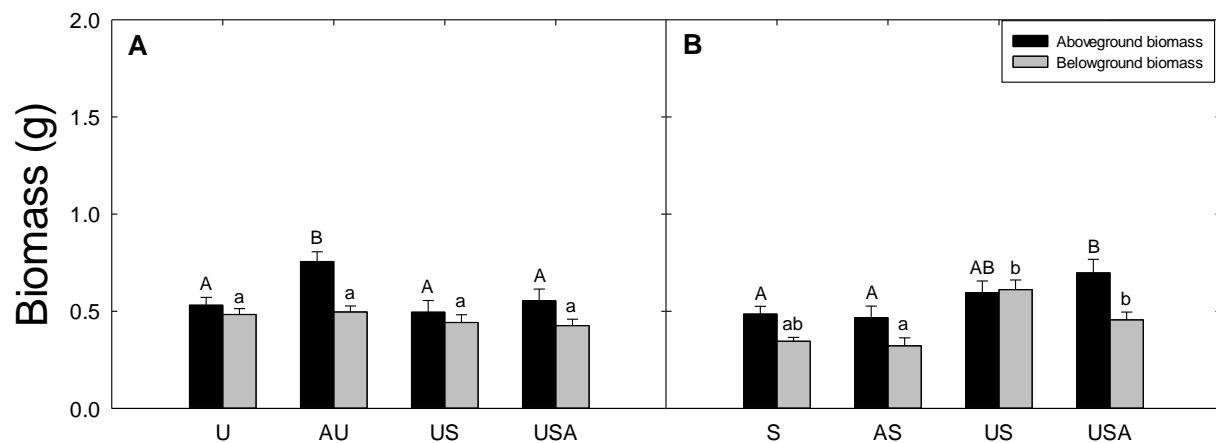
Figure 3. **A)** Above- and belowground biomass measurements (g) of *U. paniculata* L. across competition levels, not segregated by treatment ± 1 SE. **B)** Above- and belowground biomass measurements (g) of *S. patens* Muhl. across all competition levels, not segregated by treatment ± 1 SE. Where S = *S. patens*, U = *U. paniculata*, US = *U. paniculata + S. patens*, AS = *A. breviligulata + S. patens*, AU = *A. breviligulata + U. paniculata*, and USA = *U. paniculata + A. breviligulata + S. patens*. Bars not connect be letters are significantly different (*P* < 0.05).

Figure 4. A) Elongation and MRL (cm) of *A. breviligulata* Fern. across all competition levels, not segregated by treatment ± 1 SE. **B)** Elongation and MRL (cm) of *U. paniculata* L. across all competition levels, not segregated by treatment ± 1 SE. Where A = *A. breviligulata*, U = *U. paniculata*, US = *U. paniculata* + *S. patens*, AS = *A. breviligulata* + *S. patens*, AU = *A. breviligulata* + *U. paniculata*, and USA = *U. paniculata* + *A. breviligulata* + *S. patens*. Bars not connect be letters are significantly different (*P* < 0.05).
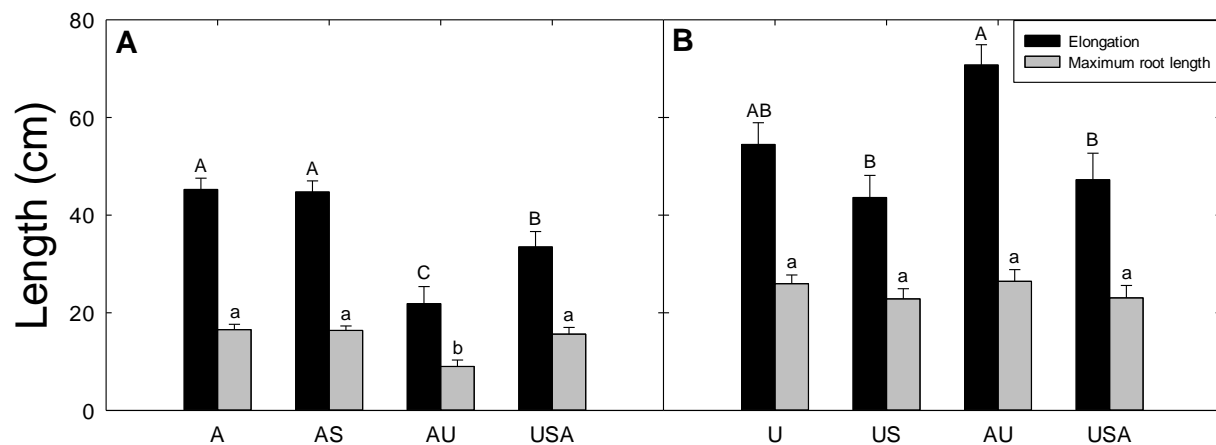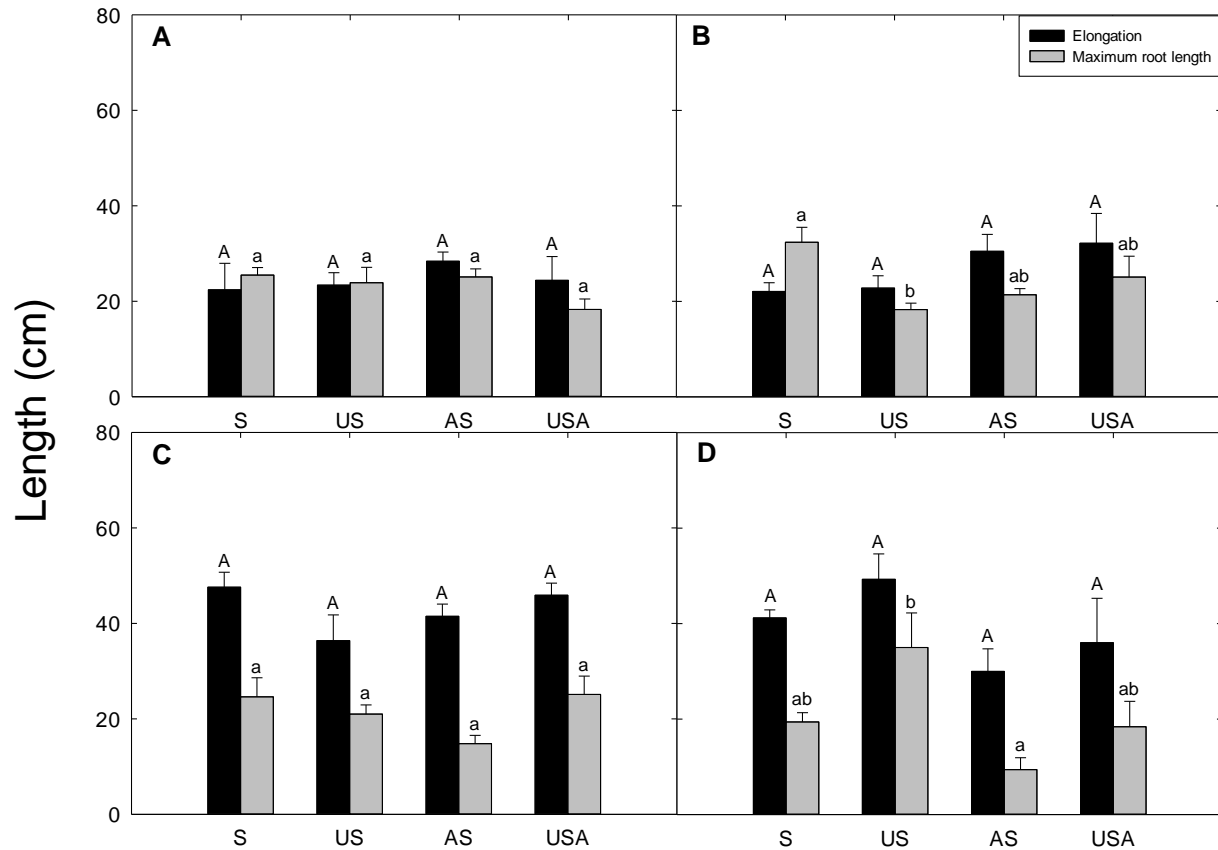
Figure 5. A) Elongation and MRL measurements (cm) of *S. patens* Muhl. under control conditions across all competition levels ± 1 SE. **B)** Elongation and MRL measurements (cm) of *S. patens* Muhl. under sea-spray (no burial) treatments across all competition levels ± 1 SE. **C)** Elongation and MRL measurements (cm) of *S. patens* Muhl. under burial (no sea-spray) conditions across all competition levels ± 1 SE. **D)** Elongation and MRL measurements (cm) of *S. patens* Muhl. under burial + sea-spray conditions across all competition levels ± 1 SE. Where S = *S. patens*, US = *U. paniculata + S. patens*, AS = *A. breviligulata + S. patens*, and USA = *U. paniculata + A. breviligulata + S. patens*. Bars not connect be letters are significantly different (*P* < 0.05).
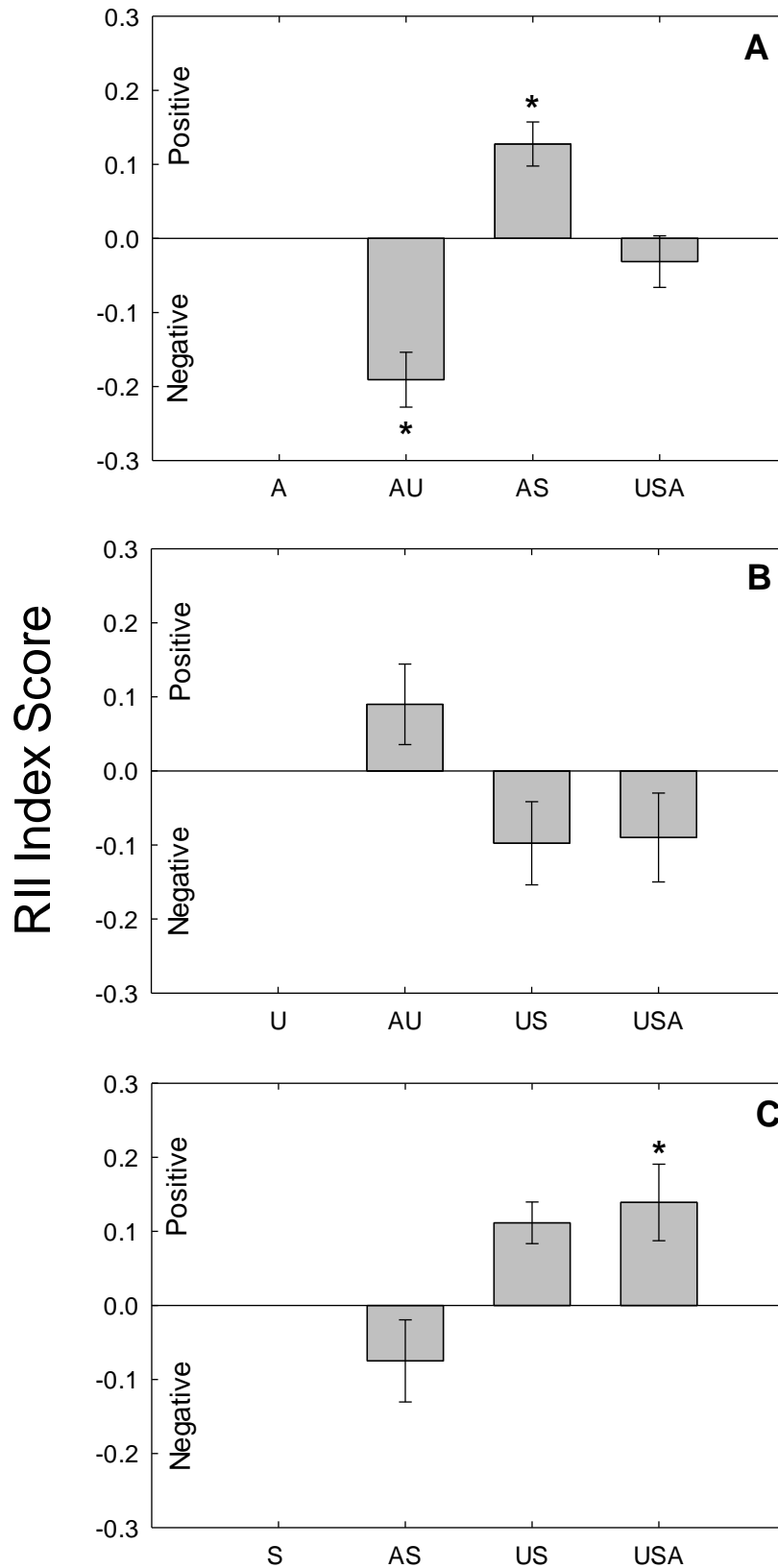
**Figure 6.** **A**) Average RII index scores for *A. breviligulata* Fern. in each biotic mixture ± 1 SE. **B**) Average RII index scores for *U. paniculata* L. in each biotic mixture ± 1 SE. **C**) Average RII index scores for *S. patens* Muhl. in each biotic mixture ± 1 SE. Monoculture growth measurements used to create baseline (0.0); indicates no interaction. Interactions are considered significant if RII index is significantly different than that of monoculture (0.0). Where A = *A. breviligulata*, S = *S. patens*, U = *U. paniculata*, US = *U. paniculata + S. patens*, AS = *A. breviligulata + S. patens*, AU = *A. breviligulata + U. paniculata*, and USA = *U. paniculata + A. breviligulata + S. patens*. *$P < 0.05$
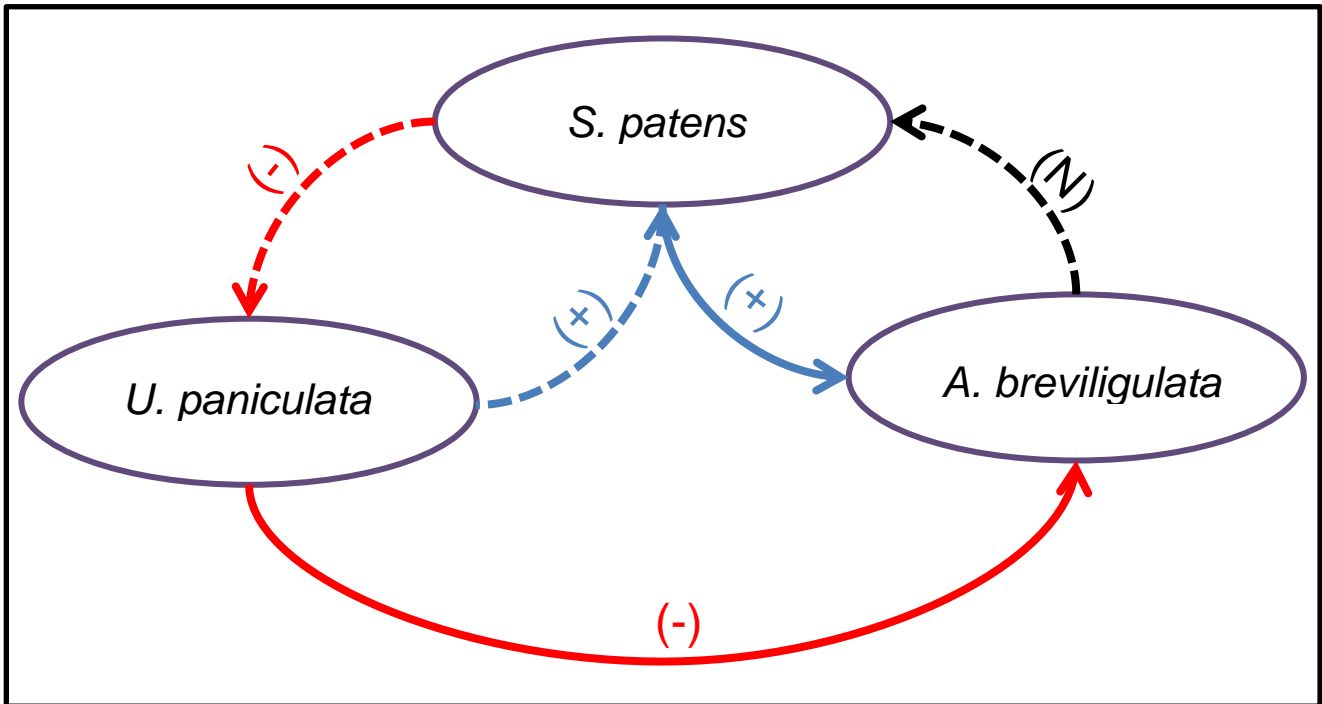
Figure 7. Intransitive biotic network describing complex interplay between species supporting coexistence of these species. Solid lines indicate interactions that are supported by significant data. Dashed lines indicate interactions exhibited by trends in data. Blue lines (+) indicate possible positive interactions, Red lines (-) indicate the presence of possible competition, and black lines indicate no direct effect of species *a* on species *b*. Species names are in purple circles symbolizing intransitivity.
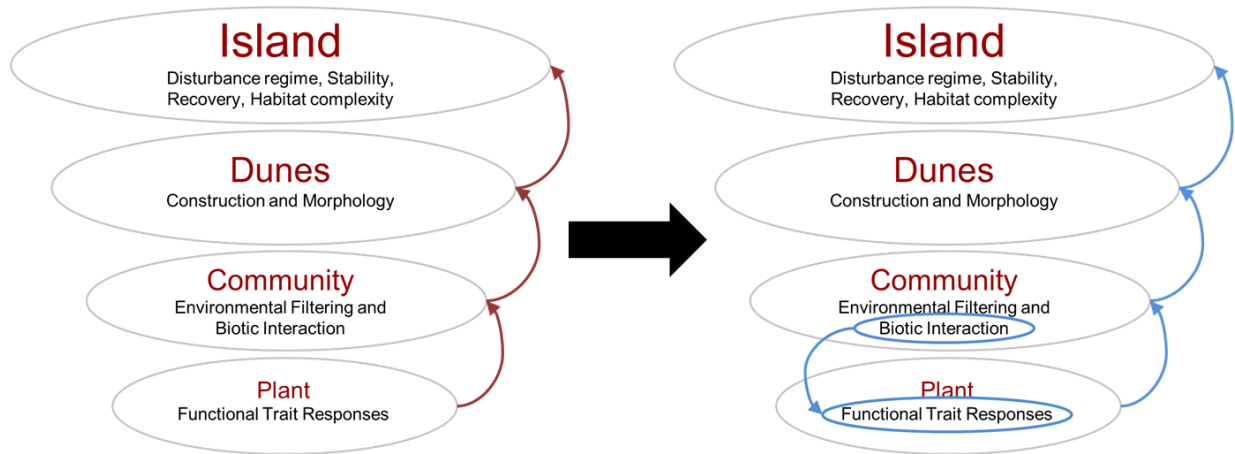
Figure 8. Influence of biotic interactions on cross-scale effects. Novel biotic interactions can cause shifts in functional trait responses at the plant level leading to subsequent changes at larger spatial scales.

Table 1. Tukey HSD summary of results for *A. breviligulata* Fern. aboveground biomass, where A = *A. breviligulata*, AS = *A. breviligulata + S. patens*, AU = *A. breviligulata + U. paniculata*, and USA = *U. paniculata + A. breviligulata + S. patens.*

Control	
Comparison	**P value**
A vs AS	0.6807
A vs AU	0.3008
A vs USA	0.9200
AS vs AU	**0.0427***
AS vs USA	0.9600
AU vs USA	0.1070

Sea-spray	
Comparison	**P value**
A vs AS	0.2491
A vs AU	**0.0111***
A vs USA	0.9357
AS vs AU	**0.0002***
AS vs USA	0.0931
AU vs USA	**0.0356***

Burial	
Comparison	**P value**
A vs AS	0.2550
A vs AU	0.1973
A vs USA	0.4442
AS vs AU	**0.0049***
AS vs USA	**0.0154***
AU vs USA	0.9413

Burial + Sea-spray	
Comparison	**P value**
A vs AS	**0.0301***
A vs AU	0.9044
A vs USA	0.8021
AS vs AU	**0.0077***
AS vs USA	0.1611
AU vs USA	0.4156

Table 2. Tukey HSD summary of results for *U. paniculata* L. aboveground biomass, U = *U. paniculata*, US = *U. paniculata + S. patens*, AU = *A. breviligulata + U. paniculata*, and USA = *U. paniculata + A. breviligulata + S. patens.*

Comparison	P value
U vs US	0.9423
U vs AU	**0.0048***
U vs USA	0.9849
US vs AU	**0.0008***
US vs USA	0.7962
AU vs USA	**0.0134***

Table 3. Tukey HSD summary of results for *S. patens* Muhl. biomass, where S = *S. patens*, US = *U. paniculata + S. patens*, AS = *A. breviligulata + S. patens*, and USA = *U. paniculata + A. breviligulata + S. patens.*

Aboveground	
Comparison	**P value**
S vs US	0.2378
S vs AS	0.9881
S vs USA	**0.0030***
US vs AS	0.1274
US vs USA	0.3117
AS vs USA	**0.0011***

Belowground	
Comparison	**P value**
S vs US	0.1059
S vs AS	0.7140
S vs USA	0.0605
US vs AS	**0.0068***
US vs USA	0.9947
AS vs USA	**0.0032***

Table 4. Tukey HSD summary of results for *A. breviligulata* Fern. elongation and maximum root length, where A = *A. breviligulata*, AS = *A. breviligulata + S. patens*, AU = *A. breviligulata + U. paniculata*, and USA = *U. paniculata + A. breviligulata + S. patens*.

Elongation	
Comparison	**P value**
A vs AS	0.9991
A vs AU	**< 0.0001***
A vs USA	**0.0200***
AS vs AU	**< 0.0001***
AS vs USA	**0.0285***
AU vs USA	**0.0221***
MRL	
Comparison	**P value**
A vs AS	0.9995
A vs AU	**< 0.0001***
A vs USA	0.9320
AS vs AU	**< 0.0001***
AS vs USA	0.9620
AU vs USA	**0.0004***

Table 5. Tukey HSD summary of results for *U. paniculata* L. elongation, where U = *U. paniculata*, US = *U. paniculata + S. patens*, AU = *A. breviligulata + U. paniculata*, and USA = *U. paniculata + A. breviligulata + S. patens.*
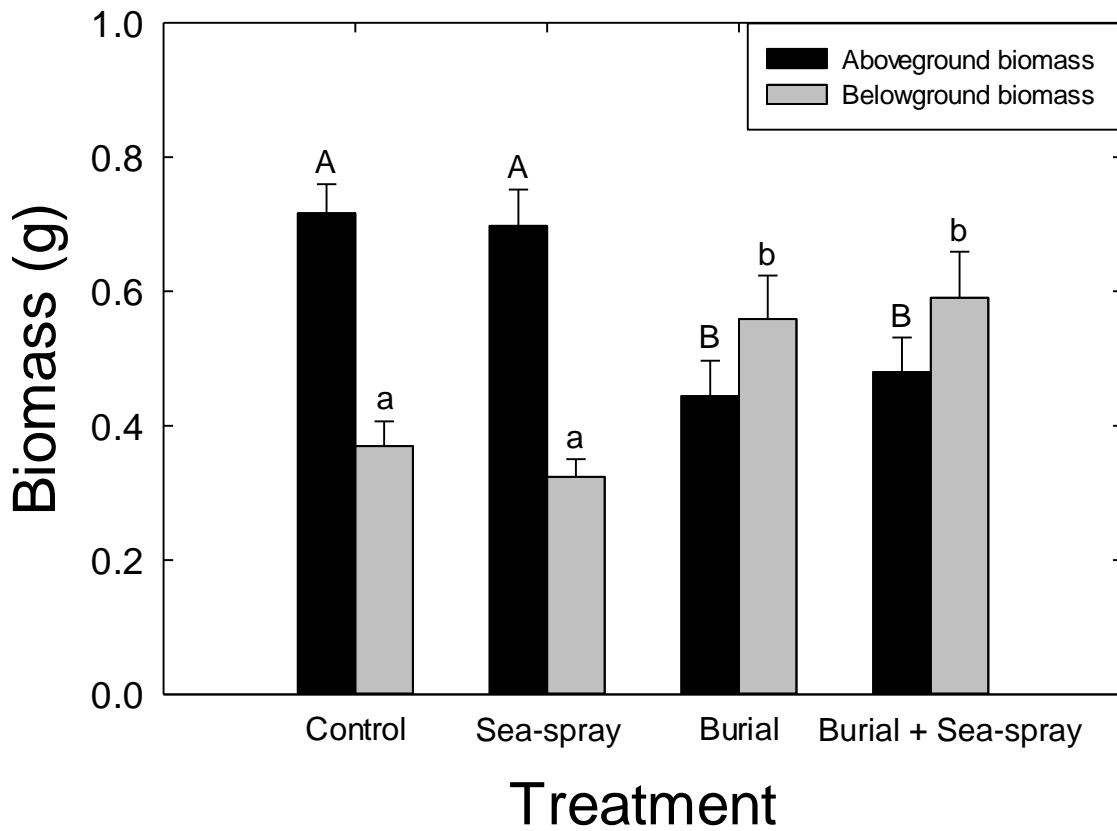
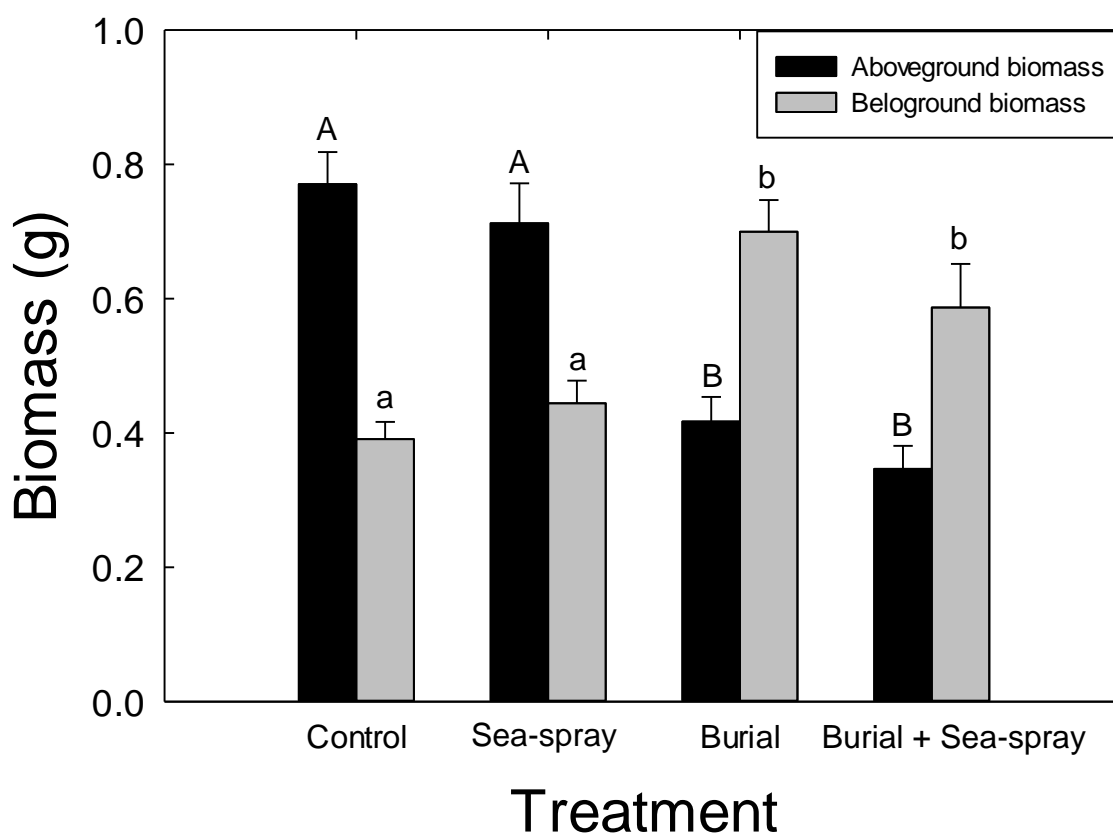| Elongation	
Comparison	**P value**
U vs US	0.0825
U vs AU	0.1032
U vs USA	0.2874
US vs AU	**< 0.0001***
US vs USA	0.9204
AU vs USA	**0.0006***

Treatment effects on above- and belowground biomass of *U. paniculata.*

Supplementary Figure 1. *U. paniculata* above- and belowground biomass (g) across all treatment groups ± 1 SE. Grey bars indicate belowground dry weight while black bars indicate aboveground dry weight.
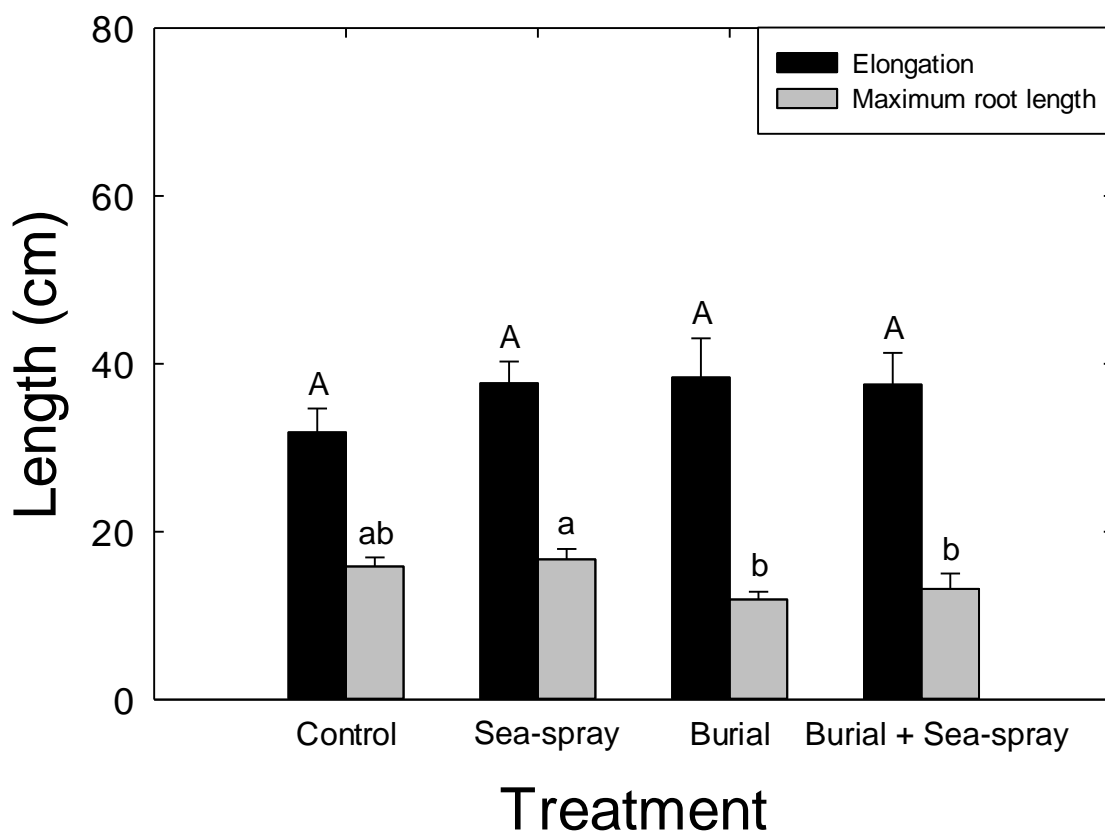
Treatment effects on above- and belowground biomass of *S. patens*.

Supplementary Figure 2. *S. patens* above- and belowground biomass (g) across all treatment groups ± 1 SE. Grey bars indicate belowground dry weight while black bars indicate aboveground dry weight.

Treatment effects on elongation and maximum root length of *A. breviligulata.*

Supplementary Figure 3. *Ammophila breviligulata* elongation and MRL (cm) across all treatment groups ± 1 SE. Grey bars indicate MRL while black bars indicate plant elongation.
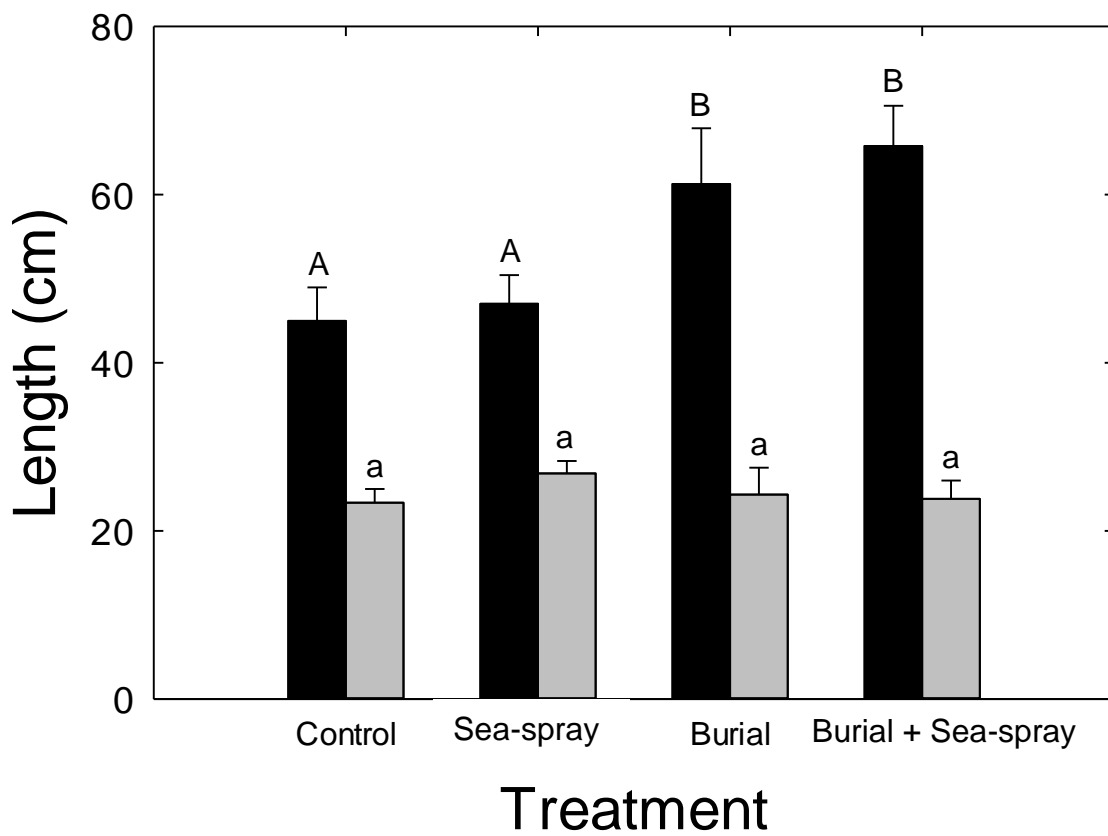
Treatment effects on elongation and maximum root length of *U. paniculata.*

Supplementary Figure 4. *Uniola paniculata* elongation and MRL (cm) across all treatment groups ± 1 SE. Grey bars indicate MRL while black bars indicate plant elongation.

Results of Species × treatment × competition three factor ANOVA for elongation.

Supplementary Table 1. Results of three-way ANOVA test of elongation between species, treatment, and competition type.

Effect	F value	P value
Species	33.16	**< 0.0001***
Treatment	10.91	**< 0.0001***
Competition	1.97	0.0708
Species x Treatment	2.87	0.0588
Species x Competition	2.17	0.0742
Treatment x Competition	3.03	**0.0073***
Species x Treatment x Competition	0.90	0.5442

Results of species × treatment × competition three factor ANOVA for MRL.

Supplementary Table 2. Results of three-way ANOVA test of maximum root length between species, treatment, and competition type.

Effect	F value	P value
Species	26.51	**< 0.0001***
Treatment	2.59	0.0538
Competition	0.61	0.7255
Species x Treatment	2.92	0.0564
Species x Competition	0.63	0.6441
Treatment x Competition	3.03	0.0658
Species x Treatment x Competition	0.81	0.6402

Results of species × treatment × competition three factor ANOVA for aboveground biomass

Supplementary Table 3. Results of three-way ANOVA test of aboveground biomass between species, treatment, and competition type.

Effect	F value	P value
Species	29.55	**< 0.0001***
Treatment	96.15	**< 0.0001***
Competition	19.56	**< 0.0001***
Species x Treatment	1.49	0.2271
Species x Competition	2.42	**0.0498***
Treatment x Competition	0.49	0.8128
Species x Treatment x Competition	0.88	0.5635

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Results of species × treatment × competition three factor ANOVA for belowground biomass

Supplementary Table 4. Results of three-way ANOVA test of belowground biomass between species, treatment, and competition type.

Effect	F value	P value
Species	7.15	**0.0010***
Treatment	32.94	**< 0.0001***
Competition	2.19	**0.0451***
Species x Treatment	0.07	0.9321
Species x Competition	2.32	0.0586
Treatment x Competition	0.94	0.4688
Species x Treatment x Competition	1.50	0.1265

<u>Vita</u>

Joseph Karl Brown was born to Stephen and Lisa Brown on November 4, 1990, in Alexandria, VA. He grew up in Fredericksburg, VA and graduated from Massaponax High School, Fredericksburg, VA in 2009. He received his Bachelor of Science in Biology from Virginia Commonwealth University, Richmond, VA in 2013. He then briefly worked for The Great Basin Institute in Wells, NV, mapping and treating invasive, noxious weeds of the west. He moved on to teach at Post Oak Middle School in Spotsylvania County, VA, as a remedial math instructor, while also coaching the boy's spring track team. He will be receiving a Master of Science in Biology with a focus on Plant Ecology from Virginia Commonwealth University in 2016, and will be continuing his education in a PhD program in Integrative Life Sciences at Virginia Commonwealth University.