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Hierarchical organization of a Sardinian sand dune plant community

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Coastal sand dunes have attracted the attention of plant ecologists for over a century, but they have largely relied on correlations to explain dune plant community organization. We experimentally examined longstanding hypotheses that sand binding, interspecific interactions, abiotic factors and seedling recruitment are drivers of sand dune plant community structure in Sardinia, Italy. Removing foundation species from the fore, middle and back dune habitats over 3 years led to erosion and habitat loss on the fore dune and limited plant recovery that increased with dune elevation. Reciprocal species removals in all zones suggested that interspecific competition is common, but that dominance is transient, particularly due to sand burial disturbance in the middle dune. A fully factorial 2-year physical factor manipulation of water, nutrient availability and substrate stability revealed no significant proximate response to these abiotic factors in any dune zone. In the fore and middle dune, plant seeds are trapped under adult plants during seed germination, and seedling survivorship and growth generally increase with dune height in spite of increased herbivory in the back dune. Sand and seed erosion lead to limited seed recruitment on the fore dune while high summer temperatures and allelopathy lead to competitive dominance of woody plants in the back dune. Our results suggest that Sardinian sand dune plant communities are hierarchically organized, structured by sand binding foundation species on the fore dune, sand burial in the middle dune and increasingly successful seedling recruitment, growth and competitive dominance in the back dune.

Hierarchical organization of a Sardinian sand dune plant community

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Abstract (246 words). Coastal sand dunes have attracted the attention of plant ecologists for over a century, but they have largely relied on correlations to explain dune plant community organization. We experimentally examined longstanding hypotheses that sand binding, interspecific interactions, abiotic factors and seedling recruitment are drivers of sand dune plant community structure in Sardinia, Italy. Removing foundation species from the fore, middle and back dune habitats over 3 years led to erosion and habitat loss on the fore dune and limited plant recovery that increased with dune elevation. Reciprocal species removals in all zones suggested that interspecific competition is common, but that dominance is transient, particularly due to sand burial disturbance in the middle dune. A fully factorial 2-year physical factor manipulation of water, nutrient availability and substrate stability revealed no significant proximate response to these abiotic factors in any dune zone. In the fore and middle dune, plant seeds are trapped under adult plants during seed germination, and seedling survivorship and growth generally increase with dune height in spite of increased herbivory in the back dune. Sand and seed erosion lead to limited seed recruitment on the fore dune while high summer temperatures and allelopathy lead to competitive dominance of woody plants in the back dune. Our results suggest that Sardinian sand dune plant communities are hierarchically organized, structured by sand binding foundation species on the fore dune, sand burial in the middle dune and increasingly successful seedling recruitment, growth and competitive dominance in the back dune.

42

INTRODUCTION

43 Understanding the biotic and abiotic mechanisms that generate spatial patterns in natural
44 communities is a major goal of ecology and is critical for developing ecology into a
45 predictive science that can inform ecosystem management and contribute to conservation
46 (Morin 2011). Many natural communities are structured and defined by foundation species,
47 *sensu* Dayton (1975). Foundation species are operationally defined as common, abundant
48 species that build and maintain habitats, ameliorating potentially limiting physical and
49 biological stressors, thus providing habitat for other species (Jones et al. 1994, Bruno and
50 Bertness 2001, Ellison et al. 2005, Angelini et al. 2011). Examples of foundation species-
51 dependent ecosystems include forests, coral reefs, salt marshes, mangroves, mussel and
52 oyster reefs, which are all built and maintained by numerically dominant habitat forming
53 foundation species (Bruno and Bertness 2001). Amelioration of potentially limiting physical
54 and/or biotic conditions is a hallmark of foundation species-based ecosystems. Coastal sand
55 dunes are physically harsh habitats for the halophytic plants that dominate them and are best
56 understood as foundation species-based ecosystems (Olff et al. 1993).

57 Recently, it has been suggested that foundation species-based ecosystems are commonly
58 hierarchical, where the amelioration of potentially limiting stresses is responsible for
59 ecosystem establishment and maintenance, but that other species interactions are often
60 responsible for generating the most conspicuous, but superficial spatial patterns in these
61 communities (Bruno and Bertness 2001, Bruno et al. 2003, Altieri et al. 2007, Angelini et al.
62 2011). While this model of community organization appears to be widespread (Bruno and
63 Bertness 2001), most evidence for hierarchical organization is anecdotal or correlative with
64 few explicit experimental tests (for exceptions see Altieri et al. 2007, Angelini and Silliman
65 2014). This is the case in spite of the potential importance of hierarchical community
66 organization to conservation and management strategies. Plant communities that occur at the

land/sea interface, like sand dune, salt marsh, sea grass and mangrove communities, provide important ecological services including stabilizing shorelines from erosion and storm damage, harboring animal diversity, providing nursery habitats to threatened avifauna, marine turtles, and shellfish, as well as processing nutrient-rich terrestrial runoff (Barbier et al. 2013). Provisioning of these services relies on informed management facing increasing threats. Sand dunes, however, are generally not conservation priorities and not managed to protect their ecosystem services (Ehrenfeld 1990, Everard et al. 2010).

Most research on sand dune communities has been descriptive and reliant on dated correlative literature (e.g. Cowles 1899, Oosting and Billings 1942, Mack and Harper 1977). Experimental work has been restricted to small scale sand burial, seed dispersal and disturbance studies (Maun and Perumal 1999, Franks and Peterson 2003, Miller et al. 2010), field studies of annuals, nurse plant effects and comparisons among chronosequences as a proxy for successional shifts in plant species interactions (Lichter 2000, Franks 2003, Cushman et al. 2010), experimental grazer studies (Huntzinger et al. 2008) and greenhouse studies of the importance of plant/mycorrhizal associations (Gemma et al. 1989). Experimental studies of the roles of the dominant foundation species that have long been hypothesized to build and maintain sand dune plant communities by binding sand and ameliorating potentially limiting physical conditions such as water and nutrient limitation are notably absent. Consequently, the critical interactions between biological and physical processes that have been assumed to generate the organization of sand dune communities have not been rigorously tested.

Descriptive and correlative studies of sand dune communities suggest that substrate stabilization, water and nutrient limitation, and plant facilitation and competition are the main drivers of sand dune community structure and organization (Barbour et al. 1985, Ehrenfeld 1990, Lichter 1998, Isermann 2011). Typically, distinct plant zonation occurs in coastal sand

dune systems (Hesp 1991, Lortie and Cushman 2007, Acosta 2009). On the seaward border of sand dunes, the fore dune, a limited number of clonal pioneer plant species with deep roots trap and bind sand, initiating dune formation. These plants stabilize substrate, trap seeds, and grow vertically and horizontally as sand accumulates, building the seaward border of sand dunes (Cowles 1899, Oosting and Billings 1942). At higher elevations in the middle dune, plant species diversity increases, but unvegetated free space remains common. At these elevations, substrate stabilization remains important, but interspecific plant interactions, including facilitation (Franks 2003, Castanho et al. 2015) and competition (Lichter 2000), and water and nutrient limitation also appear to mediate plant success. In the back dune, furthest from ocean winds and salt spray, the dominant physical stresses of substrate instability and low soil nutrients and moisture are less severe, plant cover typically reaches 100%, and numerically dominant woody shrubs or trees, appear to competitively displace plants that dominate lower dune elevations (Lichter 2000). This descriptive structure of dune communities is consistent with a hierarchical community organization model and the stress gradient hypothesis of community assembly.

The hierarchical model of community organization (Bruno and Bertness 2001, Ellison et al. 2005, Altieri et al. 2007) hypothesizes that within communities built by biogenic foundation species, dependent species are only able to persist through positive interactions and feedbacks initiated by primary foundation species. The stress gradient hypothesis (Bertness and Callaway 1994, Maestre et al. 2009, He and Bertness 2014) proposes that the biological processes controlling community development shift from positive, facilitative interactions in physically and biologically harsh environments to negative, competitive interactions in benign environments. Many shoreline communities built and maintained by foundation species, such as salt marshes, coral reefs, mangrove forests, mussel reefs, and seagrass beds,

have been hypothesized to be hierarchically organized by the general principles of the stress gradient hypothesis (Bruno and Bertness 2001).

In this paper we examine long-standing assumptions of sand dune plant community ecology. We hypothesize that sand dunes are hierarchically organized, initially built by facilitation, but ultimately structured by spatially and temporally predictable shifts from facilitative interactions to competitive interactions and seedling recruitment across decreasing stress gradients. Many of these patterns were initially theorized in classic descriptive work (Cowles 1899), but have never been tested experimentally. We take a field experimental approach to test these underlying assumptions and elucidate the sand dune community assembly by examining the hypotheses that: 1) foundation species are responsible for stabilizing the seaward border of the dune from erosion and habitat loss, 2) at intermediate dune elevations, interspecific plant interactions, plant resource availability, and seedling recruitment dictate plant abundance and distribution, and 3) at high dune elevations, reduced physical stresses lead to increased plant abundance, interspecific competitive dominance and displacement. Our results support a hierarchical model of sand dune community organization and confirm many, but not all of the assumptions of early work on the organization of these communities.

MATERIALS AND METHODS

Our study was carried out on the Badesi dunes (40° 56' 45.571'' N, 8° 49' 41.048'' E) on the North coast of Sardinia, in the Mediterranean Sea. It is a wide dune system at the mouth of Coghinas River and has a plant community similar to other dune systems in Sardinia (Cusseddu personal observations), it is within a Site of Community Importance (SIC) and the Town of Badesi gave us the field permit to undertake the field experiments (approval number: 3343 - 23/03/2012). The Coghinas dunes are almost three kilometers long overlooking the Asinara Gulf, with a width of approximately five hundred meters. Sardinia

has a warm temperate Mediterranean climate with hot, dry summers and cooler, wet winters. As in other Mediterranean habitats, most plant growth and reproduction occurs during the winter months (Blondel and Aronson 1999). Previous studies of Sardinian sand dune plant communities reveal that they have high species richness, endemism (Bacchetta et al. 2008, Prisco et al. 2012) and adaption to physical stress (Fenu et al. 2013).

Zonation survey

To quantify the plant distribution across the Badesi dune, we surveyed transects in each distinct plant zone. We defined three major dune plant zones: the fore dune, located on the seaward border of the dune and characterized by a steep lower border of stabilized sand and low plant diversity; the middle dune, characterized by high but patchy plant species diversity and 40-60% bare sand cover; and the back dune, characterized by total woody plant cover (Fig. 2). In addition to these zones we will also refer to the front of the fore dune, or the seaward border of the fore dune where embryo dunes (Cowles 1899) form and transition areas between major vegetation zones. Our objective was to develop a site description of the plant community.

To assess the plant zonation, we quantified vegetation along 120 m transects parallel to the water in each major dune zone: the embryonic fore dune, the top fore dune on the first ridge of the dune formation, the middle dune with high plant species richness and bare substrate, and the back dune dominated by woody plants. In each zone we ran a transect line, starting from a random point, parallel to the shoreline and every 2 m placed a 0.5x0.5 m sampling quadrat subdivided into a 25 5x5 cm sampling grid to estimate plant species and unvegetated substrate cover. Sixty quadrats were sampled/zone. We characterized species diversity for each zone with the Shannon diversity index (H). All plants, after introduced, will be referred to by genus names.

In addition to vertical zonation, a conspicuous feature of plants on the fore and middle dunes were that they generally occurred on elevated sand mounds. Since sand in these zones is mobile and windblown, we hypothesized that these mounds were created by the presence of plants rather than plant establishment on transient dune features (Cowles 1899). We used an auto level and stadia rod to quantify the elevation of plants in each zone ($n=20/\text{zone}$) and bare sand adjacent to (25-cm away) each plant. In each zone we randomly selected 20 adult individuals of the most common plant species, measured their elevation and the elevation of unvegetated sand 10-20 cm away. Plant and adjacent sand elevation differences were calculated, tested for homogeneity of variances with Cochran's test, transformed as necessary (Underwood 1997) and analyzed by species with a one-way ANOVA and by a zone x plant ANOVA for *Armeria*, the only species in all zones.

Foundation species removal experiment

To test the hypothesis that foundation dune plants bind sand and build the dune habitat, as well as to quantify secondary succession, we performed a foundation species removal experiment. In each of the 3 major dune zones in March 2012, we marked the corners of 24 randomly placed 1x1 m plots each separated by at least 10 m. Each plot was individually labeled and then randomly assigned in each zone as controls, total species removals, or procedural controls ($n=8/\text{treatment}/\text{zone}$). The 4 corners of each plot were marked with 2 cm diameter rebar driven to refusal into the sand and cut to initially extend 10-15 cm above the sand surface. Control plots were otherwise untouched. In plant removal replicates, plots were perimeter trenched to 30 cm with straight edged shovels and all vegetation in the plots was sprayed weekly for 3 weeks with a systemic herbicide (Roundup, Monsanto) until all vegetation was dead. Dead aboveground vegetation was left to simulate natural death. Roundup is widely used in ecological research, has localized effects if plots are trenched to avoid translocation outside of the target area, and we have used it successfully in the past in

189 shoreline habitats (Bertness and Hacker 1994). No herbicide effects on plants outside
190 herbicided plots were observed. Procedural controls were trenched, but not herbicided.
191 Surface Elevation Table (SET) posts (Cahoon et al. 2000) modified for experimental
192 replication were installed in the center of all plots to measure sand erosion/accretion as a
193 function of the presence/absence of foundation species and dune zone. A 2 cm diameter rebar
194 rod was driven to refusal in the center of each plot, cut 10-15 cm above the sand surface and
195 fitted with a 30 cm long horizontal PVC bar with four evenly spaced locations to measure the
196 height of the sediment (see Brisson et al. 2014). Elevations were taken in October and March
197 of each year at each SET post for three years. Corner post heights were simultaneously
198 measured to supplement SET data and assess spatial patterns in sand erosion/accretion.
199 Sediment height (the sand erosion/deposition balance) data were analyzed with a treatment x
200 zone ANOVA followed by post-hoc testing (SNK test).

201 In the spring of 2013, we began monitoring temperature after noting apparent summer heat
202 death of some high middle dune plants during the first year of this experiment. We deployed
203 24 thermistors (Econorma S.a.s. FT-800/System) to plots of this first experiment (n=8/dune
204 zone), attaching them under the canopy in control plots and plant removal plots with wire
205 staples. This allowed us to quantify plant heat exposure during the summer, and quantify
206 differences between control and removal treatments. Thermistors were left in the field from
207 the mid-June until the first week of September 2013 measuring temperature hourly. We
208 pooled the data between 01:30 a.m. and 05:30 a.m. and between 13:30 p.m. and 17:30 p.m. to
209 estimate the lowest and the highest daily temperature, respectively. These temperature ranges
210 gave us mean minimum (Min) and mean maximum temperatures (Max) for each zone and
211 treatment. Data were analyzed with a three-way ANOVA, considering zone, temperature
212 (Min vs Max), and treatment (removal vs control) as orthogonal and fixed. A one-way
213 ANOVA was used to analyze mean maximum temperature by zone.

214 Reciprocal species removal experiment (Interspecific plant interactions)

215 To test the hypothesis that interspecific plant interactions shifted from facilitative to
 216 competitive across the sand dune, being especially important in the middle dune, we
 217 performed reciprocal species removal experiments in all dune zones with dominate species
 218 pairs. On the fore dune, we chose *Armeria pungens* (sea rose) and *Lotus cytisoides*; in the
 219 middle dune we chose *Armeria*, *Lotus*, and *Carpobrotus acinaciformis* (ice plant); and in the
 220 back dune, we chose *Armeria*, *Carpobrotus*, and *Pinus* spp. (*Pinus halepensis* and *Pinus*
 221 *pinea*). For each species pair in each zone, we located 24 0.5x0.5 m plots with mixtures of the
 222 two target species. All plots were marked with numbered rebar corner posts driven to refusal
 223 into the sand and labeled with a unique numbered plastic tag. For each zone and species pair
 224 (species a and b), we randomly assigned control plots, “species a” removal plots, and
 225 “species b” removal plots (n=8/treatment/species pair/dune zone). Species assigned for
 226 removal were pulled manually with minimal disturbance. Treatments were maintained
 227 monthly as needed for two years. During this time, plots were photographed in the spring and
 228 fall of each year and analyzed for percent plant cover. The height of the corner posts was also
 229 measured to quantify sand deposition/erosion. Plant cover was analyzed by zone with a two-
 230 way ANOVA, only *Carpobrotus* data were Sqrt (X+1) transformed. Separate ANOVAs were
 231 run for each species at 12 and 18 months, to accommodate the loss of plots to sand burial
 232 over time. For the same reason, we also analyzed plant cover and sand deposition on plot
 233 plant cover data pooled by zone with a t test and plant x sediment cover with linear
 234 regression.

235 Boardwalk shadow effect sampling

236 During the first year we noticed that in the summer months, temperatures in the middle and
 237 back dune, protected from on shore winds by the fore dune berm, were extremely high and

coincided with the death of the ice plant *Carpobrotus* at high dune elevations. To examine this hypothesis, we quantified the long-term effect of shading on the Badesi dune as a proxy for high temperature impacts. In February 2015, we quantified shaded and unshaded vegetation adjacent to boardwalks (1.5 m wide, elevated 50 cm over the substrate) perpendicular to the shoreline that extended from the fore dune to the highest point of the back dune, and 4 m on both sides of the boardwalks. We hypothesized that shading by the boardwalk would decrease solar stress on ice plants. We sampled 2 boardwalks that had been in place 5 years. At each boardwalk we quantified live and dead ice plant % cover in 0.5x0.5 m quadrats every 2 m from the beginning of the fore dune to the back dune. For analysis we excluded plots levels without ice plants and pooled the data from adjacent and control plots. Dead/alive ratio of *Carpobrotus* % cover and dead *Carpobrotus* % cover were transformed to meet the assumptions of parametric statistics and analyzed with a one-way ANOVA.

Physical stress alleviation experiment

To test the hypothesis that physical stress limits dune plant recovery across the dune, we ran a fully factorial experiment across all zones manipulating all combinations of nutrient limitation (with 33 ml of slow release Osmocote NPK pellets spread on them every 6 months and without nutrient additions), water limitation (with 2 liters of tap water every 2-3 weeks and without water additions) and substrate stability (with and without substrate stabilizing fish net attached flush to the surface with wire staples). In each zone we located 72 0.5x0.5 m plots with bare sand substrate. Every combination of nutrient, water, substrate limitations and controls were marked and labeled with numbered rebar corner posts (n=8/treatment combination/zone). Replicate treatments were maintained for two years and all plots were monitored for % plant cover initially and then for 2 years in the spring and fall. Plant cover in the plots was transformed to meet parametric statistic assumptions and analyzed with a treatment x dune zone ANOVA.

263

Seedling recruitment

264 To examine the distribution of dune plant seeds and test the hypothesis that like sand, seeds
265 are deposited and trapped under adult plants, we sampled sand under plants and in adjacent
266 bare sand >25 cm from plants in all dune zones. This was done in October 2013, after the
267 summer when seed dispersal and germination was most pronounced on the dune (Bakker et
268 al. 2008). In each zone (fore, middle and back) we sampled sand under and adjacent to the
269 most common plants in each zone by taking 100 ml surface sand samples (1 cm deep;
270 n=10/species/habitat/zone). Samples were returned to the laboratory and sorted under a
271 dissecting microscope. Seed density was analyzed with an experimental treatment (under and
272 adjacent to adult plants) one-way ANOVA separately by zone and on conspecific seeds of the
273 target plant and seeds of all other plants combined.

274 To examine seedling survivorship across the dune and test the hypothesis that seedling
275 survivorship increased with dune elevation and association with adult plants, we marked
276 natural seedlings in all dune zones that were under adult plants and in bare sand >30 cm from
277 an adult plant. 450 seedlings were marked and monitored monthly for survivorship over three
278 seasons. Seedling survivorship was analyzed with a non-parametric log rank test to compare
279 survival among zones (fore vs middle vs back) and species survival by location (next to adult
280 vs adjacent bare sand). In both cases the whole follow up period was taken into account
281 (Bland and Altman 2004) and a χ^2 test was done on the log rank data of $\ln(X+1)$
282 transformed *Armeria* seedling data.

283 To examine the hypothesis that seed supply is a determinant of the distribution and
284 abundance of plants across the dune, we performed a seed transplant experiment with the 5
285 most common Badesi sand dune plants (*Armeria*, *Lotus*, *Carpobrotus*, *Cakile maritima*, and
286 *Panocratium maritimum*). For each species, we collected dehiscing seed heads and dissected

out and separated the seeds of each species. We then sorted them into aliquots of 4-10 seeds depending on species seed availability and placed them by species into polyester mesh bags that would retain the seeds, but would allow germination and seedling growth. For each species we planted 21 seed bags in each zone (fore, middle and back) under conspecific adults and in unvegetated sand >30 cm from adult vegetation and marked their location with color-coded wire markers. We monitored seed transplants weekly for germination and seedling survivorship for 3 months. Germination and seedling survivorship were analyzed separately by species with non-parametric log rank data, using a χ^2 test.

To test the hypothesis that loose seeds were eroded away on the fore dune, we also transplanted seeds of *Pancratium* in mesh bags (n=10), loose in the sand (n=10), and loose in the sand covered on the sand surface with nylon mesh (2 mm mesh, 5x5 cm cover pinned to the sand surface with garden fabric staples; n=10). We only did this experiment in the fore dune since the fore dune was the only zone that showed significant erosion. Seed species were marked and identified by color-coded wires (2 mm). All seeds were planted 3 cm below the sand surface. We monitored these transplants for germination and survivorship weekly for the first 3 months and monthly for one year. Germination and seedling survivorship data were analyzed with a non-parametric log rank data, using a χ^2 test.

Back dune competitive release experiment

It was not possible to do *Pinus* removal experiments similar to the reciprocal plant species removal experiments or foundation species removals in other zones because of the size of *Pinus* trees. Since the Badesi dune is a protected conservation area, removing entire *Pinus* trees would have been destructive and not permitted. To test the hypothesis that *Pinus* domination of the terrestrial high dune border is due to competitive dominance, we did a competitive release experiment by removing large (~2m long) *Pinus* branches and estimated

natural plant recruitment by following seed germination and cover in 1.5x1.5 m plots under *Pinus* canopies (n=10), where *Pinus* shading was alleviated by branch removal (n=10) and in areas where *Pinus* shading was removed but replaced by a similar level of shading by shade cloth (n=10), as procedural controls. A central 0.5x0.5m quadrat in each plot was monitored photographically monthly for a year.

To test the hypothesis that allelopathy contributes to the dominance of *Pinus* and ice plant in the back dune, we also manipulated sediment in the *Pinus* plots described above. Allelopathy was suggested because in dense *Pinus* and ice plant stands, natural substrate is covered by *Pinus* needles and ice plant leaves, but seedlings of all species are extremely rare (Ceccherelli, personal observation). In 100 ml plastic greenhouse seedling pots we planted seeds of *Armeria*, *Pancratium*, and *Lotus* (plus no seed controls) with either 1) *Pinus* soil, 2) middle dune bare soil, 3) middle dune ice plant soil, 4) potting soil mixed with sand, 5) potting soil with a 2 cm layer of *Pinus* needles and 6) potting soil with a 2 cm layer of ice plant leaves (n=10/*Pinus* treatment/soil type) and scored them monthly for germination. Seed germination and survivorship of *Armeria*, and *Lotus* were transformed with $\ln(X+1)$ and all were analyzed with *Pinus* treatment x soil type ANOVA.

RESULTS

Zonation survey

Elevational zonation of plants across the Badesi dune is striking (Fig. 1). The fore dune has low plant richness and cover (35%) and 65% unvegetated sand cover. *Armeria* (sea rose), *Otanthus maritimus* (cotton weed) and *Lotus* (trefoil of the cliffs) are the numerically dominant plant species on the fore dune and all are clonally spreading, deep-rooted perennials. The width of the fore dune varies at Badesi from ~20-25 m. The middle dune has over 28% higher plant cover and 53% higher species richness than the fore dune, but still has

considerable bare sand substrate (Fig. 2, 38%). *Armeria*, *Carpobrotus* (ice plant) and *Ephedra distachya* (joint pine) are the most common middle dune plant species and are all clonally spreading perennials. The middle dune is ~30-35m wide.

The transition from the middle to back dune is more gradual (Fig. 1). The seaward border of the back dune has the highest plant species richness on the dune, and is dominated by the ice plant, *Carpobrotus*, a perennial succulent, that can be seen overgrowing other back dune plants like *Pinus* on the terrestrial border of the dune (33%). At higher elevations of the back dune, *Pinus* dominates the landscape as a solitary evergreen species that has a prostrate morphology at lower elevations, an arborescent morphology at higher elevations and an understory of bare substrate in the dense *Pinus* canopy that dominates high elevations.

Substrate topography in relation to plants varied across the dune. In dune zones closest to the water, plants were found on elevated sand mounds that decreased in elevation with distance from the water, while in the back dune, furthest from the water, plants were not associated with sand mounds. On the front dune, *Armeria* and *Otanthus* were found on sand mounds 53 ± 2.16 cm (mean $\pm$ SE) and 56 ± 2.51 cm higher than adjacent substrate, respectively. For the front dune, the one-way ANOVA did not reveal any significant difference between the two species ($F_{1,38}=0.36$ $p>0.05$). On the fore dune, *Lotus* and *Armeria* were similarly found on mounds 17 ± 4.64 and 24 ± 1.92 cm high, respectively, and no significant effect was detected with the ANOVA ($F_{1,38}=2.07$ $p>0.05$). In contrast, in the middle dune, *Armeria*, *Carpobrotus*, *Lotus*, *Helicrysum microphyllum* and *Ephedra* were found on smaller sand mounds 22 ± 1.47 , 12 ± 2.06 , 11 ± 1.06 , 16 ± 1.27 , and 12 ± 2.48 cm high, respectively. In this zone the difference among species was significant ($F_{4,95}=7.51$ $p<0.0001$, SNK: *Armeria*>all others). These front, fore and middle dune plants occur on elevated sand mounds that appear to be actively trapped by passive deposition of wind-blown sand. *Armeria*, a robust perennial shrub, was found on higher sand mounds than all other species. One-way ANOVA on

Armeria revealed differential *Armeria* sand binding by zone ($F_{3,76}=111.36$ $p<0.0001$, SNK: front>fore top=middle>back). In the back dune, buffered from winds and sand transport by the lower dune zones, *Armeria* = 9 ± 1.65 cm, *Carpobrotus* = 4 ± 1.31 cm, *Pinus* = 4 ± 2.76 cm, *Acacia cyanophylla* = 0 ± 3.8 cm, and *Juniperus* spp. (*Juniperus macrocarpa* and *Juniperus phoenicea*) = -2 ± 4.72 cm were not found on elevated locations and there were no significant species effects ($F_{4,95}=1.93$ $p>0.05$).

Foundation species removal experiment

Removing foundation species had different effects across zones (Fig. 3). We performed a two-way ANOVA of zone and treatment, both considered as orthogonal fixed factors. The analyses revealed a significant zone x treatment interaction ($F_{4,63}=2.67$ $p<0.05$). In the fore dune, foundation species removal led to sand erosion of >2 cm/year, in contrast to control and procedural control plots that had annual sand accretion rates of >4 cm/year (Fig. 3, $p<0.05$, SNK test fore: removal<procedural control=control). Sand erosion in the fore dune foundation species removal plots was dramatic and led to the collapse of the fore dune edge in just two years. This was reflected in the erosion of the seaward corner posts in the fore dune, where, while the seaward edge corner post of procedural control (5.29 ± 1.3 cm/year) and control plots (3.66 ± 1.77 cm/year) showed net sand accretion, when foundation species were removed, erosion on the seaward edge corner posts increased over 60% leading to an amount of sand dispersion of -2.8 ± 1.6 cm/year (Fig. 3).

In the middle dune, sediment variation resulted in net sand accretion that occurred in all treatments (Fig. 3, $p<0.05$, SNK test: fore=back<middle), but was nearly twice as high in control and procedural control plots with live vegetation to bind and trap sand than in removal plots (SNK middle: removal<procedural control=control).

In the back dune, annual sediment accretion was more than an order of magnitude less than middle dune plots with foundation species (Fig. 3). Sand accretion in the back dune was also similar among foundation removal, control and procedural control treatments (Fig. 3, $p < 0.05$, SNK back: removal=procedural control=control). In all treatments, annual accretion was < 1 cm/year and when foundation species were removed there was no sand accretion.

Mean minimum temperature (Min) did not differ among treatments or zones (fore $18.73 \pm 0.45^\circ \text{C}$ and $18.50 \pm 0.4^\circ \text{C}$, middle $19.11 \pm 0.28^\circ \text{C}$ and $19.12 \pm 0.18^\circ \text{C}$, back $16.59 \pm 0.77^\circ \text{C}$ and $17.07 \pm 0.87^\circ \text{C}$ for removal and control, respectively; $F_{2,228}=1.77$ $p > 0.05$ for the interaction zone x temperature range x treatment). In contrast, mean maximum temperature (Max) differed among zones (fore $33.73 \pm 1.40^\circ \text{C}$ and $32.83 \pm 2.46^\circ \text{C}$, middle $41.86 \pm 2.74^\circ \text{C}$ and $38.31 \pm 1.34^\circ \text{C}$, back $44.89 \pm 3.61^\circ \text{C}$ and $40.26 \pm 3.45^\circ \text{C}$; $F_{2,114}=39.85$ $p < 0.0001$, SNK: fore<middle=back) peaking in back zone removal treatments ($F_{1,114}=10.72$ $p < 0.002$, SNK: Removal>Control). Maximum temperatures peaked around 70°C , and averaged 45°C in the removal plots, 5°C lower than when vegetation was present.

Reciprocal species removal experiments (Interspecific plant interactions)

Evaluation of interspecific interactions of common plant species in all dune zones with reciprocal species removal experiments initially revealed significant interspecific interactions (Fig. 4). Over time, however, these interactions were lost due to sand burial and erosion disturbance, particularly in the fore and middle dune zones as evidenced by a decrease in plant cover over time in the species interaction plots (Fig. 5, middle $p < 0.001$, t test). After 18 months, 22% of the middle dune reciprocal species interaction plots had been completely lost due to sand burial, and an additional 10% of the plots were still recognizable but almost totally buried, while no plots were lost in the fore or back dune. To analyze this experiment, we ran separate two-way ANOVAs for each species after 12 months and 18 months of

treatments, taking into account species interaction (comparison of each pair in which the species is involved) and treatment (reciprocal species removal vs control) as orthogonal and fixed factors. Insignificant interaction p values will always refer to the last sampling date (18 months). In the fore dune after 6, 12, and 18 months, there was no evidence of reciprocal effects between *Armeria* and *Lotus* (Fig. 4, interaction x treatment $F_{4,70}=1.96$ $p>0.05$ and $F_{2,42}=2.27$ $p>0.05$, respectively).

In the middle dune, after one year of treatment, there was evidence of *Armeria* and *Lotus* competitively depressing *Carpobrotus*, but after two seasons this competitive effect was no longer apparent (Fig. 4, treatment $F_{3,56}=12.12$ $p<0.03$, SNK reciprocal species removal>control at 12 months, interaction x treatment $F_{3,56}=1.23$ $p>0.05$ at 18 months). At the same time, there was no evidence of interactions affecting *Armeria* or *Lotus* (Fig. 4, interaction x treatment $F_{4,70}=1.96$ $p>0.05$ and $F_{2,42}=2.27$ $p>0.05$, respectively). In all the interspecific interaction plots in the middle dune there was, over time, a general decrease in plant cover associated with sand burial disturbance (Fig. 5, $p<0.001$, t test).

In the back dune sand burial was not prevalent, but a summer die off of *Carpobrotus* was seen every year (see below). *Carpobrotus* and *Pinus* had a facilitating effect on *Armeria* after 12 months but not at 18 months (Fig. 4, interaction x treatment $F_{4,70}=3.31$ $p<0.02$, SNK test for both Back pairs: reciprocal species removal<control at 12 months, $F_{4,70}=1.96$ $p>0.05$ at 18 months). While, after one year of removal, the opposite was found for *Carpobrotus*, which was competitively depressed by both *Armeria* and *Pinus*, but after another two seasons of observation these interactions were no longer detectable (Fig. 4, treatment $F_{3,56}=12.12$ $p<0.03$, SNK reciprocal species removal>control at 12 months, interaction x treatment $F_{3,56}=1.23$ $p>0.05$ at 18 months). Reciprocal removal of *Pinus* in the back dune did not reveal significant species interactions (Fig. 4, interaction x treatment $F_{1,28}=0.18$ $p>0.05$). In the back dune species removal plots, the most conspicuous trend over time was the decrease in the

cover of *Carpobrotus* during summer. This left large areas of dead, desiccated *Carpobrotus* dominating the seaward border of the back dune at the end of the summer (Cussedu, personal observation).

Boardwalk shadow effect sampling

Analysis of *Carpobrotus* cover data (dead/alive ratio) adjacent to and 2 m away from the shade of boardwalks revealed almost twice as much live ice plant cover under the shade of the boardwalks ($46 \pm 3.24\%$) than in adjacent unshaded habitats ($25 \pm 2.84\%$; $F_{1,190}=5.58$ $p<0.02$). There was also nearly 10% more dead ice plant in unshaded habitats ($16 \pm 1.76\%$) than under the shade of boardwalks ($7 \pm 1.10\%$, $F_{1,190}=18.76$ $p<0.0001$). Since ice plant death occurs almost exclusively in summer (Cussedu, personal observation), ice plant mortality at high dune elevations appears to be driven by heat and desiccation from direct sun exposure leaving desiccated ice plant skeletons marking their initial distribution.

Physical stress alleviation experiment

Experimentally manipulating water availability, nutrient availability and substrate stability across dune zones (fore, middle and back) in a fully factorial 2 year experiment at a 0.5×0.5 m spatial scale revealed that these factors, long thought to be critical to sand dune plant communities, had no effect on plant recovery, as evidenced for each zone using ANOVA with treatment as orthogonal and fixed factor (Fig. 6, fore $F_{9,70}=1.95$ $p>0.05$, middle $F_{9,70}=2.16$ $p<0.04$ but not significant post hoc SNK, back $F_{9,70}=0.57$ $p>0.05$). There were no significant responses in plant biomass or species identity to treatments within zones, but a two-way ANOVA (zone and treatment orthogonal and fixed), showed that recovery increased from the fore to back dune (Fig. 6, $F_{2,210}=18.30$ $p<0.0001$, SNK fore<mid<back), although treatment and the zone x treatment interaction were not significant ($F_{9,210}=1.70$ $p>0.05$ and $F_{18,210}=0.87$ $p>0.05$, respectively). No effect of the environmental manipulations was found

at these levels, but this does not mean that other levels would not have an effect, or that water and nutrient availability are unimportant for the structure of dune communities.

Seedling recruitment: seed and seedling quantification

Seeds of the most common dune plants were most abundant under adult plants independent of species identity and dune zone (Fig. 7). For each zone, we performed a three-way ANOVA considering the species, the substrate (under adult vs bare sand) and the seeds category (conspecific vs other seeds) all as orthogonal and fixed factors. We will present only the factors and/or the interactions that were significant.

On the front of the fore dune, seeds of *Armeria* and *Otanthus* were almost exclusively found under conspecific adults, whereas the seeds of other species were rare and not found associated with adult plants (Fig. 7, substrate x seeds interaction $F_{1,72}=0.11$ $p<0.02$). On the top of the fore dune, seeds of *Armeria* were more common under *Armeria* adults than in nearby bare sand, but for *Lotus* and *Pancratium*, seeds of conspecifics were just as common in bare sand than under adults (Fig. 7). For *Lotus* and *Pancratium*, conspecific seeds were equally distributed under and away from adults, but seeds of other species were significantly more common under *Lotus* and *Pancratium* than in adjacent bare sand (Fig. 7, species x seed category interaction, $F_{2,108}=4.43$ $p<0.02$ and species x substrate x seeds category $F_{2,108}=3.45$ $p<0.04$, respectively).

In the middle dune, *Armeria* seeds were more common under *Armeria* adults than in adjacent bare sand, but seeds of other species were not (Fig. 7), while for *Carpobrotus*, conspecific seeds were rare, but seeds of other species were more than an order of magnitude more abundant under *Carpobrotus* than in bare sand (Fig. 7, species x substrate x seeds interaction, $F_{1,72}=12.59$ $p=0.0007$). In the back dune, *Armeria* seeds were 20 times more common under conspecifics than in adjacent bare sand, while for *Carpobrotus*, seeds of other species were

significantly more abundant under *Carpobrotus* than in adjacent bare substrate (Fig. 7, species x substrate x seeds interaction $F_{1,72}=8.34$ $p<0.006$).

The survivorship of marked seedlings was analyzed with a log rank test comparing the survival of the seedlings of each species comparing the proximity with adult (next to adult vs adjacent bare sand) and of the seedlings in general pooled in three groups corresponding to the three zones (fore vs. middle vs. back seedlings), on the times (weeks) of survival events. The percent of seedlings survivorship increased dramatically with dune zone elevation but was not affected by neighboring plants (Fig. 8, $p<0.05$, χ^2). In the fore dune, no *Lotus* seedlings survived (independent of the proximity to adult neighbors), no *Pancratium* seedlings survived on bare sand, and less than 5% of *Pancratium* seedlings survived next to adults, but this result was not significant ($p>0.5$, χ^2). In the middle zone only 5% of *Lotus* seedlings survived with or without adult neighbors ($p>0.5$, χ^2), while for *Armeria* 25% of marked seedlings in bare sand survived, nearly three times (10%) the number of seedlings that survived next to adults ($p<0.025$, χ^2). No *Pancratium* seedlings survived. In the back dune survivorship of *Armeria* and *Carpobrotus* seedlings was over 80%, far higher than any other zone, and was not influenced by the presence or absence of neighbors (Fig. 8).

Seedling recruitment: seed transplant experiments

Of the six dune plant species seeds used in transplant studies, three species, *Pancratium*, *Lotus*, and *Cakile* germinated. Only 2 and 3 *Armeria* and *Otanthus* germinated from back and fore dune planted seeds respectively, while *Astragalus thermensis* and *Carpobrotus* did not germinate in any location or treatment. For each of the three species that had sufficient germination, we compared the germination and survival in each of the three zones. 30-40% of *Pancratium* seed transplants germinated, but germination was similar among zones ($p>0.1$, χ^2). Survivorship of seedlings from the seed transplant experiment showed that all species had

the highest survivorship in the back dune, but this pattern was only significant for *Pancratium*, which had the highest sample size (Fig. 9, $p < 0.005$, χ^2). Less than 10% of the transplanted *Lotus* seeds germinated and *Lotus*, found ubiquitously across the dune, had higher germination in the back dune, and lowest germination in the middle dune (Fig. 9, $p < 0.025$, χ^2), while survivorship did not differ among zones ($p > 0.5$, χ^2). For *Cakile*, a pioneer species found naturally in the fore and middle dune, 12-18% of transplanted seeds germinated and among zones had higher germination in the middle than in fore and back dune (Fig. 9, $p < 0.05$, χ^2). Survivorship, however, did not differ among zones ($p > 0.5$, χ^2).

In the fore dune seed stabilization experiment with *Pancratium*, germination and survivorship were analyzed comparing germination and survival among the three treatments (seeds in bags vs loose seeds vs. loose seeds with net covers). Due to erosion, germination was highest in the bagged seed treatment, second highest in the seed treatment with stabilized substrate (net cover), and lowest in the loose seed treatments (Fig. 10, $p < 0.005$, χ^2). The difference in germination between loose seeds and the secured seed treatments was $> 10\%$, suggesting that sand erosion in the fore dune can lead to seed loss. Seedling survivorship in this experiment was similar among stabilization treatments ($p > 0.25$, χ^2).

Back dune competitive release experiment

Natural plant recruitment was not observed under *Pinus*, irrespectively of canopy removal treatments. However, germination of experimentally planted seeds under *Pinus* occurred, although it was generally lower in control plots, especially for *Armeria* and *Lotus* (Fig. 11). We ran a two-way ANOVA by species with canopy treatment and soil type as fixed and orthogonal factors. Under the *Pinus* canopy, *Armeria* seeds attracted small animals as they were often removed or eaten. *Armeria* germination, however, was significantly higher in the *Pinus* removal treatment in middle dune soil, and very little germination occurred in any soil

type under *Pinus* canopy and in procedural control plots (Fig. 11, canopy treatment x soil type interaction, $F_{12,126}=2.54$ $p<0.005$). *Pancratium* and *Lotus* germination was not influenced by the *Pinus* treatment ($F_{2,126}=2.11$ $p>0.05$ and $F_{2,126}=0.79$ $p>0.05$, respectively), but they were significantly affected by soil type (Fig. 11, $F_{6,126}=5.02$ $p=0.0001$ and $F_{6,126}=7.06$ $p<0.0001$, respectively), even if, for both species, there were no significant post hoc comparisons. The interaction *Pinus* treatment x soil type was not significant for both species ($F_{12,126}=0.96$ $p>0.05$ and $F_{12,126}=0.84$ $p>0.05$, respectively).

DISCUSSION

Our experiments testing the basic assumptions of sand dune community organization generally support earlier descriptive and correlative studies. This is unusual. Experimental examination of community assemblages typically has not upheld the findings of earlier correlative conclusions. For example, rocky intertidal, salt marsh, kelp forest, and coral reef community dynamics once thought to be driven by physical forces (e.g. Odum 1953, Lewis 1964, Mann 1982), were later shown to be strongly regulated by interactions between physical and biological factors once community level experiments were carried out (Dayton 1971, Estes and Palmisano 1974, Bertness and Ellison 1987). Our results suggest that fore dune foundation species are responsible for binding, stabilizing and maintaining sand dune communities, and that interspecific plant interactions occur across the dune, but are generally overshadowed by physical stresses, particularly sand burial in the middle dune. Additionally, they indicate that while physical stresses limit plant distributions across the dune, their impacts are largely stochastic, except in the back dune where competitively dominant woody plants competitively displace other sand dune plants.

Biogenic Communities, Foundation species and Hierarchical organization

Like most communities in physically and/or biologically stressful habitats, the Badesi sand dune plant community is dependent on foundation species that ameliorate stress and facilitate community development (Angelini et al. 2011). Our foundation plant species removals reveal that plant species on the fore dune bind and stabilize sand, building the habitat that supports all the dune plants living at higher elevations, making them foundation species-dependent (Fig. 3). Without habitat-modifying foundation species that initially colonize potential fore dune habitats, sand dune plant communities would not develop or be maintained. Biogenic communities that are dependent on foundation species often display this type of hierarchical organization (Bruno and Bertness 2001, Angelini et al. 2011) and include salt marshes (Angelini et al. 2011), terrestrial forests (Ellison et al. 2005), seagrass meadows (Duarte et al. 2000), as well as smaller scale associations within communities (Angelini and Silliman 2014). This common dependency of communities on foundation species habitat modification needs to be incorporated into ecological theory (Bruno et al. 2003). It is a major organizing force in community assembly, confirmed by the overwhelming evidence that it is the template for communities in physically and biologically stressful habitats due to habitat amelioration and associational defenses, respectively (Bertness and Callaway 1994, Ellison et al. 2005, Crotty and Bertness 2015).

Competitive dominance

Pairwise quantification of the interaction of the numerically common plants in each dune zone revealed that interspecific plant competition was common across the dune landscape (Fig. 4). In the middle dune, however, burial disturbance over the 18-months time course of our experiments revealed that, while interspecific plant interactions occurred, their importance was transient and not of long term consequence in generating species distribution patterns. Rather, sand burial disturbance limited the role of interspecific competition and competitive displacement, but promoted plant species diversity and coexistence in the middle

dune (Fig. 4). Similar results have been found on rocky shores, intertidal boulder fields, mussel beds, salt marshes, and grasslands (see Dayton 1971, Sousa 1979, Paine and Levin 1981, Bertness and Ellison 1987, and Platt 1975, respectively). In the back dune, where sand burial is less common, competitive dominance by woody plants emerges as a major factor leading to competitive dynamics that determine the prevalence of shrub and woody species like *Pinus* and *Armeria* and exclusion of other dune species. Due to the dense *Pinus* canopy, germination of other middle dune species is precluded and in some cases soil type prevents seedling recruitment. Moreover, high summer temperatures in the back dune limit ice plant competitive dominance, because it suffers drying and summer die-off limiting its dominance, favoring *Pinus*.

Sand burial disturbance

While our interspecific plant interaction experiments initially revealed significant interactions (Fig. 4), sand burial, particularly in the middle dune, was ultimately the most pervasive and powerful driver of plant community patterns at lower dune elevations, rendering competitive dominance and facilitative interactions inconsequential. Thus, the same physical force that is responsible for building the dune, sand mobility, is responsible for plant mortality and diversity, particularly in the middle dune. Burial in sand is recognized as a major aspect shaping the arrangement and composition of vegetation in coastal sand dune communities (Ranwell 1958, van der Valk 1974, Maun and Lapierre 1986). Sand deposition has been renowned as a main selective force in the evolution of seeds, in survivorship of seedlings and adult plants and, to a larger scale, in zonation and succession of vegetation (Maun 1994 and 1998).

Seedling Recruitment

Sexual recruitment is generally problematic in physically and biologically stressful communities like salt marshes (Pennings and Callaway 1996) and coral reefs (Hughes and Jackson 1985), respectively. As a result, asexual reproduction and clonal growth play a leading role in the community dynamics of communities in physically or biologically stressful habitats. Sexual recruitment is limited in Sardinian sand dune plant communities exposed to severe substrate mobility and strong wind and salt spray exposure. Dispersed seeds of dune plants are trapped near substrate stabilizing adult plants and are uncommon in unvegetated substrate (Fig. 7). The survivorship of marked seedlings was low, but generally increased from the fore to the back dune and was higher when seedlings were associated with adult plants than when on bare sand substrate without neighbors.

Field germination experiments with 3 common species had low germination rates and poor survivorship in all zones except the back dune. Seedlings of back dune grew the best, but suffered from desiccation, due to high temperature and herbivory (Fig. 9). Like in other stressful environments, the low success of seed reproduction appears to lead to nurse plant effects (Franks 2003) and strong reliance of dune plants on clonal reproduction, particularly at low elevations (Maun 1998). Nurse plant effects have been identified as a strong generator of pattern in sand dune plant communities in general (Martinez 2003).

CONCLUSIONS

Lessons from Sardinian sand dunes

Our results reveal that sand dune plant communities are hierarchically organized and dependent on the establishment of fore dune foundation plants that bind sand above and belowground, leading to sand dune initiation, development, and maintenance (Fig. 3). Without foundation plant species, mechanisms such as sand binding, erosion, mobility, and burial act as barriers to plant community development (Fig. 3) and are more pronounced than

at higher elevations that are less exposed to sand mobility. On the fore dune, removing the foundation species led to habitat loss, while competitive and facilitative plant species interactions were not detectable due to sand erosion and burial disturbance (Fig. 4 and 5). Manipulating physical factors thought to be critical in sand dune communities (i.e. water additions, nutrient additions, substrate stabilizations) in well replicated plots of all these factors alone and in combination also did not affect plant colonization at the small 0.25m² spatial scale manipulated. Seed and seedling success were also rare on the fore dune suggesting that asexual clonal expansion and colonization was more common in the highly disturbed fore dune habitat. This also suggests that disturbance on the fore dune trumps all other biological and physical factors. Since all of these physical and biological factors have been shown to correlatively impact sand dune communities at regional spatial scales, our results imply that spatial scale is important in understanding process and pattern in sand dune ecosystems since they are so fundamentally shaped by disturbance processes and their interaction with foundation plant species that stabilize the substrate.

Plant species cover and richness increased from 35% in the fore dune to 63% in the middle dune (Fig. 1). Like the fore dune, however, recovery from foundation removal was minimal, being less than 1% in 3 years. Moreover, while the middle dune initially revealed interspecific plant interactions, it is subjected to heavy sand burial that limited the role of species interactions, seed germination (Fig. 9), and seedling success (Fig. 8). Therefore, frequent sand burial disturbances have a large influence over the middle dune zone (Fig. 5).

In the back dune we see nearly 100% plant cover and the emergence of competitive dominants (Fig. 1 and 2c). On the seaward border of the high dune the ice plant is common and appears to be competitively dominant to middle dune plant species, but, at higher dune elevations, the evergreen *Pinus* competitively displaces most other dune plants, forming a back dune *Pinus* monoculture. Seedling germination and survivorship were higher in the back

dune than at lower dune elevations (Fig. 9), as was the recovery of bare space (Fig. 6). The competitive dominance of *Pinus* in the back dune was shown by a competitive release experiment in which removing *Pinus* canopy in the back dune lead to the success of important foundation species such as *Armeria*, especially favored by middle dune soil (Fig. 11). In general, however, seeds of plants characteristic of the middle and fore dune planted in *Pinus* zone showed a quite good capability of germination. This suggests that they are excluded from the back dune because *Pinus* acts as a physical barrier, having a dense prostrate morphology (Fig. 11).

These results are summarized in a conceptual model of the assembly rules of the Badesi sand dune plant community (Fig. 12). The gradient of stress in this dune system acts crosswise, from land to sea and back again, creating an area of aggregation of the various factors in the middle of the dune, in which the living conditions are particularly difficult. It is for this reason that facilitation mechanisms are determined among species, however they are transient in nature due to the high instability of the conditions. Physical stress on the vascular plants of the dune, including wind exposition, sand scouring, and other stressors of terrestrial origins, decreases with increasing dune elevation exposure. Conversely, the role of biological factors in shaping the dune plant community increases with increasing dune elevation. These factors include interspecific plant competition and facilitation, increasing seedling recruitment and survival, and increasing herbivory. The Badesi dune plant community is a biogenic community built and maintained by foundation plant species that stabilize sand on the fore dune. On the middle dune, sand burial disturbance is the dominant structuring force that limits the role of sexual recruitment and interspecific competition in structuring the middle dune zone. In the back dune, ice plant and *Pinus* competitively dominate all other dune plants, but high summer temperatures limit ice plant dominance in the back dune, resulting in *Pinus* competitively dominating the back dune terrestrial border.

Ecosystems, like sand dunes, where the establishment of foundation species ameliorates stresses allow community development that would not occur without foundation species. Coral reefs (Hughes and Jackson 1985), salt marshes (Bruno and Bertness 2001), mangroves (Ellison and Farnsworth 2001), seagrass (Duarte et al. 2000), and forest ecosystems (Ellison et al. 2005) have all been explicitly or implicitly described as hierarchically organized systems. In all of these ecosystems, once foundation species enabled community establishment, while other biotic factors like competition, recruitment, and trophic dynamics became important community structuring forces (Bruno and Bertness 2001). These findings and their implications are important for understanding community organization well enough to make it a more predictive science and for conservation since they stress that managing, protecting, and restoring foundation species should often be the first step in many conservation efforts, rather than focusing on charismatic species.

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REFERENCES

693 Acosta, A., M. L. Carranza, and C. F. Izzi. 2009. Are there habitats that contribute best to
694 plant species diversity in coastal dunes? *Biodiversity and Conservation* 18: 1087-1098.

695 Altieri, A. H., B. R. Silliman, and M. D. Bertness. 2007. Hierarchical Organization via a
696 Facilitation Cascade in Intertidal Cordgrass Bed Communities. *American Naturalist* 169:
697 195-206.

698 Altieri, A. H., et al. 2010. Facilitation cascade drives positive relationship between native
699 biodiversity and invasion success. *Ecology* 91: 1269-1275.

700 Angelini, C., et al. 2011. Interactions among Foundation Species and their Consequences for
701 Community Organization, *Biodiversity and Conservation*. *BioScience* 61: 782-789.

702 Angelini, C., and B. R. Silliman. 2014. Secondary foundation species as drivers of trophic
703 and functional diversity: evidence from a tree-epiphyte system. *Ecology* 95: 185-196.

704 Bacchetta, G., et al. 2008. Systematics, phylogenetic relationships and conservation of the
705 taxa of *Anchusa* (Boraginaceae) endemic to Sardinia (Italy). *Systematics and Biodiversity*
706 6: 161-174.

707 Bakker, J. P., L. G. Bravo, and A. M. Mouissie. 2008. Dispersal by cattle of salt marsh and
708 dune species into salt marsh and dune communities. *Plant Ecology* 197: 43-54.

709 Barbier, E. B., et al. 2013. The Value of Wetlands in Protecting Southeast Louisiana from
710 Hurricane Storm Surges. *PLoS ONE* 8(3): e58715. doi:10.1371/journal.pone.0058715.

711 Barbour, M. G., T. D. de Jong, and B. M. Pavlik. 1985. Marine beach and dune plant
712 communities. Pages 296-322 in *Physiological ecology of North American plant*
713 *communities*. B. F. Chabot and H. A. Mooney, editors. Chapman and Hall, New York.

- 714 Bertness, M. D., and A. M. Ellison. 1987. Determinants of Pattern in a New England Salt
715 Marsh Plant Community. *Ecological Monographs* 57: 129-147.
- 716 Bertness, M. D., and R. Callaway. 1994. Positive interactions in communities. *Trends in*
717 *Ecology and Evolution* 9: 191-193.
- 718 Bertness, M. D., and S. D. Hacker. 1994. Physical Stress and Positive Associations Among
719 Marsh Plants. *The American Naturalist* 144: 363-372.
- 720 Bland, J. M., and D. G. Altman. 2004. The log rank test. *BMJ* 328: 1073.
- 721 Blondel, J., and J. Aronson. 1999. *Biology and wildlife of the Mediterranean region*. Oxford
722 University Press, New York.
- 723 Brisson, C. P., T. C. Coverdale, and M. D. Bertness. 2014. Salt marsh die-off and recovery
724 reveal disparity between the recovery of ecosystem structure and service provision.
725 *Biological Conservation* 179: 1-5.
- 726 Bruno, J. F., and M. D. Bertness. 2001. Habitat Modification and Facilitation in Benthic
727 Marine Communities. Pages 201-218. in *Marine Community Ecology*. M. D. Bertness, S.
728 D. Gaines and M. E. Hay, editors. Sinauer Associates, Sunderland, Massachusetts, USA.
- 729 Bruno, J. F., J. J. Stachowicz, and M. D. Bertness. 2003. Inclusion of facilitation into
730 ecological theory. *Trends in Ecology and Evolution* 18: 119-125.
- 731 Cahoon, D. R., et al. 2000. A method for measuring vertical accretion, elevation, and
732 compaction of soft, shallow-water sediments. *Journal of Sedimentary Research* 70: 1250-
733 1253.

- 734 Castanho de Toledo, C., et al. 2015. A meta-analysis of plant facilitation in coastal dune
735 systems: responses, regions, and research gaps. PeerJ 3:e768
736 <https://dx.doi.org/10.7717/peerj.768>.
- 737 Cowles, H. C. 1899. The Ecological Relations of the Vegetation on the Sand Dunes of Lake
738 Michigan. Geographical Relations of the Dune Floras. Botanical Gazette 27: 95-117.
- 739 Crotty, S. M., and M. D. Bertness. 2015. Foundation Species Expansion of Habitat Use and
740 the Realized Niches of Sympatric Species. Ecology. In press
- 741 Cushman, J. H., J. C. Waller, and D. R. Hoak. 2010. Shrubs as ecosystem engineers in a
742 coastal dune: influences on plant populations, communities and ecosystems. Journal of
743 Vegetation Science 21: 821-831.
- 744 Dayton, P. K. 1971. Competition, Disturbance, and Community Organization: The Provision
745 and Subsequent Utilization of Space in a Rocky Intertidal Community. Ecological
746 Monographs 41: 351-389.
- 747 Dayton, P. K. 1975. Experimental evaluation of ecological dominance in a rocky intertidal
748 algal community. Ecological Monographs 45:137-159.
- 749 Duarte, C.M., et al. 2000. An experimental test of the occurrence of competition among SE
750 Asian seagrasses. Marine Ecology Progress Series 197: 231-240.
- 751 Ehrenfeld, J. G. 1990. Dynamics and processes of barrier-island vegetation. Reviews in
752 Aquatic Sciences 2: 437-480.
- 753 Ellison, A. M., and E. J. Farnsworth. 2001. Mangrove Communities. Pages 424-442. In
754 Marine Community Ecology. M. D. Bertness, S. D. Gaines and M. E. Hay, editors.
755 Sinauer Associates, Sunderland, Massachusetts, USA.

- 756 Ellison, A. M., et al. 2005. Loss of foundation species: consequences for the structure and
757 dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* 9: 479-486.
- 758 Estes, J. A., and J. F. Palmisano. 1974. Sea otters: Their role in structuring nearshore
759 communities. *Science* 185: 1058-1060.
- 760 Everard, M., L. Jones, and B. Watts. 2010. Have we neglected the societal importance of
761 sand dunes? An ecosystem services perspective. *Aquatic Conservation: Marine and*
762 *Freshwater Ecosystems* 20: 476-487.
- 763 Fenu, G., et al. 2013. Environmental Factors Influencing Coastal Vegetation Pattern: New
764 Insights from the Mediterranean Basin. *Folia Geobotanica* 48:493-508.
- 765 Franks, S. J. 2003. Competitive and facilitative interactions within and between two species
766 of coastal dune perennials. *Canadian Journal of Botany* 81: 330-337.
- 767 Franks, S. J. 2003. Facilitation in multiple life-history stages: evidence for nucleated
768 succession in coastal dunes. *Plant Ecology* 168: 1-11.
- 769 Franks, S. J., and C. J. Peterson. 2003. Burial disturbance leads to facilitation among coastal
770 dune plants. *Plant Ecology* 168: 13-21.
- 771 Gemma, J. N., R. E. Koske, and M. Carreiro. 1989. Seasonal dynamics of selected species of
772 V-A mycorrhizal fungi in a sand dune. *Mycological Research* 92: 317-321.
- 773 He, Q., and M. D. Bertness. 2014. Extreme stresses, niches, and positive species interactions
774 along stress gradients. *Ecology* 95: 1437-1443.
- 775 Hesp, P. A. 1991. Ecological processes and plant adaptations on coastal dunes. *Journal of Arid*
776 *Environments* 21: 165-191.

- 777 Hughes, T. P., and J. B. C. Jackson. 1985. Population dynamics and life histories of
778 foliaceous corals. *Ecological Monographs* 55: 141-166.
- 779 Huntzinger, M., R. Karban, and J. H. Cushman. 2008. Negative effects of vertebrate
780 herbivores on invertebrates in a coastal dune community. *Ecology* 89: 1972-1980.
- 781 Isermann, M. 2011. Patterns in Species Diversity during Succession of Coastal Dunes.
782 *Journal of Coastal Research* 27: 661-671.
- 783 Jones, C. G., J. H. Lawton, and M. Shachak. 1994. Organisms as ecosystem engineers. *Oikos*
784 69: 373-386.
- 785 Lewis, J. R. 1964. *The Ecology of Rocky Shores*. English Universities Press, London, UK.
- 786 Lichter, J. 1998. Primary Succession and Forest Development on Coastal Lake Michigan
787 Sand Dunes. *Ecological Monographs* 68: 487-510.
- 788 Lichter, J. 2000. Colonization constraints during primary succession on coastal Lake
789 Michigan sand dunes. *Journal of Ecology* 88: 825–839.
- 790 Lortie, C. J., and J. H. Cushman. 2007. Effects of a directional abiotic gradient on plant
791 community dynamics and invasion in a coastal dune system. *Journal of Ecology* 95: 468-
792 481.
- 793 Mack, R. N., and J. L. Harper. 1977. Interference in dune annuals: spatial pattern and
794 neighbourhood effects. *Journal of Ecology* 65: 345-363.
- 795 Maestre, F. T., et al. 2009. Refining the stress-gradient hypothesis for competition and
796 facilitation in plant communities. *Journal of Ecology* 97: 199-205.
- 797 Mann, K. H. 1982. *Ecology of Coastal Waters: A Systems Approach*. University of
798 California Press.

- 799 Martinez, M. L. 2003. Facilitation of seedling establishment by an endemic shrub in tropical
800 coastal sand dunes. *Plant Ecology* 168: 333-345.
- 801 Maun, M. A. and Lapierre, J. 1986. Effects of burial of sand on seed germination and
802 seedling emergence of four dune species. *American Journal of Botany* 73: 450-455.
- 803 Maun, M. A. 1994. Adaptation enhancing survival and establishment of seedlings on coastal
804 dune systems. *Vegetatio* 111: 59-70.
- 805 Maun, M. A. 1998. Adaptations of plants to burial in coastal sand dunes. *Canadian Journal of*
806 *Botany* 76: 713-73.
- 807 Maun, M. A., and J. Perumal. 1999. Zonation of vegetation on lacustrine coastal dunes:
808 effects of burial by sand. *Ecology Letters* 2: 14-18.
- 809 Miller, T. E., E. S. Gornish and H. L. Buckley. 2010. Climate and coastal dune vegetation:
810 disturbance, recovery, and succession. *Plant Ecology* 206: 97-104.
- 811 Morin, P. J. 2011. *Community Ecology*, Second Edition. Wiley Blackwell, Oxford, UK.
- 812 Odum, E. P. 1953. *Fundamentals of Ecology*. W. B. Saunders Company, Philadelphia, USA.
- 813 Olff, H., J. Huisman, and B. F. Van Tooren. 1993. Species dynamics and nutrient
814 accumulation during early primary succession in coastal sand dunes. *Journal of Ecology*
815 81: 693-706.
- 816 Oosting, H. J., and W. D. Billings. 1942. Factors Effecting Vegetational Zonation on Coastal
817 Dunes. *Ecology* 23: 131-142.
- 818 Paine, R. T., and S. A. Levin 1981. Intertidal Landscapes: Disturbance and the Dynamics of
819 Pattern. *Ecological Monographs* 51: 145-178.

- 820 Pennings, S. C., and R. M. Callaway. 1996. Impact of a Parasitic Plant on the Structure and
821 Dynamics of Salt Marsh Vegetation. *Ecology* 77: 1410-1419.
- 822 Platt, W. J. 1975. The Colonization and Formation of Equilibrium Plant Species Associations
823 on Badger Disturbances in a Tall-Grass Prairie. *Ecological Monographs* 45: 285-305.
- 824 Prisco, I., A. T. R. Acosta, and S. Ercole. 2012. An overview of the Italian coastal dune EU
825 habitats. *Annali di Botanica* 2: 39-48.
- 826 Ranwell, D. S. 1958. Movement of vegetated sand dunes at Newborough Warren, Anglesey.
827 *Journal of Ecology* 46: 83-100.
- 828 Sousa, W. P. 1979. Disturbance in Marine Intertidal Boulder Fields: The Nonequilibrium
829 Maintenance of Species Diversity. *Ecology* 60: 1225-1239.
- 830 Underwood, A. J. 1997. Experiments in ecology: their logical design and interpretation using
831 analysis of variance. Cambridge University Press.
- 832 van der Valk, A. G. 1974. Environmental factors controlling the distribution of forbs on
833 coastal foredunes in Cape Hatteras National Seashore. *Canadian Journal of Botany* 52:
834 1057-1073.

Figure 1

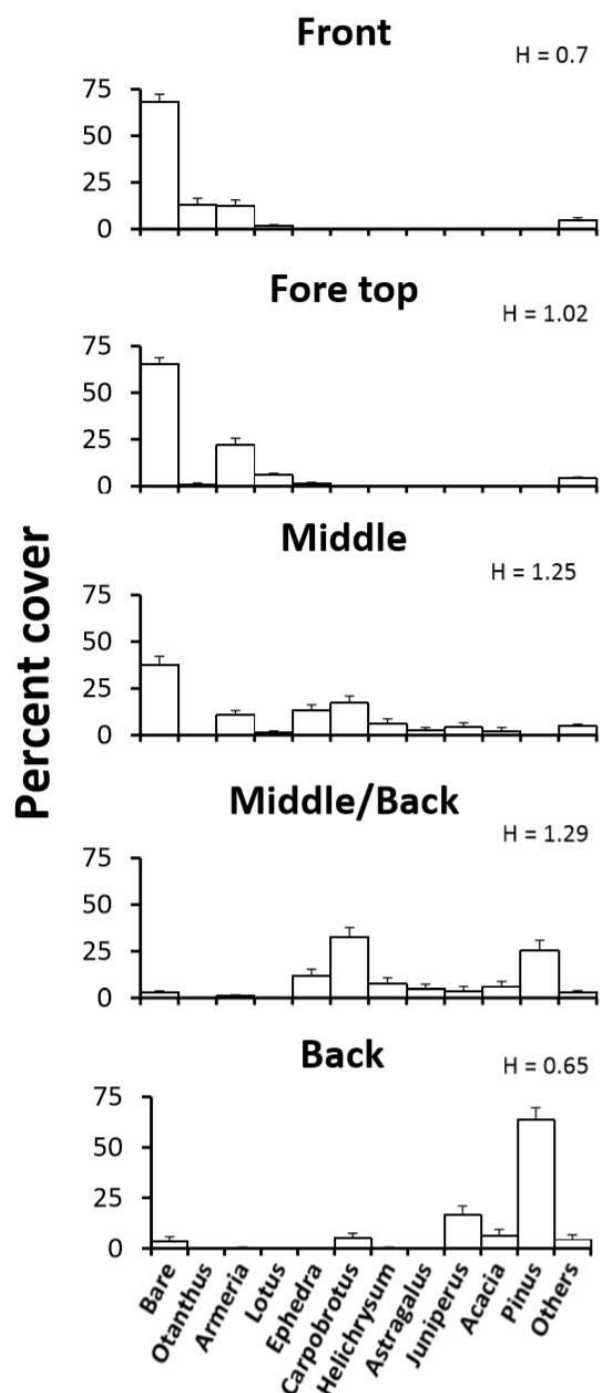


FIG. 1. Plant zonation (mean percent cover + SE) at the Badesi dune. Transects parallel to the shoreline were sampled at 2 m intervals with 0.5x0.5 m quadrats (60/zone) to estimate percent cover. H is the Shannon diversity index.

Figure 2

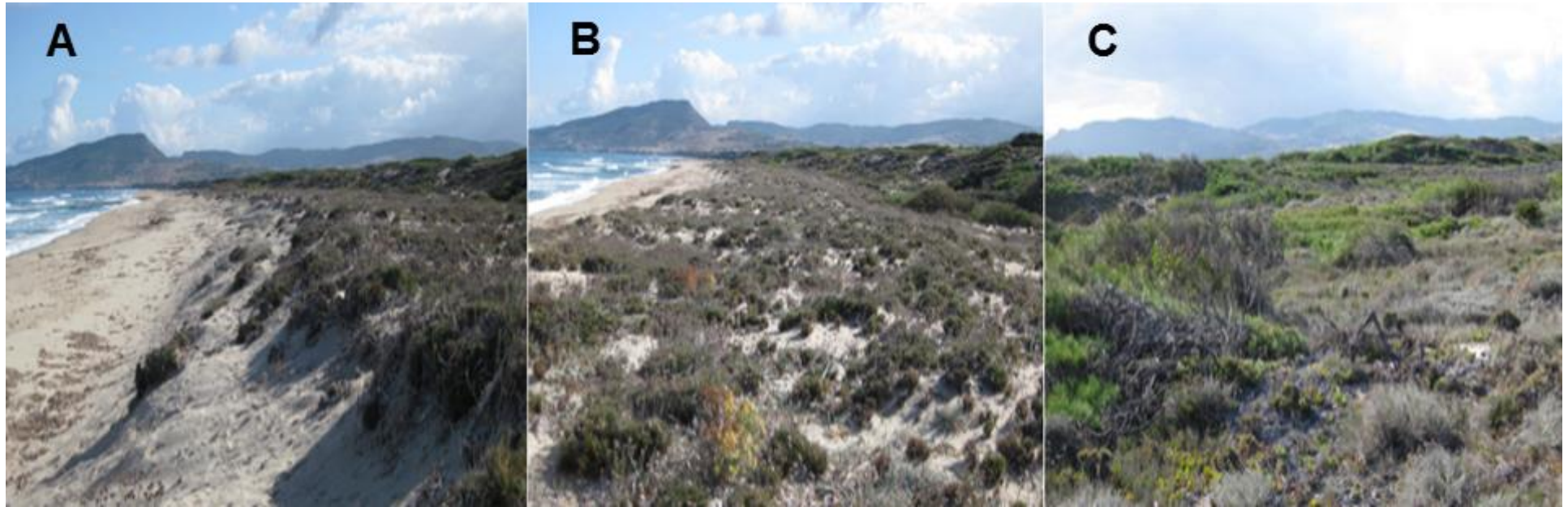


FIG. 2. Photograph of the dune zones at Badesi (Sardinia, Italy). (A) fore dune, (B) middle dune and (C) back dune.

Figure 3

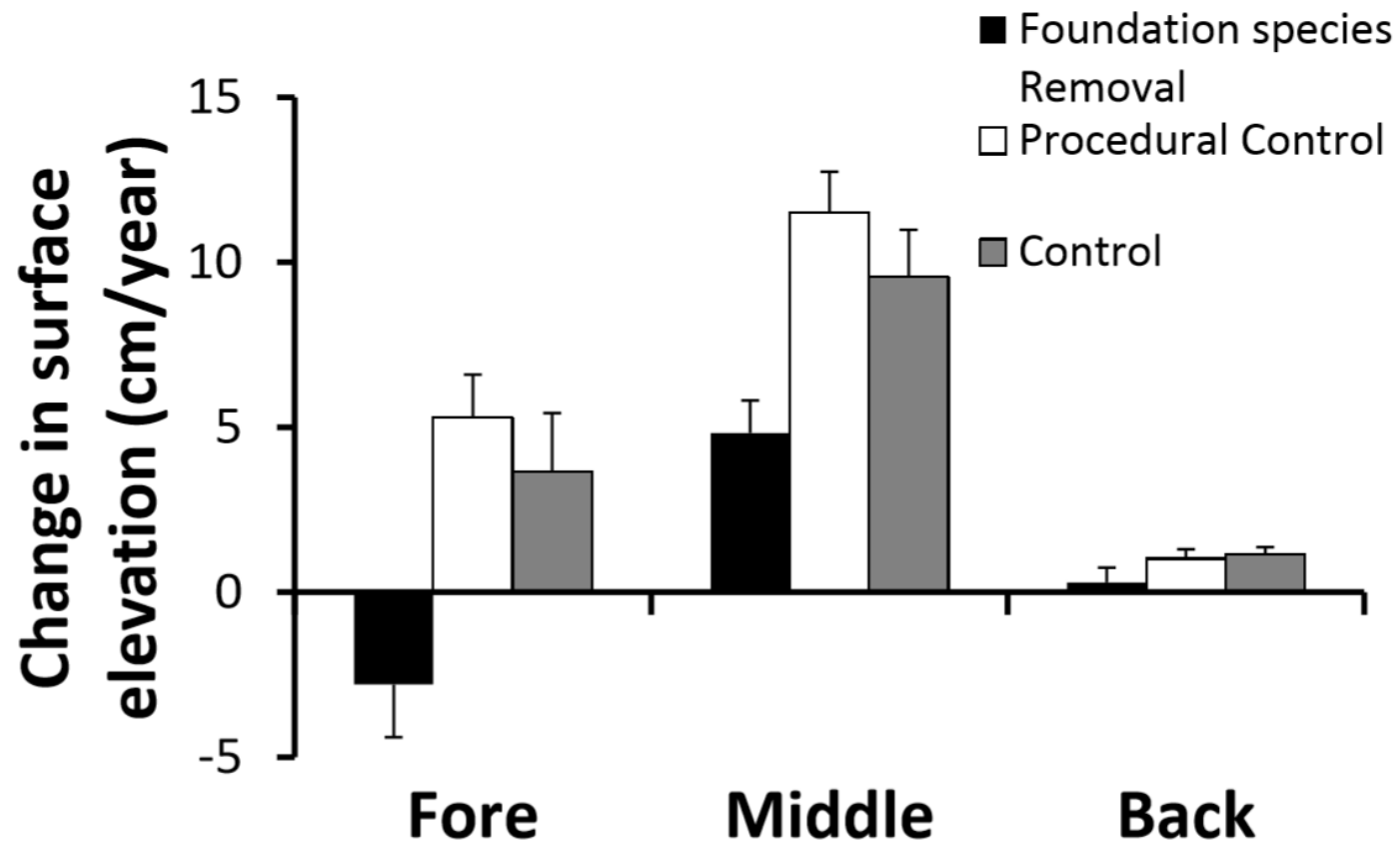


FIG. 3. Surface elevation or sand erosion/deposition balance (mean + SE) of the foundation plant species removal experiment in the three dune zones.

Figure 4

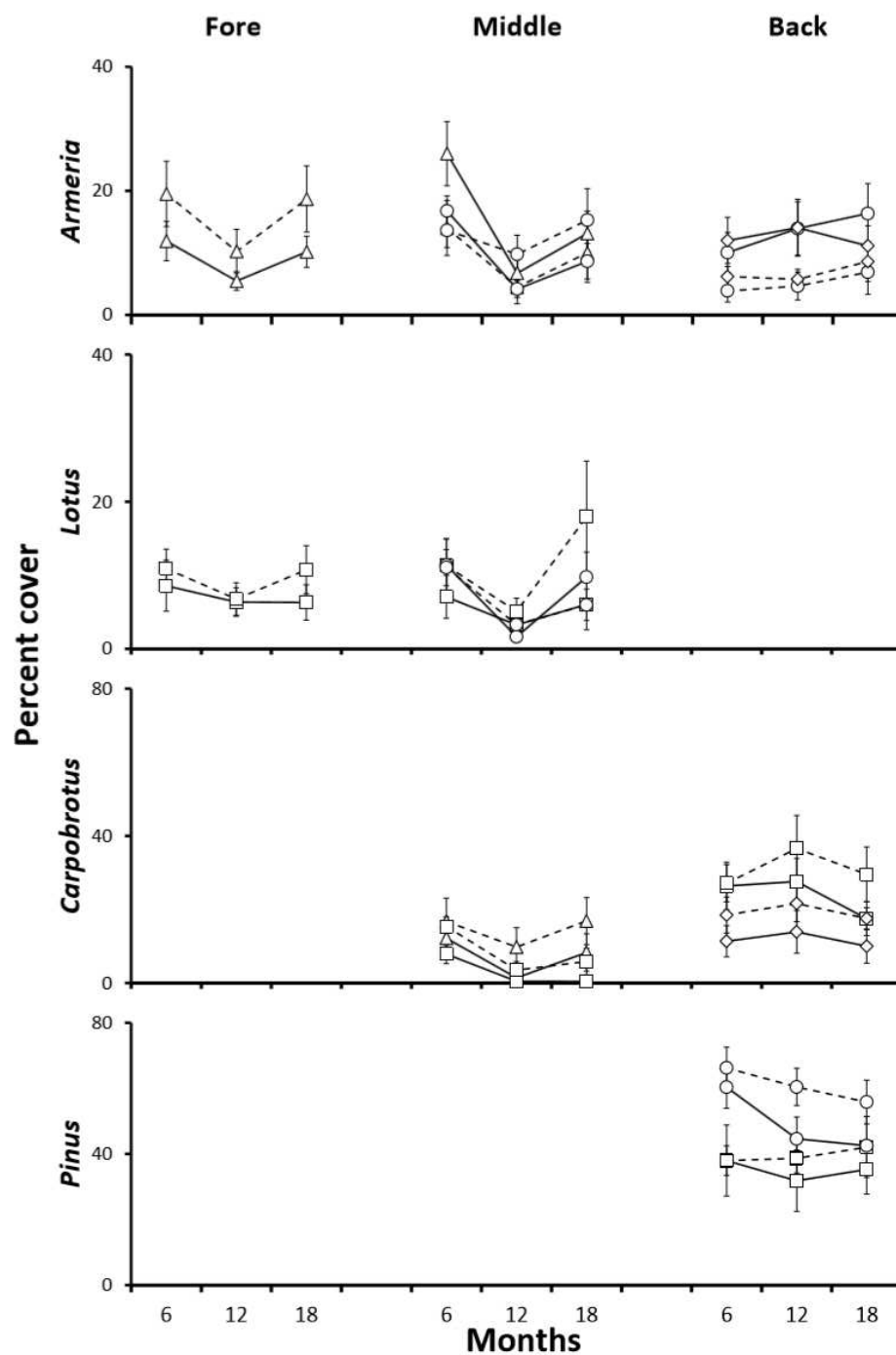


FIG. 4. Reciprocal species removal: *Armeria* (□), *Lotus* (△), *Carpobrotus* (○), *Pinus* (◇), Species control (—), Reciprocal species removal (- - -). Results of pair-wise reciprocal species removal experiments after 18 months in the fore, middle and back dune.

Figure 5

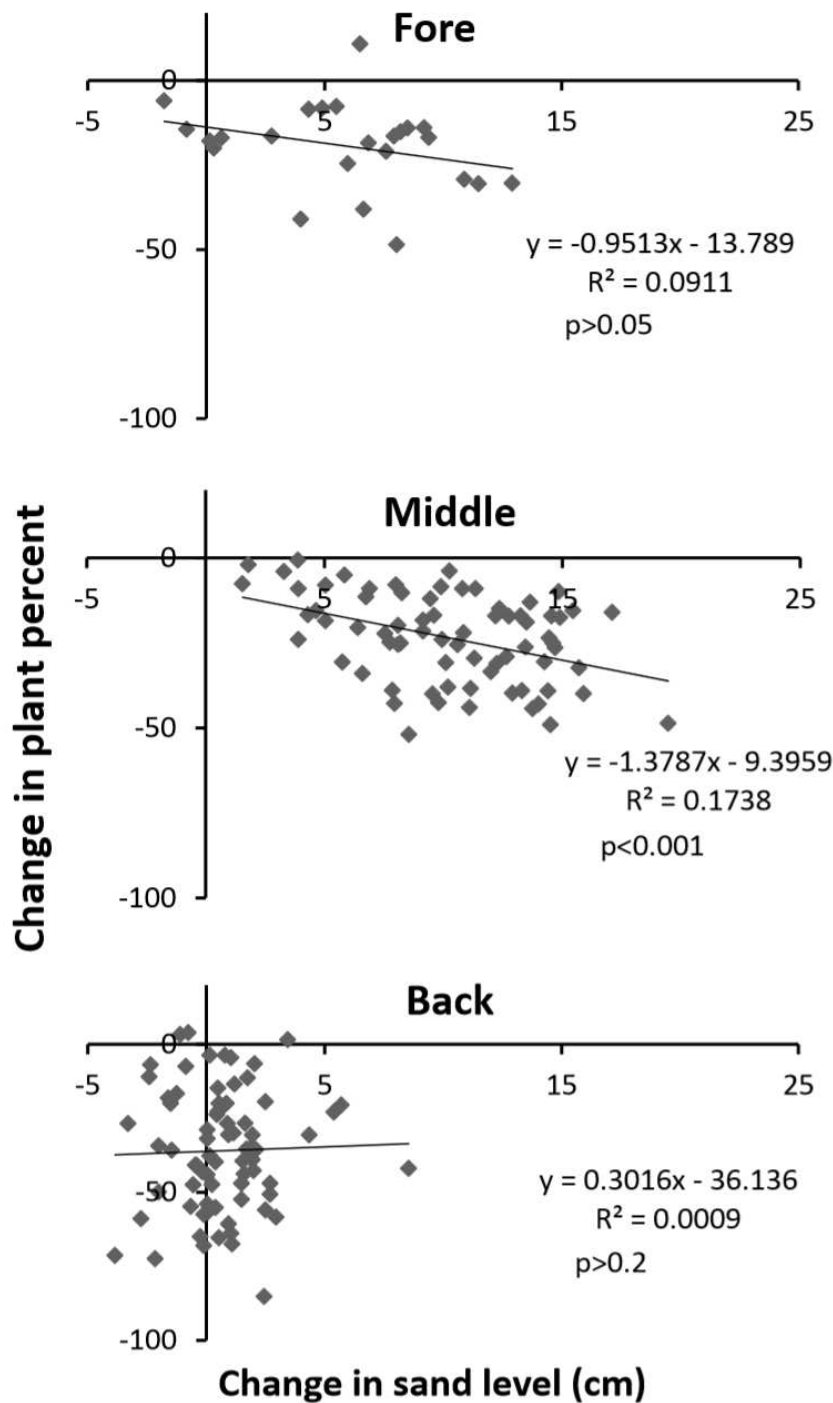


FIG. 5. Relationship between sand burial and percent plant cover in the pair-wise reciprocal species removal experiments after 12 months in the fore, middle and back dune.

Figure 6

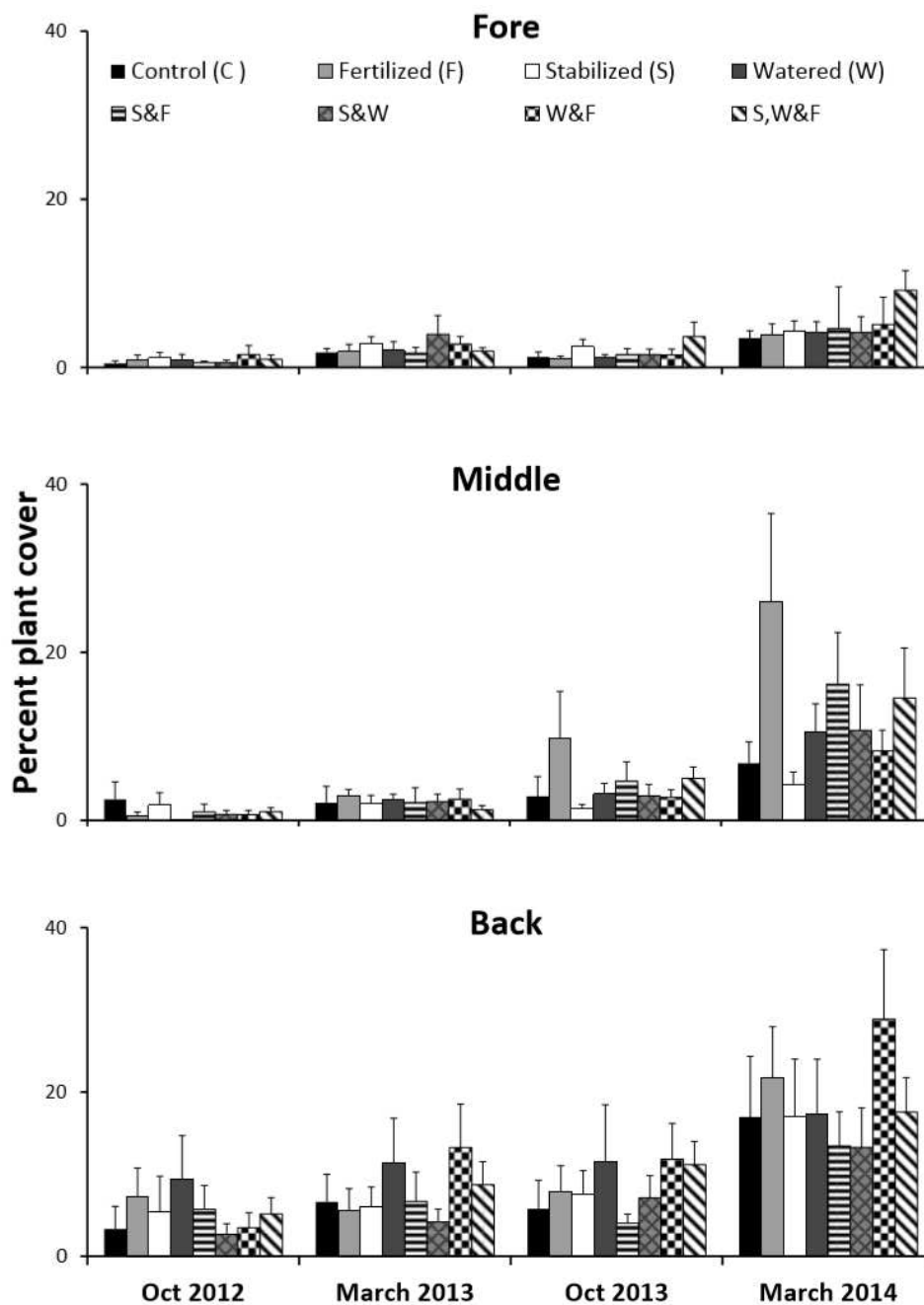


FIG. 6. Physical stress alleviation experiment. Percent plant cover (mean + SE) of the fully factorial manipulation of water availability, nutrient availability and substrate stability during 2 years in the fore, middle and back dune. No single or combination of stress alleviation treatments affected plant cover, but higher recovery occurred in back zone.

Figure 7

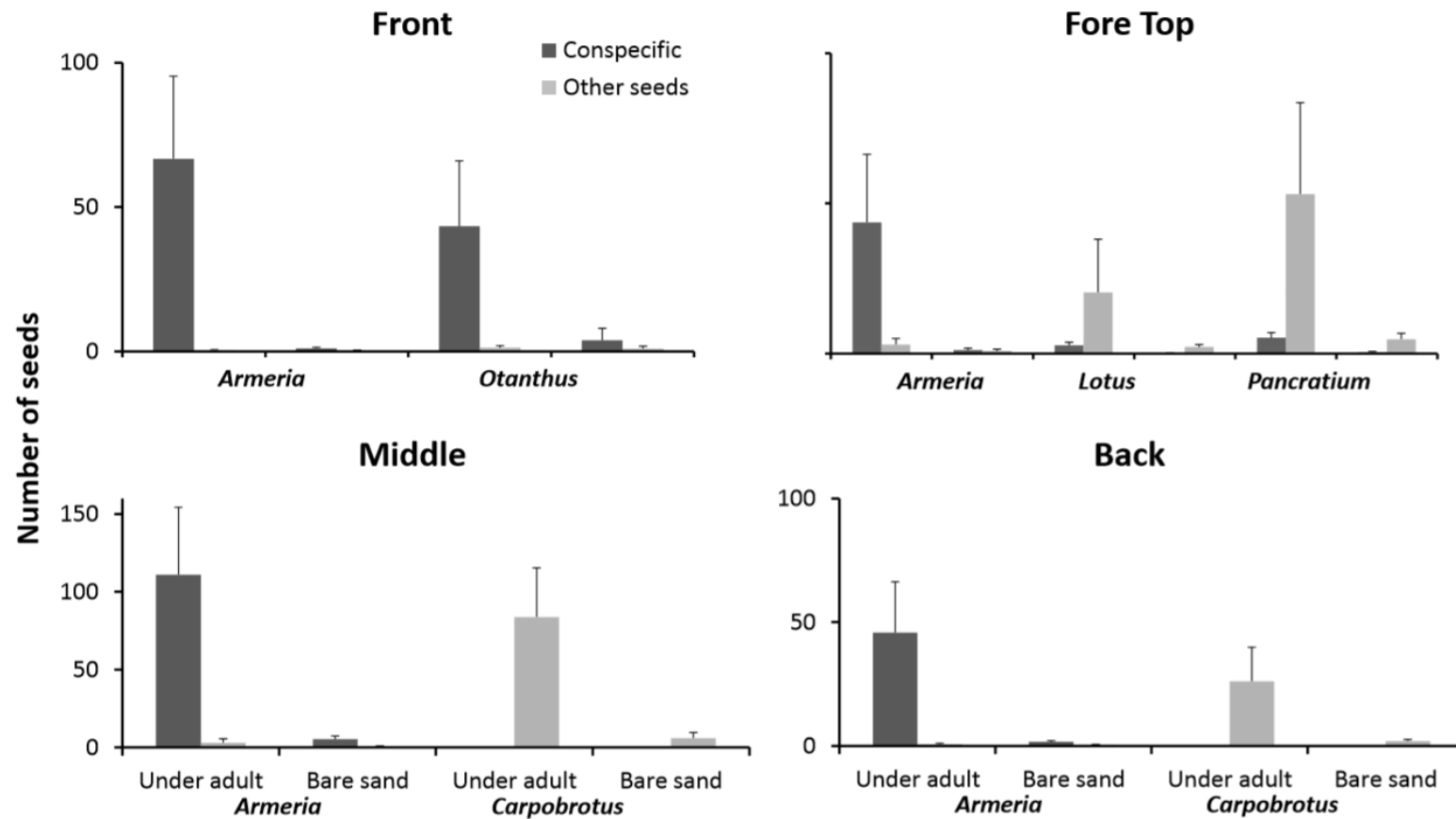


FIG. 7. Seeds distribution (mean + SE) of common dune plants across zones under adult plants and in adjacent unvegetated sand. Data are given separately for conspecific and non-conspecific seeds.

Figure 8

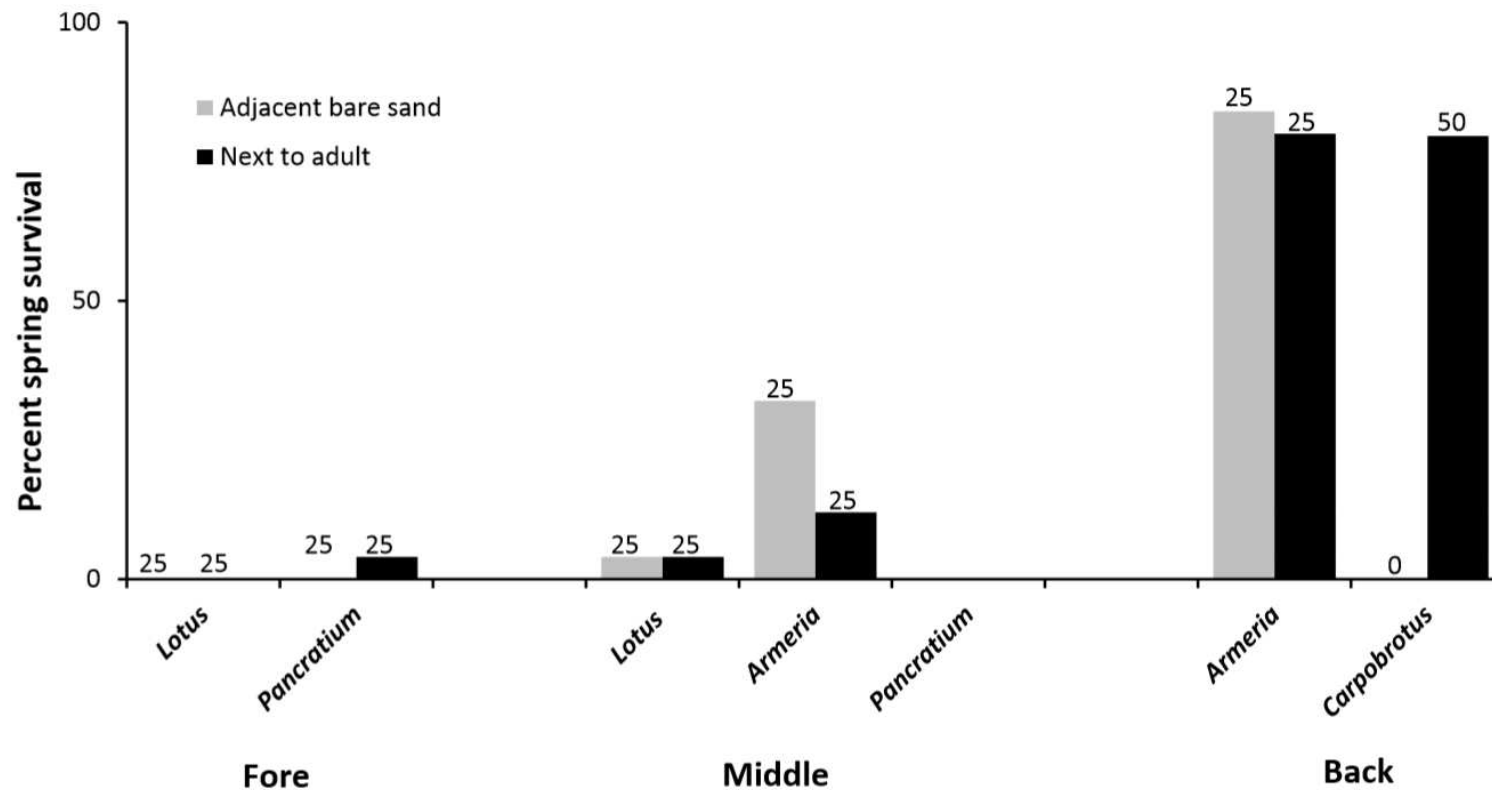


FIG. 8. Survivorship of natural seedlings marked in October 2013. For each zone and species, 25 seedlings were marked associated with conspecific adults and 25 seedlings were marked in bare substrate >30 cm from adult vegetation. Ice plant seedlings were only found under adult ice plants, so all 50 marked ice plant seedlings were with conspecifics. Data are percent of seedlings survived after 3 seasons.

Figure 9

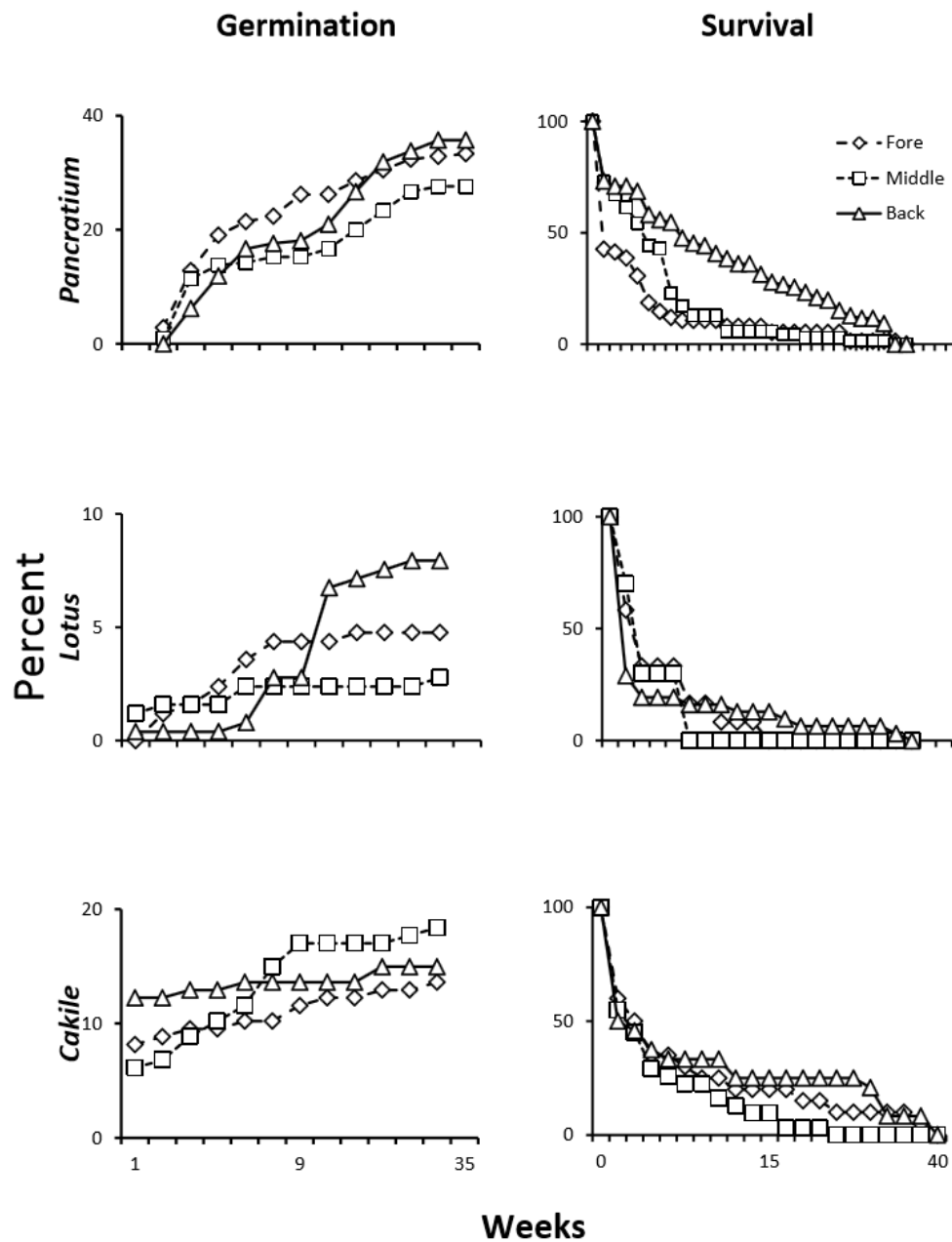


FIG. 9. Results of the seed transplant experiment where germination permeable net bags of locally collected seeds ($n=21/\text{zone}/\text{species}$) from dehiscing seed heads were transplanted to the fore, middle and back dune with and without plant neighbors and scored for germination and seedling survivorship. Data are percent survivorship and germination of the total potential.

Figure 10

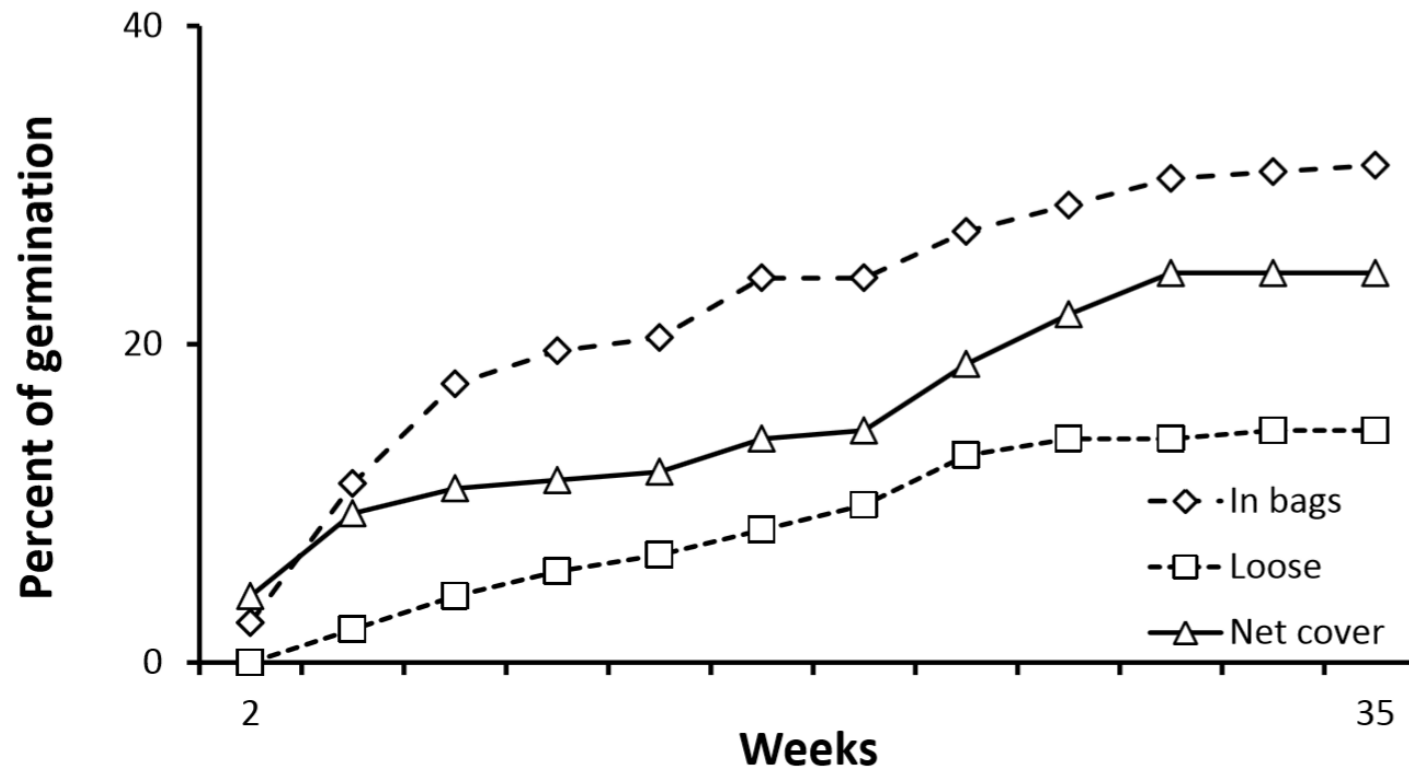


FIG. 10. Seeds erosion experiment. Germination in the fore dune where *Pancratium* seeds were transplanted in germination permeable net bags, loose in the soil and loose in the soil but covered at the surface with nylon mesh to limit erosion (n=8 treatment). Data are percent germination of the total potential.

Figure 11

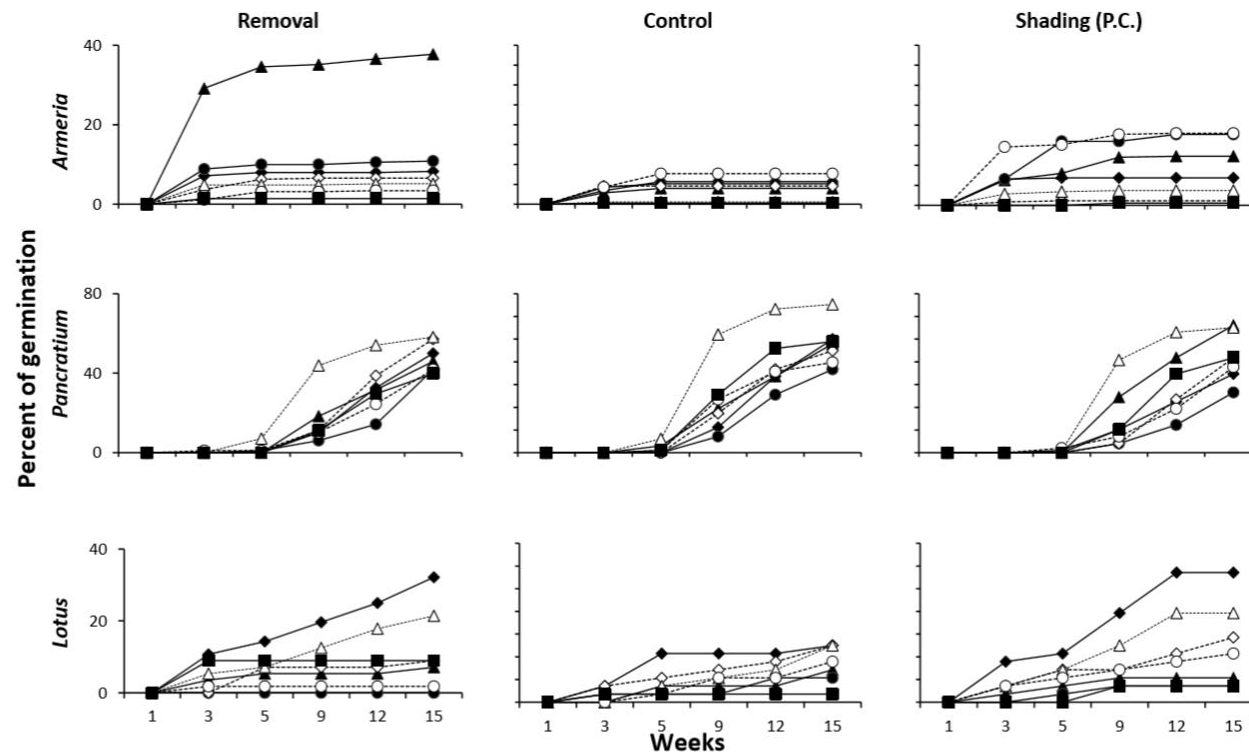


FIG. 11. Back dune competitive release experiment. Germination of *Armeria*, *Pancratium* and *Lotus* seeds sowed in different soils: *Pinus* soil (◆), middle dune soil (▲), *Carpobrotus* soil (●), potting with *Pinus* needles (◇), potting with middle dune soil (△), potting with *Carpobrotus* soil (○) and field (■) (no canopy), Control (under canopy) and Procedural controls (*Pinus* removal with shade cloth to simulate canopy shading, P.C.). N=8/treatment. Data are percent of seeds germinated out of the total potential.

Figure 12

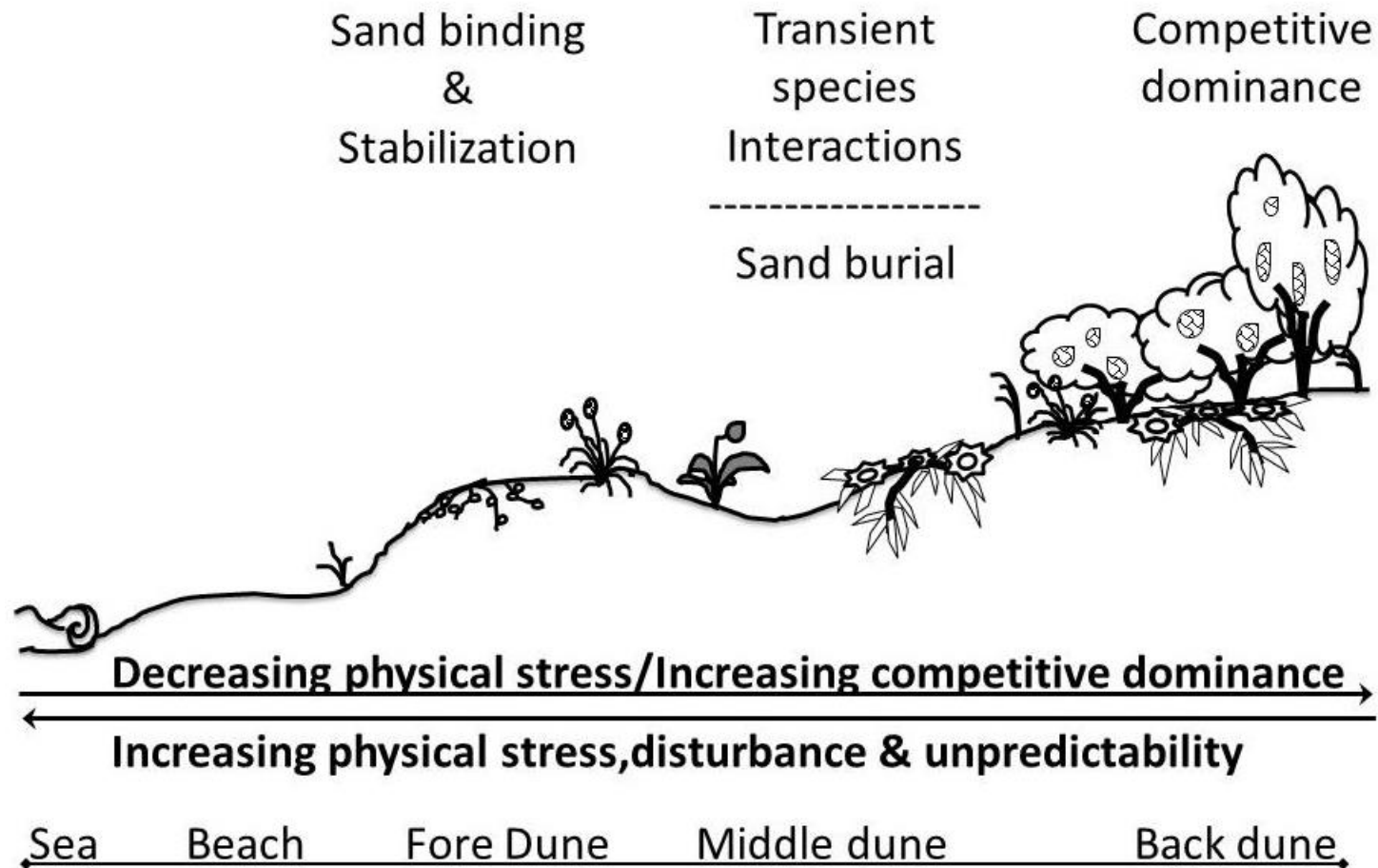


FIG. 12. Conceptual model of the assemble rules of the Badesi sand dune plant community.