



University of Sassari
Ph.D. School in Natural Sciences and Environmental resources
Via Piandanna 4, 07100 Sassari, Italy

*Dissertation for the Degree of Doctor of Philosophy in Environmental Biology
presented at Sassari University in 2015*

XXVIII cycle

***“Structure, organization and dynamics of a
Sardinian sand dune plant community”***

Candidate:

Valentina Cusseddu

Director of the School:

Prof. Marco Curini Galletti

Tutor:

Dr. Giulia Ceccherelli

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Prof. Mark D. Bertness



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To my island

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SUMMARY (English and Italian)

Coastal sand dunes have attracted the attention of plant ecologists for over a century, but have largely relied on correlations to explain striking 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 over 3 years led to erosion and habitat loss on the fore dune and limited plant recovery that was enhanced 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 and high summer temperatures in the back 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, competitive dominance and allelopathy in the back dune.

Key words: community assembly; community structure; disturbance; facilitation; hierarchical organization; plant competition; shoreline ecology.

Le dune costiere sabbiose hanno attirato l'attenzione degli ecologi vegetali per oltre un secolo, ma maggiormente riguardo alla correlazione per spiegare l'aspetto suggestivo dell'organizzazione di comunità delle piante dunali. Noi abbiamo esaminato sperimentalmente le ipotesi a lungo termine che l'intrappolamento del sedimento, le interazioni interspecifiche, i fattori abiotici e il reclutamento dei germogli siano fattori guida della struttura della comunità vegetale di dune sabbiose in Sardegna, Italia. La rimozione di specie fondatrici dalle zone frontale, intermedia e retrostante della duna nel corso di 3 anni ha portato all'erosione e alla perdita di habitat nella zona del fronte della duna e ha limitato il recupero della vegetazione che aumenta col progredire della duna. La rimozione reciproca di specie in tutte le zone ha suggerito che la competizione interspecifica è comune, ma questa proprietà è transitoria, in particolar modo a causa del disturbo dovuto all'insabbiamento nella zona intermedia della duna e alle alte temperature estive nel retro della duna. Un esperimento biennale di tipo manipolativo a fattori ortogonali su acqua, disponibilità dei nutrienti e stabilità del substrato ha restituito risultati non significativi relativamente a questi fattori abiotici, in ogni zona della duna. Nelle zone frontale e intermedia della duna, i semi vengono intrappolati sotto le piante adulte durante la germinazione, e, generalmente, la sopravvivenza e la crescita dei germogli aumenta al progredire della duna nonostante l'aumento dell'erbivoria nel retro della duna. La perdita della sabbia e dei semi porta a una ridotta disponibilità dei semi sul fronte della duna mentre le elevate temperature estive e l'allelotopia determinano la dominanza competitiva di piante legnose nella duna retrostante. I nostri risultati suggeriscono che la comunità vegetale di dune sabbiose in Sardegna è organizzata gerarchicamente, strutturata da specie fondatrici che trattengono la sabbia sul fronte della duna, dall'insabbiamento nella zona intermedia della duna e da germinazione maggiormente affermata, crescita, dominanza competitiva e allelotopia nel retro della duna.

Parole chiave: assemblaggio di comunità; struttura di comunità; disturbi; facilitazione; organizzazione gerarchica; competizione tra piante; ecologia costiera.

GENERAL INTRODUCTION

Understanding the biotic and abiotic mechanisms that generate spatial patterns in natural communities is a major goal of ecology and is critical for developing ecology into a predictive science that can inform ecosystem management and contribute to conservation (Morin 2011). Many natural communities are structured and defined by foundation species, *sensu* Dayton (1975). Foundation species are operationally defined as common, abundant species that build and maintain habitats, ameliorating potentially limiting physical and biological stressors, thus providing habitat for other species (Wilson and Agnew 1992, Jones et al. 1994, Bruno and Bertness 2001, Ellison et al. 2005, Angelini et al. 2011). Examples of foundation species-dependent ecosystems include forests, coral reefs, salt marshes, mangroves, and mussel and oyster reefs, which are all built and maintained by numerically dominant habitat forming foundation species (Bruno and Bertness 2001). Amelioration of potentially limiting physical and/or biotic conditions is a hallmark of foundation species-based ecosystems. Coastal sand dunes are physically harsh habitats for the halophytic plants that dominate them and are also best understood as foundation species-based ecosystems.

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 (Everard et al. 2010, Barbier et al. 2013). The provisioning of these services relies on targeted and informed management practices in the face of increasing human threats. Sand dunes, however, are generally not appreciated as conservation priorities and are not managed to protect their ecosystem services. Globally, shoreline sand dune

communities are a low conservation and management priority (Ehrenfeld 1990, Everard et al. 2010).

In addition to the lack of appreciation of the ecological importance of sand dune communities, most research to date on sand dune community organization has been descriptive and relies heavily on dated correlative literature (e.g. Cowles 1899, Oosting and Billings 1942, Mack and Harper 1977). Experimental work on sand dune plants has been largely restricted to comparisons among dune chronosequences (Lichter 2000), small scale sand burial, seed dispersal and disturbance studies (Maun and Perumal 1999, Franks and Peterson 2003, Miller et al. 2010), field studies of small annuals, nurse plant effects and comparisons among chronosequences as a proxy for successional shifts in plant species interactions over time (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 other 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 and services of sand dune communities have not been rigorously tested. Thus, in spite of their importance, major assumptions about the mechanisms generating the structure and biodiversity of sand dune communities remain untested.

Understanding the biotic and abiotic processes that generate and maintain the structure and function of ecosystems well enough to predict and anticipate the consequences of human activities on ecosystems is one of the major challenges for contemporary ecology. If ecology

can develop into a predictive science, it can play a more critical role in the conservation, management, and restoration of ecosystems and the services they provide.

In this paper we examine the basic 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 sand dune stress gradients. Many of these patterns were initially theorized in classic descriptive and correlative work beginning over a century ago (Cowles 1899, Oosting and Billings 1942, Mack and Harper 1977), but have never been rigorously tested experimentally. We present the results of field experiments and mensurative studies examining the roles that plant sand binding, interspecific competition and facilitation, abiotic conditions and seedling recruitment play in the structure and organization of Sardinian sand dune plant communities.

We take a hypothesis driven experimental approach to elucidate the community assembly of a sand dune plant community by examining the general 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 patterns, and 3) at high dune elevations, reduced physical stresses lead to increased plant abundance and interspecific competitive dominance and displacement. As the first experimentally based analysis of the general assembly rules of a sand dune plant community, our results support a hierarchical model for sand dune plant community organization and confirm many of the conclusions of early work on the organization of these communities based solely on correlative studies.

CHAPTER 1: Study site and its features

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1.1 Study site

Our study was carried out on the Badesi dunes ($40^{\circ} 56' 45.571''$ N, $8^{\circ} 49' 41.048''$ E) on the North coast of Sardinia, in the Mediterranean Sea (Fig. 1).



FIG. 1. Study area. The star indicates the location of the coastal zone. A satellite photograph of the dune system with its surrounding area is framed.

It is a wide dune system surrounding the mouth of Coghinas River. It has a similar plant community to other dune systems in Sardinia (Cusseddu personal observations). The "Mouths of the Coghinas" is a Site of Community Importance (SIC ITB010004) recognized at the European level, and covers approximately thirteen kilometers of coastline between the towns of Valledoria and Trinità d'Agultu. The dunes associated with the Coghinas River are

almost three kilometers long overlooking the Asinara Gulf, with an average width of approximately five hundred meters. Sardinia has a warm temperate Mediterranean climate with hot, dry summers and cooler, wet winters. As in other habitats with Mediterranean climates, most plant growth and reproduction occurs during the cooler winter months (Blondel and Aronson 1999). Descriptive studies of sand dune plant communities reveal Sardinia has relatively high species richness, island endemism (Médail and Quézel 1999, Bacchetta et al. 2008, Prisco et al. 2012) and adaption to physical stress (Fenu et al. 2012, Fenu et al. 2013).

1.1.1 Zonation survey

To quantify the plant distribution patterns across the Badesi dune system, we surveyed transects in each distinct plant zone and used surveying equipment to quantify the elevation of the plant zone transitions. 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 species 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 major vegetation zones we will also refer to the front of the fore dune, or area on the seaward border of the fore dune where embryo dunes (Cowles 1899) form as well as the transition areas between major vegetation zones. The objective of our site survey was to develop a comprehensive description of the plant community at the study site. We quantified the elevation of each zone and zonal boundaries at 2 m intervals along four randomly positioned 50 m transects perpendicular to the shoreline with an auto-level and stadia rod to measure the width of each zone. We determined the absolute tidal height of each zone relative to the elevation of a standard benchmark reference elevation at the water edge calculated from local tide tables.

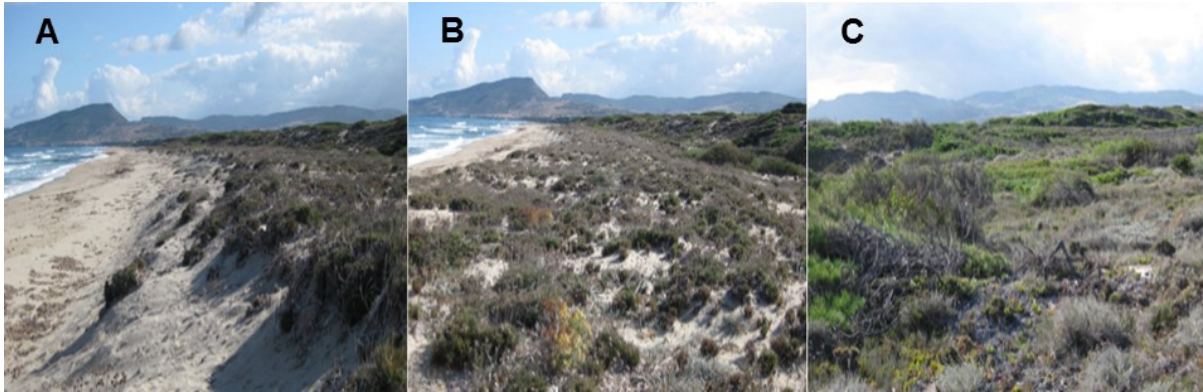


FIG. 2. Badesi dune (Sardinia, Italy). (A) fore dune, (B) middle dune and (C) back dune. Note the steep fore dune face and the decrease in unvegetated bare space with increasing dune elevation.

To quantify the plant zonation, we quantified vegetation along 120 m transects parallel to the water in each major dune zone: the embryonic fore dune closest to the water, 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 random transect line parallel to the shoreline and every 2 meters 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 in each zone. To characterize species diversity in the community, for each zone (front, fore top, middle, middle/back and back) we calculated the Shannon diversity index (H). All plant species, after introduced in the text, will be referred by genus names.

In addition to shoreline vertical zonation, one of the most conspicuous features of the plants on the fore and middle dunes were that they generally occurred on elevated mounds of sand. Since sand in these zones is highly 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). To quantify adult plant effects on the deposition and/or erosion of sand we used an auto level and stadia rod to quantify the elevation of dominant 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, including plants with different morphologies (e.g. solitary arborescent and clonal prostrate), and measured the elevation of the sand at their base and of adjacent unvegetated sand 10-20 cm away. Plant and adjacent sand elevation differential data were calculated, Cochran's test was used to test for homogeneity of variances, and when necessary, data were transformed (Underwood 1997) and analyzed by species with a one-way ANOVA and by a zone x plant ANOVA for *Armeria*, the only species present in every zone, to evaluate the zonal differences.

1.1.2 Zonation survey results

Elevational zonation of plants across the Badesi dune is striking (Fig. 3 and 4). The fore dune has low plant richness and cover (35%) and 65% unvegetated sand cover. *Armeria pungens* (sea rose), *Otanthus maritimus* (cotton weed) and *Lotus cytisoides* (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. 4, 38%). *Armeria*, *Carpobrotus acinaciformis* (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. 3). 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* spp. (*Pinus halepensis* and *Pinus pinea*) on the terrestrial border of the dune (33%).

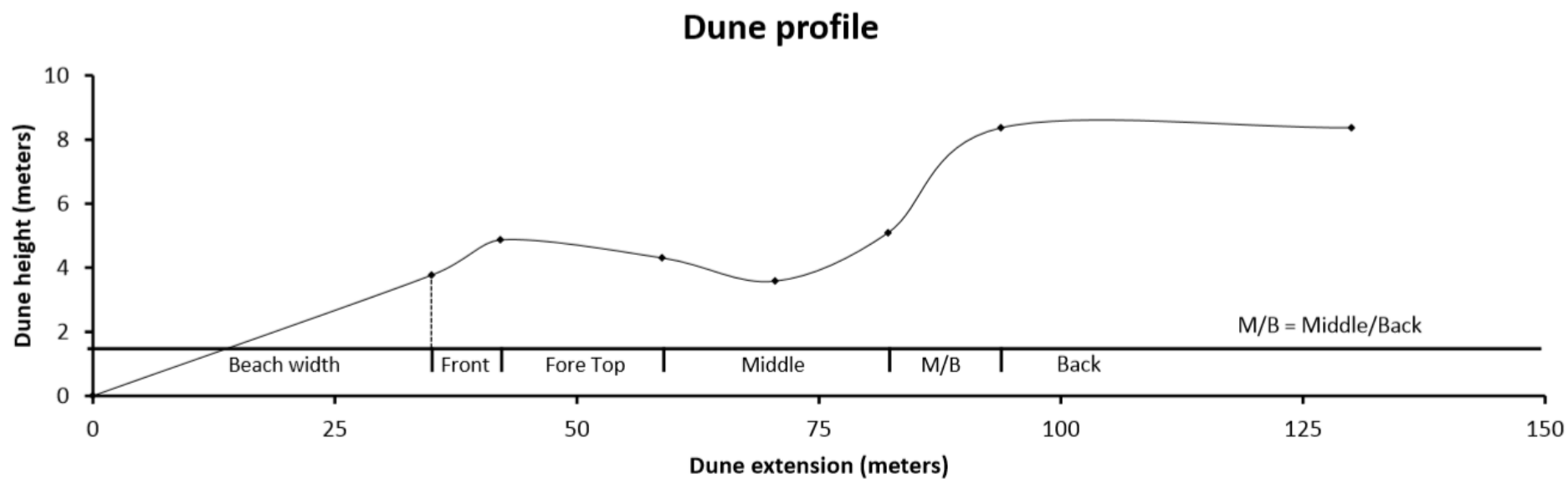


FIG. 3. Elevation and extent of vegetation zones of Badesi dune.

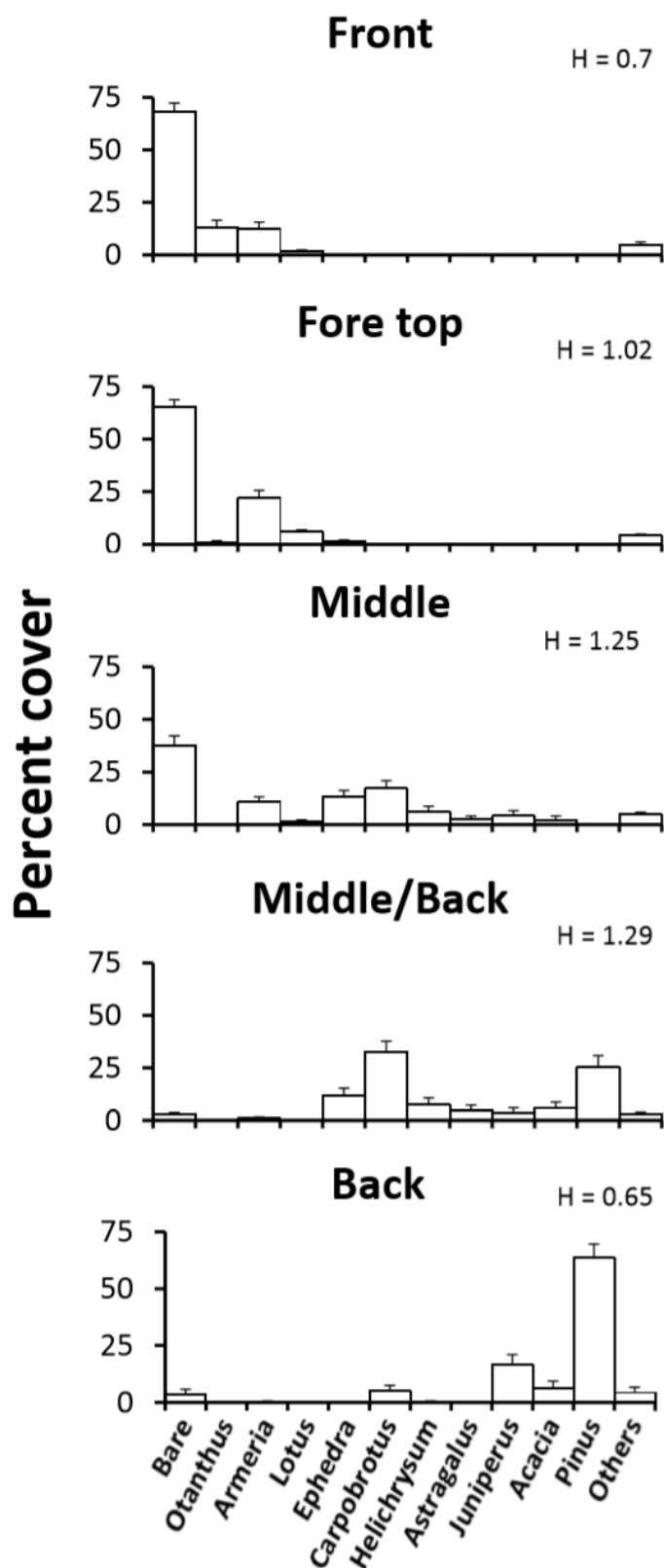


FIG. 4. Plant zonation (mean percent cover + SE) at the Badesi dune. Transects parallel to the shoreline were sampled at 2 m intervals with 0.5x0.5 m quadrats (60/zone) to estimate percent cover. H is the Shannon diversity index.

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At higher elevations of the back dune, however, *Pinus* dominates the landscape as a solitary evergreen species that has a prostrate morphology at lower elevations and an arborescent morphology at higher elevations (Fig. 5). The understory of the dense *Pinus* canopy that dominates high back dune elevations is bare substrate.



FIG. 5. Back dune with *Pinus* dominating the landscape.

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, hereafter) and 56 ± 2.51 cm higher than adjacent substrate, respectively. For the front dune, the one-way ANOVA didn't 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 run only for *Armeria* revealed a different capability of *Armeria* to bind sand by zone ($F_{3,76}=1111.36$ $p<0.0001$, 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 ($F_{4,95}=1.93$ $p>0.05$).

1.2 Discussion

Previous descriptive studies highlighted the importance of the coastal dune zonation in Mediterranean species conservation because species richness gets progressively higher across dune (Acosta et al. 2009). The severe environmental gradients across coastal dune systems is an important feature that determines the co-occurrence of different plant communities in a relatively small area (Barbour et al. 1985). Species richness and density at Badesi varied with

zonation, being more abundant moving inland, reaching its peak in the middle dune. A set of species shared by two or more coastal zones was evidenced at this site, because the vegetation of neighbour zones tends to be intermingled. Nevertheless, our results showed that substrate topography in relation to plants varied across the dune. Farthest from the seaside the vegetation was not associated with sand mounds, but approaching the coastline the capability of the plants of actively trap sand was higher, reaching its peak in the front dune, nearby the beach.

To effectively analysis the vegetation spatial and temporal variability of coastal dune environments it is essential the knowledge of the coastal floristic composition of the whole system. The difference among dune zones in species richness and composition highlights the importance of properly considering the variability at the local scale as a management priority to address regional conservation of vegetation. The deep knowledge of each dune species performance is fundamental for a successful conservation of these habitats, as the same species may have different dynamics and roles in different zones.

CHAPTER 2: Biogenic Communities, Foundation species and Hierarchical organization

Valentina Cusceddu

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2.1 Introduction

In this chapter we investigate how the habitat-forming foundation species (*sensu* Dayton 1972) can substantially affect environmental conditions in dune systems.

Recently, it has been suggested that foundation species-based ecosystems are commonly hierarchical, where the amelioration of potentially limiting stresses are responsible for ecosystem establishment and maintenance, but that other species interactions are often responsible for generating the most conspicuous, but superficial spatial patterns in these communities (Bruno and Bertness 2001, Bruno et al. 2003, Altieri et al. 2007, Angelini et al. 2011). While this model of community organization appears to be widespread (Wilson and Agnew 1992, Bruno and Bertness 2001), most evidence for hierarchical organization is anecdotal or correlative with few explicit experimental tests (for exceptions see Altieri et al. 2007, Angelini and Silliman 2014). This is the case in spite of the potential importance of hierarchical community organization to conservation and management strategies.

Several hierarchically organized communities are regulated by facilitative relationships. In these communities, the positive interaction mediated by a foundation species (e.g., mangroves, mussels, seagrasses) is the main interaction that allows the succeeding species to inhabit a given environment by reducing stress conditions, for example mitigating the tidal action, cooling the soil or oxygenating the habitat. The arrangement of the community within the habitat provided by a foundation species is also the result of competition, predation, disturbance, and recruitment variation.

We hypothesize firstly that in our community the vegetation traps the sediment especially in the fore dune, that is the zone of first colonization, improving the stability of the habitat and

allowing the establishment of other species, and secondary, that the recruitment is a factor that determines community development, strictly dependent on the action of the foundation species.

2.2 Material and methods

2.2.1 *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 in Sardinian dunes, we performed a foundation species removal experiment. In each of the 3 major dune zones in March of 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/treatment/dune zone). The four 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 left untouched. In plant removal replicates, plots were trenched to 30 cm around their perimeters 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. This herbicide is widely used in ecological research, has very localized effects if plots are trenched to avoid plant translocation of the herbicide outside of the target area, and we have used it successfully in the past in shoreline habitats (e.g. Bertness and Hacker 1994). No herbicide effects on plants outside herbicided plots were observed in our experiments. Procedural controls were trenched, but not herbicided.

Surface Elevation Table (SET) posts (Cahoon et al. 2000) modified for experimental replication were installed in the center of all plots to measure sand erosion/accretion as a

function of the presence/absence of foundation species and dune zone. A 2 cm diameter rebar rod was driven to refusal in the center of each plot, cut so it initially extended 10-15 cm above the sand surface and fitted with a 30 cm long horizontal PVC bar with four evenly spaced locations to measure the height of the sediment (see Brisson et al. 2014 for details). Elevations were taken in October and March of each year at the SET posts in all plots for three years. The height of the corner posts was also measured on these dates to supplement SET data and assess spatial patterns in sand erosion/accretion. Sediment height (the sand erosion/deposition balance) data were analyzed with a treatment x zone ANOVA followed by post-hoc testing.

In the spring of 2013, we began monitoring temperature after noting the apparent summer heat death of some high middle dune plants during the first year of this experiment. We deployed 24 thermistors (Econorma S.a.s. FT-800/System; Fig. 1) to plots of this first experiment (n=8/dune zone), attaching them under the canopy in control plots and pinning them to the surface with wire staples in plant removal plots. This allowed us to quantify plant heat exposure during the summer, considering differences between control and removal treatments. Thermistors were left in the field from the middle of June until the first week of September 2013 measuring temperature hourly. We 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 estimate the lowest and the highest daily temperature, respectively. These two temperature ranges allowed us to obtain mean minimum (Min) and mean maximum temperatures (Max) for each zone and treatment. Data were analyzed with a three-way ANOVA, considering the factors zone, temperature (Min vs Max), and treatment (removal vs control) as orthogonal and fixed. A one-way ANOVA was used to analyze mean maximum temperature by zone.

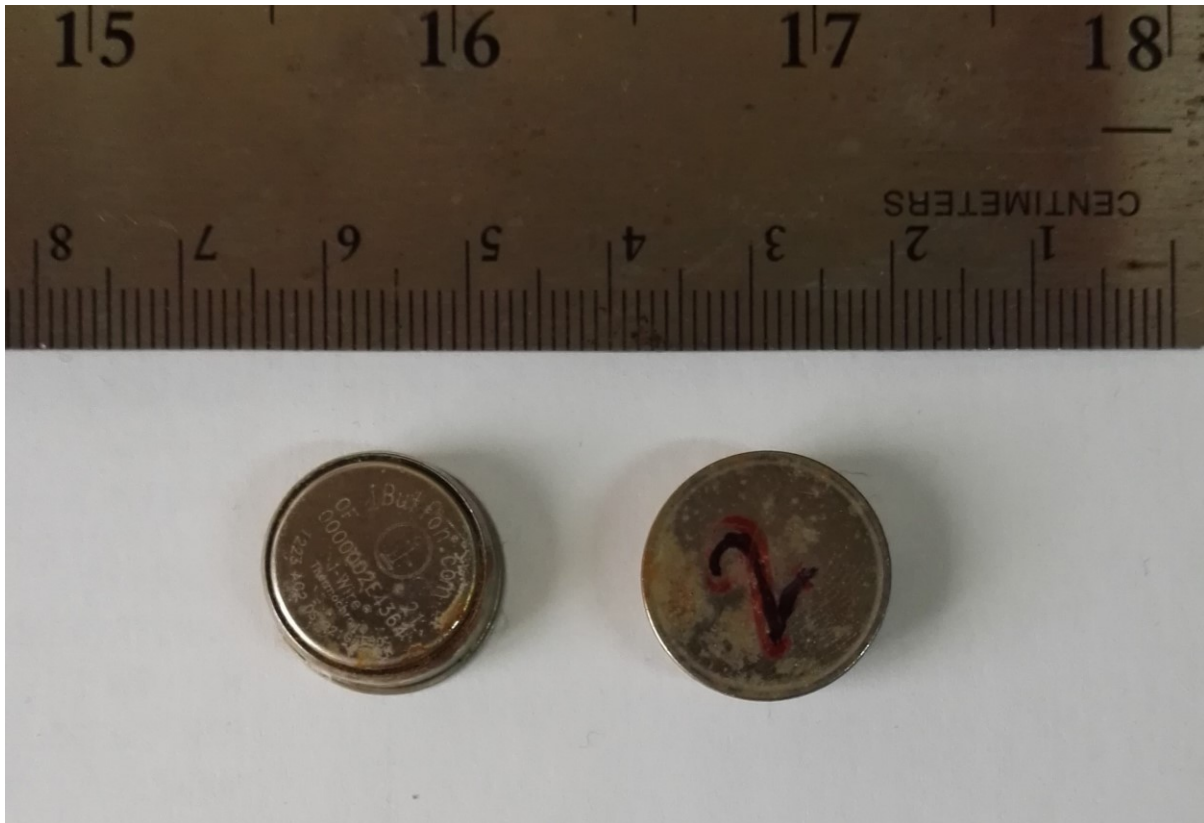


FIG. 1. Thermistors used for measuring temperature after noting the summer death of some high middle dune plants.

2.2.2 Seedling recruitment

To examine the distribution of dune plant seeds and test the hypothesis that like sand, seeds are deposited and trapped under adult plants, we sampled sand under plants and in adjacent bare sand >25 cm from plants in all dune zones. This sampling was done in October 2013, after the summer when seed dispersal and germination was most pronounced on the dune (Bakker et al. 2008). In each zone (fore, middle and back) we sampled sand under and adjacent to the most common plants in each zone by taking 100 ml surface sand samples (1 cm deep; n=10/species/habitat/zone). Samples were returned to the laboratory and sorted under a dissecting microscope for seeds. Seed density in the plots was analyzed with an

experimental treatment (under and adjacent to adult plants) one-way ANOVA separately by zone and on conspecific seeds of the target plant and seeds of all other plants combined.

To examine seedling survivorship patterns across the dune and test the hypothesis that seedling survivorship increased with dune elevation and association with adult plants, we marked natural seedlings in all dune zones that were under adult plants and in bare sand >30 cm from an adult plant. In total, 450 seedlings were marked and monitored monthly for survivorship over three seasons. Seedling survivorship data were analyzed with a non-parametric log rank test to compare the general survival among the three zones (fore vs middle vs back) and the single species survival according to species proximity (next to adult vs adjacent bare sand). In both cases the whole follow up period was taken into account (Bland and Altman 2004) and a χ^2 test was made on the log rank data of Ln (X+1) transformed *Armeria* seedling data.

To examine the hypothesis that seed supply is a determinant of the distribution and abundance of plants across the dune, we performed a seed transplant experiment with five of the most common Badesi sand dune plants (*Armeria*, *Lotus*, *Carpobrotus*, *Cakile maritima*, and *Pancreatum maritimum*). For each species, we collected dehiscent seed heads and dissected out and separated the seeds of each species (Fig. 2). 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 24 seed bags in each dune zone (fore, middle and back) under conspecific adults and in adjacent unvegetated sand >30 cm from adult vegetation and marked their location with color-coded wire markers.



FIG. 2. Seeds of *Pancratium*, dissected out and separated, ready to be sowed.

We monitored seed transplants weekly for germination and seedling survivorship for three months. Seed germination and seedling survivorship data were analyzed separately by species with a non-parametric log rank test, using a χ^2 test on the log rank data. To test the hypothesis that loose seeds were potentially 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 sand erosion (see results). Seed species were marked and identified by color-coded wire stakes (2 mm diameter, Fig. 3). Seeds were planted 3 cm below the sand surface in all treatments. We monitored these seed transplants for germination emergence and survivorship weekly for the first 3 months and monthly for

one year. Seedling germination and seedling survivorship data were processed with a non-parametric log rank test, using a χ^2 test on log rank data.



FIG. 3. Seedling of *Cakile* with its color-coded wire stake.

2.3. Results

2.3.1 *Foundation species removal experiment*

Removing foundation species had different effects across zones (Fig. 4). 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 ($F_{4,63}=2.67$ $p<0.05$).

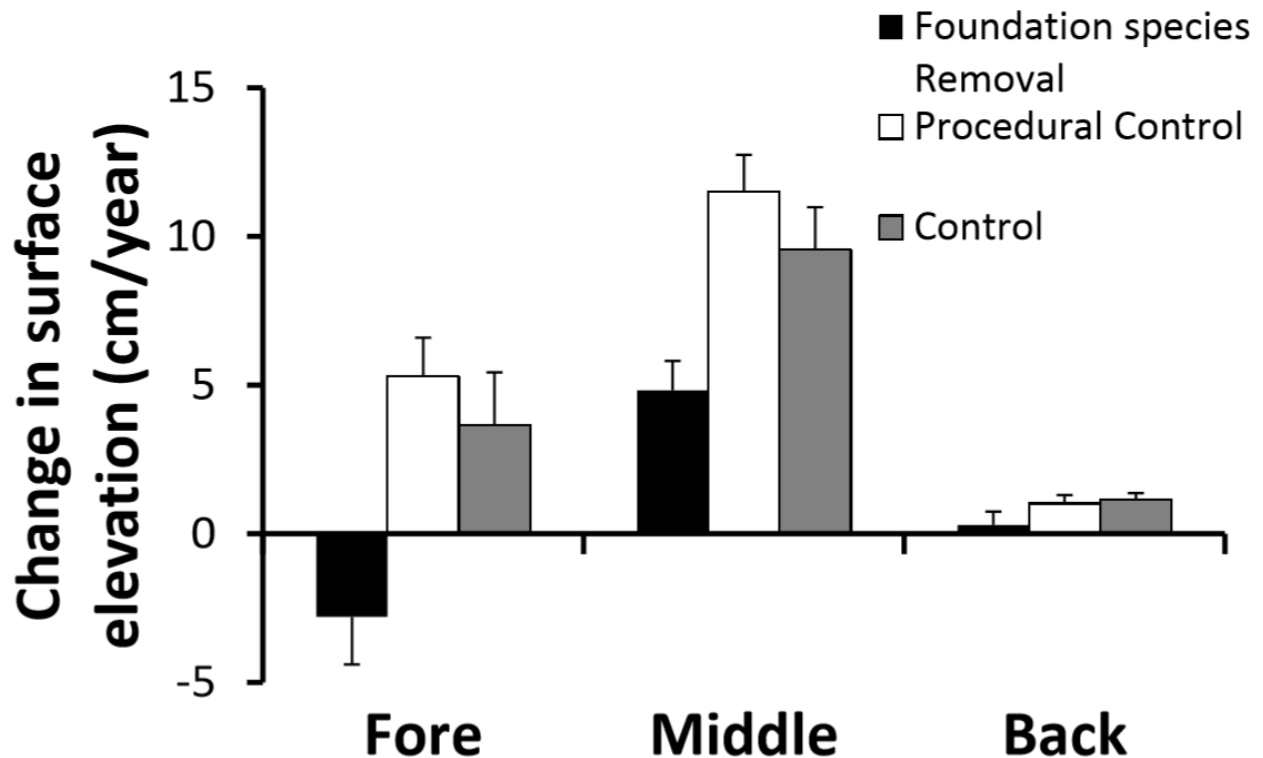


FIG. 4. Surface elevation or sand erosion/deposition balance (mean + SE) of the foundation plant species removal experiment in the three dune zones from modified Sediment Elevation Tables in each plot.

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. 4, $p < 0.05$, 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. 4).

Effects of foundation species removal, however, varied dramatically among zones (Fig. 4, $p < 0.05$, SNK test removal: fore=back<middle). In the middle dune, net sand accretion occurred in all treatments, but was nearly twice as high in control and procedural control plots with live vegetation to bind and trap sand than in foundation species removal plots (SNK test middle: removal<procedural control=control).

In the back dune, annual sediment accretion was more than an order of magnitude less than in middle dune plots with live foundation species (Fig. 4). Annual sand accretion in the back dune zone was also similar among foundation removal, control and procedural control treatments (Fig. 4, $p < 0.05$, SNK test 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 test: fore<middle=back) with a peak in removal treatments in the back zone ($F_{1,114}=10.72$ $p < 0.002$, SNK test: Removal>Control). The maximum temperatures reached a peak of around 70°C , with an overall average of 45°C in the removal plots, 5°C lower in controls where the vegetation is present.

2.3.2 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. 5). 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. 5, 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. 5). 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. 5, 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. 5), 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. 5 and 6, species x substrate x seeds category interaction, $F_{1,72}=12.59$ $p=0.0007$).

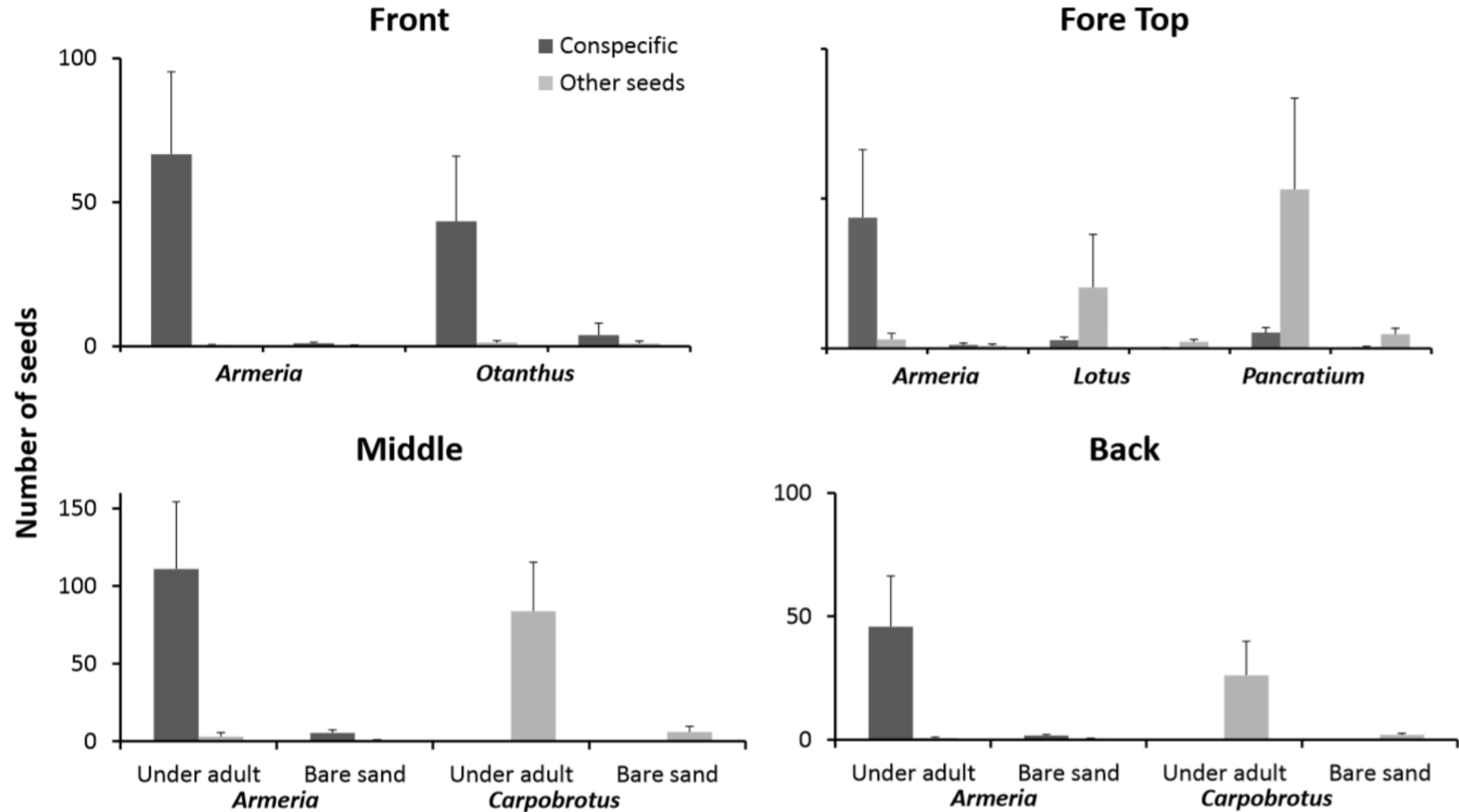


FIG. 5. Seeds distribution (mean + SE) of common dune plants across zones under adult plants and in bare, unvegetated sand adjacent to adult plants. Data are given separately for conspecific and non-conspecific seeds.

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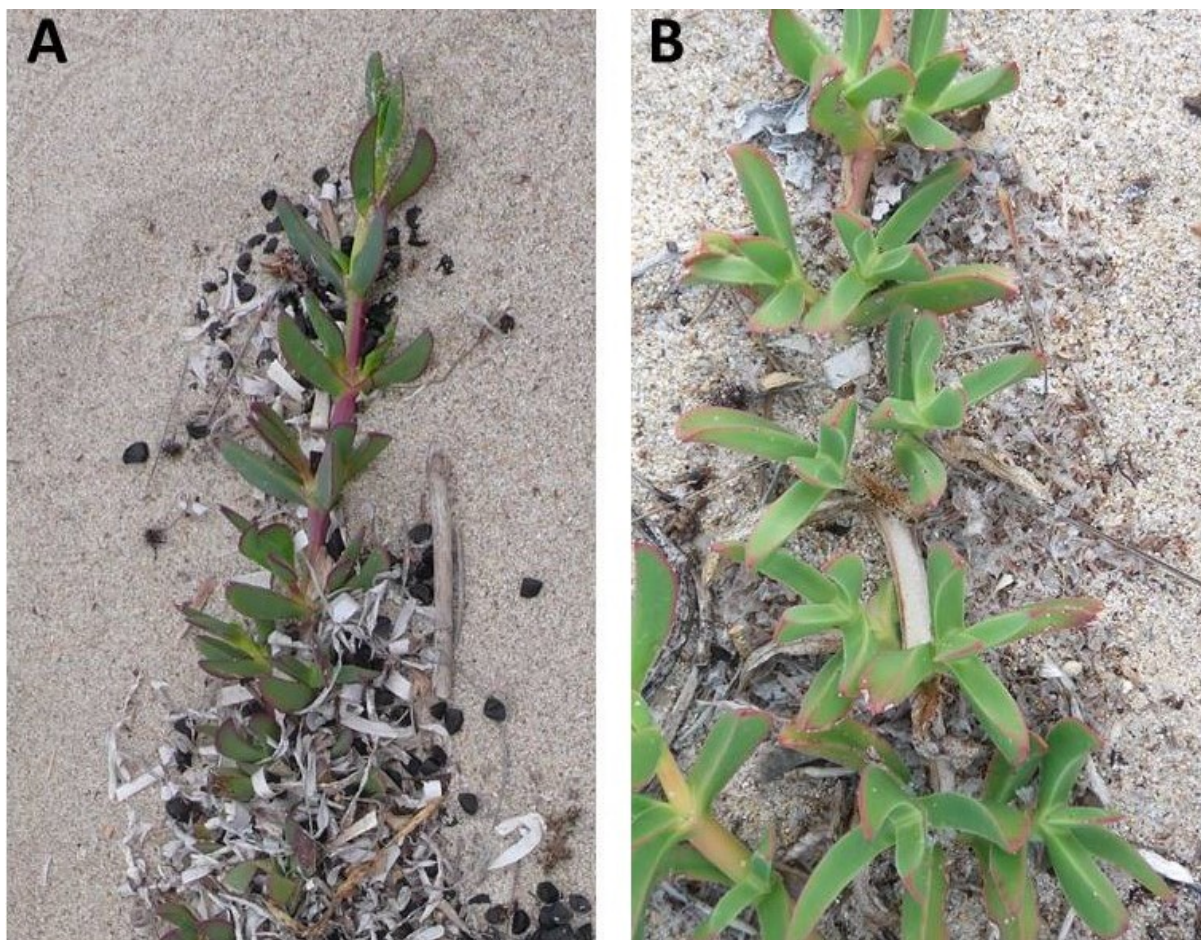


FIG. 6. (A) *Panicratium* and (B) *Armeria* seeds trapped under *Carpobrotus*.

In the back dune, *Armeria* seeds were 20 times more common under conspecific adults than in adjacent bare sand, while for *Carpobrotus*, seeds of other species were significantly more abundant under *Carpobrotus* than in adjacent bare substrate (Fig. 5 and 6, species x substrate x seeds category 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. 7, $p < 0.05$, 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 which was nearly three times (10%) the number of seedlings that survived next to adults ($p < 0.025$, χ^2) and no *Pancratium* seedlings survived. In the back dune survivorship of marked *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. 7).

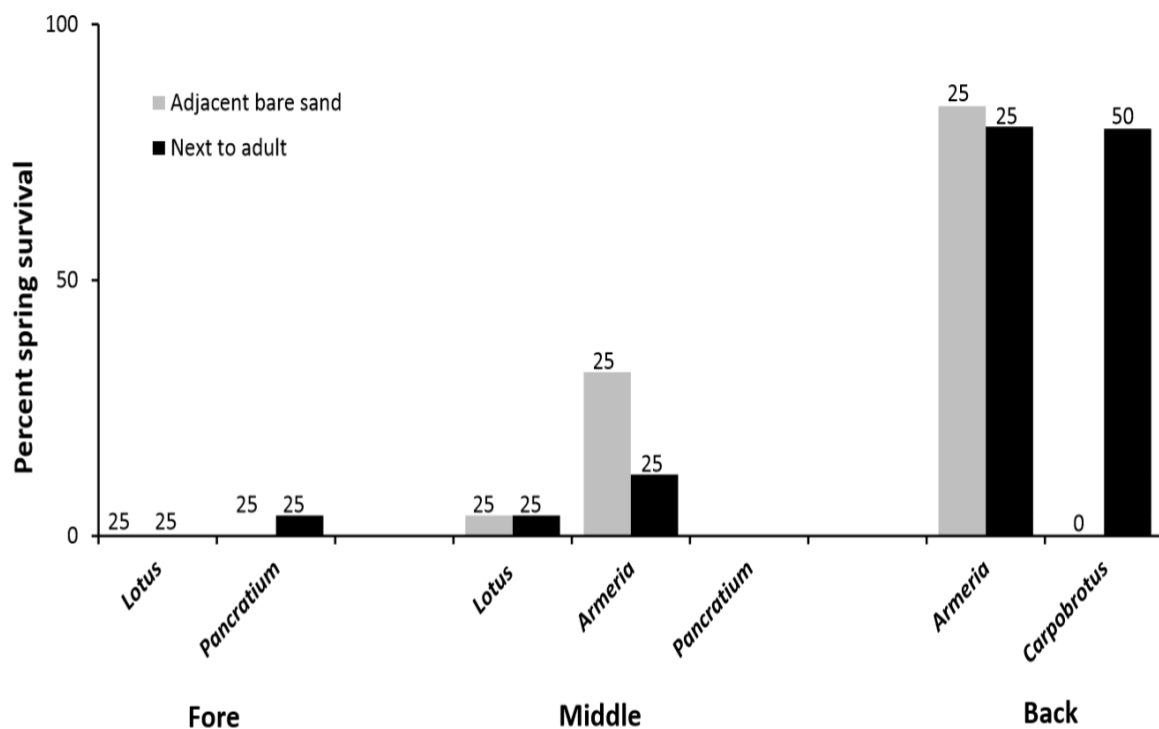


FIG. 7. Survivorship of natural seedlings marked in October 2013. For each zone and species, 25 seedlings were marked associated with conspecific adults and 25 seedlings were marked in bare substrate >30 cm from adult vegetation. The only exception was ice plant seedlings that could only be found under adult ice plants, so all 50 marked ice plant seedlings were associated with conspecifics. Data are percent of seedlings survived after 3 seasons.

2.3.3 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. 8, $p < 0.005$, 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. 8, $p < 0.025$, χ^2), while survivorship did not differ among zones ($p = 0.99$, 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. 8, $p < 0.05$, 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. 9, $p < 0.005$, χ^2).

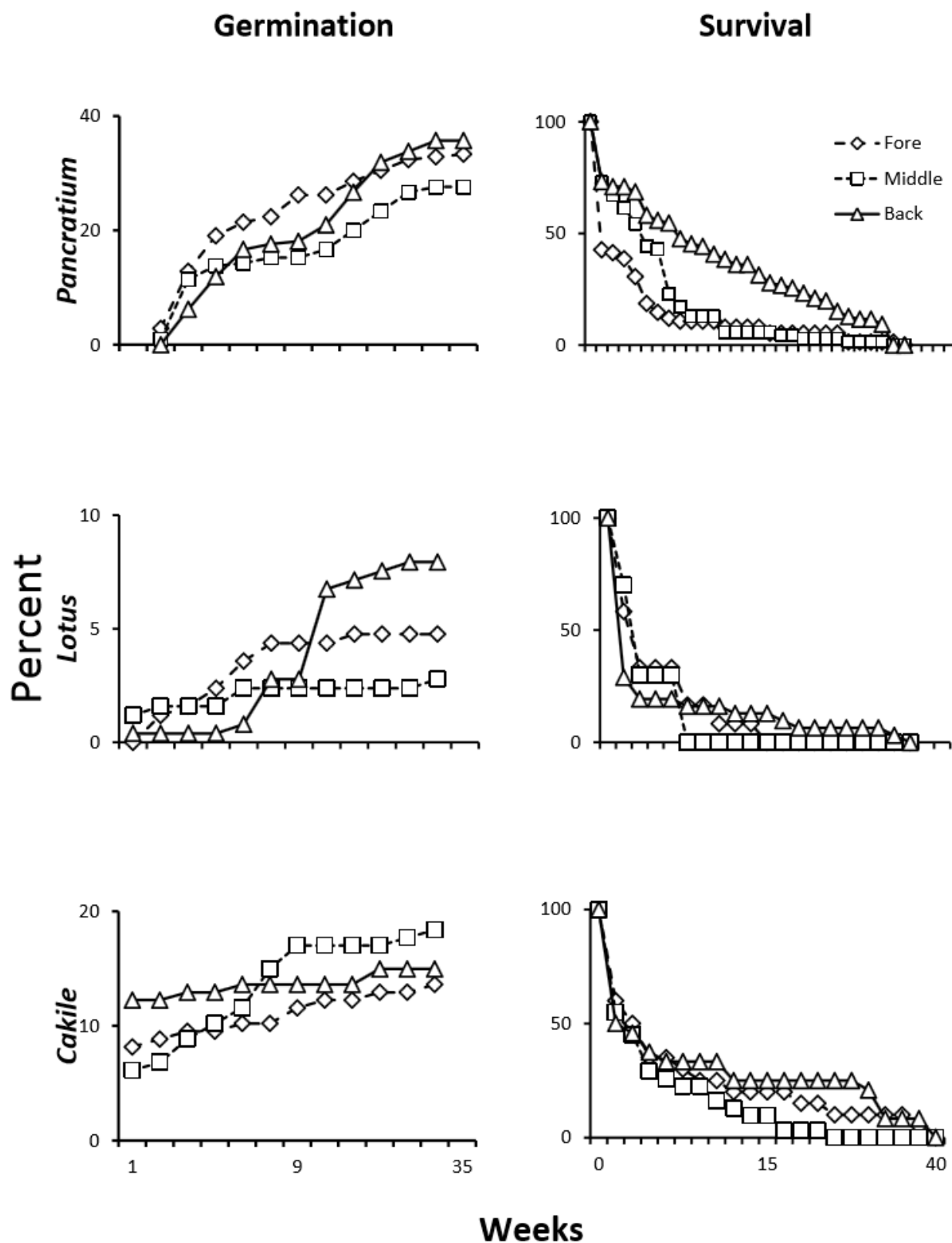


FIG. 8. Results of the seed transplant experiment where germination permeable net bags of locally collected seeds ($n=8$ /zone/treatment/species) from dehiscent seed heads were transplanted to the fore, middle and back dune with and without plant neighbors and scored for germination and seedling survivorship. Data are percent survivorship and germination of the total potential.

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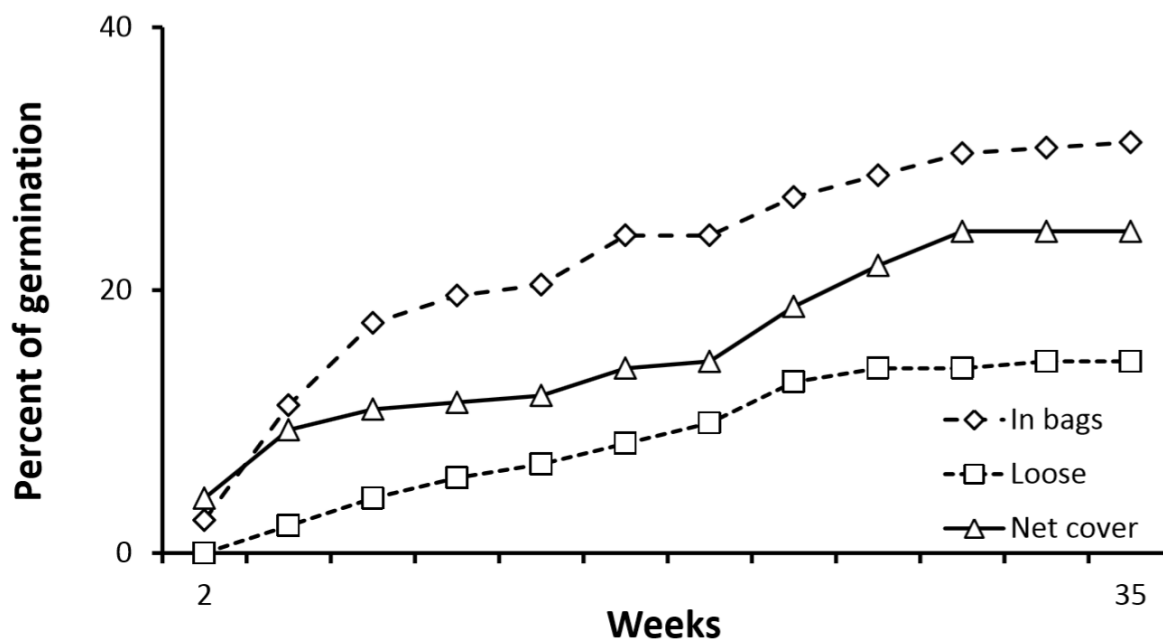


FIG. 9. Seeds erosion experiment. Germination in the fore dune where *Pancratium* seeds were transplanted in germination permeable net bags, loose in the soil and loose in the soil but covered at the surface with nylon mesh to limit erosion (n=8 treatment). Data are percent germination of the total potential.

The difference in percent germination between loose seeds and the two secured seeds treatments was higher than 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).

2.3 Discussion

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. 4). 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 (Bertness et al. 2004), terrestrial forests (Ellison et al. 2005), seagrass meadows (Duarte et al. 2000), as well as smaller scale associations within communities (Altieri et al. 2010, 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 that is largely ignored in current theory, in spite of 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, Crotty and Bertness 2015).

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 water and nutrient limitation. Dispersed seeds of dune plants are trapped near substrate stabilizing adult plants and are uncommon in unvegetated substrate (Fig. 5). 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 (Fig. 7).

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. 8). 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).

CHAPTER 3: Interspecific plants interactions

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3.1 Introduction

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 dominant biological processes controlling community development shift from positive, facilitative interactions in physically and biologically harsh environments to negative, competitive interactions in physically or biologically benign environments. It has been hypothesized that many shoreline biogenic communities built and maintained by foundation species that ameliorate limiting physical stresses, such as salt marshes, coral reefs, mangrove forests, mussel reefs, and seagrass beds, are hierarchically organized and governed by the general principles of the stress gradient hypothesis (Bruno and Bertness 2001). The trend and power of the biotic relationships along productivity-based stress gradients can be different according to the considered species, depending on their competitive ability and their stress tolerance, and to the type of related interactions if they are between or within species (Maestre et al. 2009).

The stress gradient hypothesis makes a broad prediction for general patterns across species and systems, to understand whether and in which way it may fit to dune environments we start from the original concept that “common” interactions occur along stress gradients and oscillate in the strength of pair-wise interactions. To explore this assumption we just actively manipulated the occurrence of one or more species, taken into account, and simulating their

absence it was possible to define whether a hierarchy of relations contributes to the organization and the stability of the community.

3.2 Material and methods

3.2.1 Reciprocal species removal experiment

To test the hypothesis that interspecific plant interactions shifted from facilitative to competitive across the sand dune from the fore to back dune, we performed reciprocal species removal experiments in all dune zones with dominant species pairs. On the fore dune, we chose *Armeria* and *Lotus*; in the middle dune we chose *Armeria*, *Lotus*, and *Carpobrotus*; and in the back dune, we chose *Armeria*, *Carpobrotus*, and *Pinus* (Fig. 1).

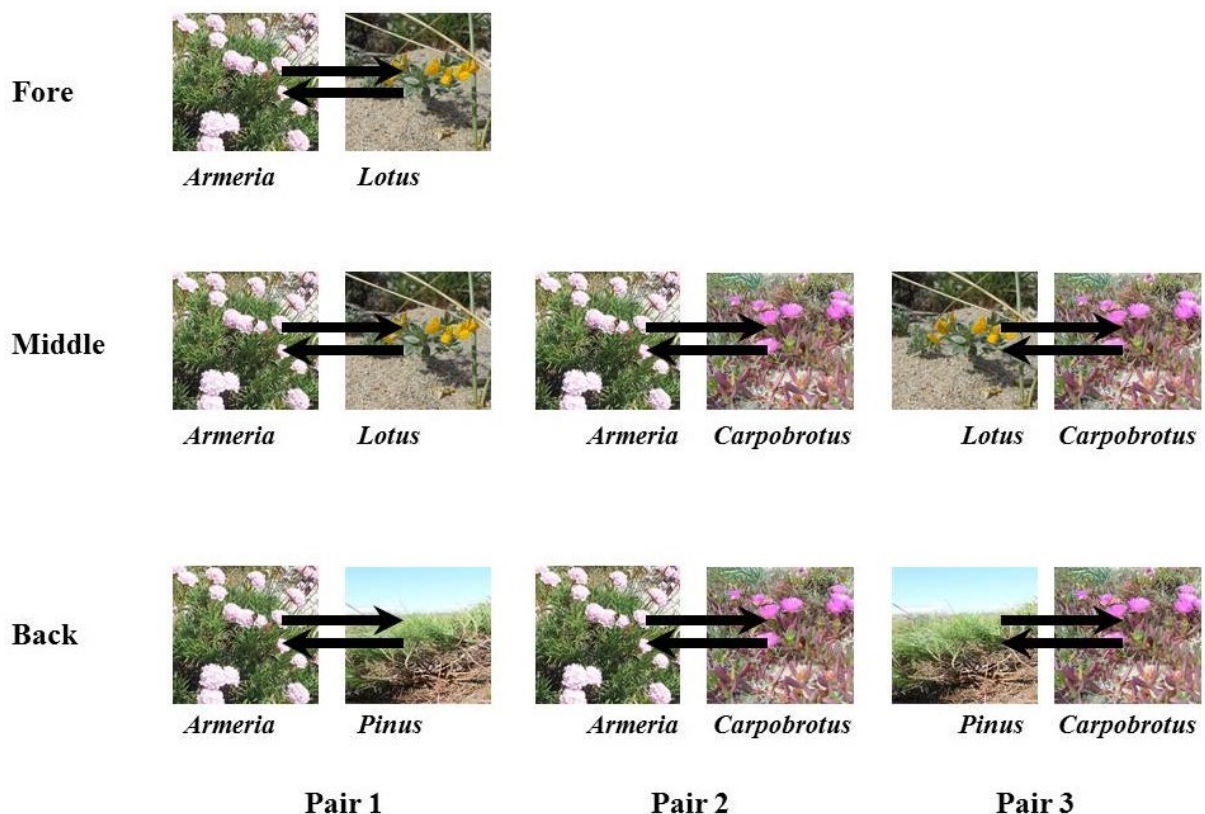


FIG.1. Species pairs considered in each dune zone.

For each species pair in each zone, we located 24 0.5x0.5 m plots with mixtures of the two target species. All plots were marked with numbered rebar corner posts driven to refusal into the sand and labeled with a unique numbered plastic tag. For each zone and species pair (species a and b), we randomly assigned control plots, “species a” removal plots, and “species b” removal plots (n=8/treatment/species pair/dune zone). Species assigned for removal were removed manually with minimal disturbance. Treatments were maintained monthly as needed for two years. During this time, plots were photographed in the spring and fall of each year and analyzed for percent plant cover. The height of the corner posts were additionally measured to quantify sand deposition/erosion. Plant cover in the plots was analyzed by zone with a two-ways ANOVA, only *Carpobrotus* data were Sqrt (X+1) transformed. Separate ANOVAs were run for each species at the time period of 12 and 18 months, to accommodate the loss of plots to sand burial over time (see results). We also analyzed the relationship between plant species cover and sand deposition on plot plant cover data pooled by zone with a t test and a plant cover x sediment cover linear regression.

3.2.2 Back dune competitive release experiment

It was not practical or 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. Furthermore, since the Badesi dune is a protected conservation area, removing entire *Pinus* trees would have been destructive and not permitted. Therefore, 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 full *Pinus* canopies (n=10), in areas 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 0.5x0.5 m marked quadrat in each plot was monitored monthly by photographs for a year (Fig. 2).



FIG. 2. Large *Pinus* branches removal with the 0.5x0.5 m marked plot used to estimate the natural recruitment and the plastic greenhouse seedling pots.

To test the hypothesis that allelopathy contributes to the dominance of *Pinus* and ice plant in the back dune, we also manipulated sediment in all *Pinus* plots described above. Allelopathy was suggested because in dense *Pinus* and ice plant stands, the 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 seeds as 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; Fig. 3) and scored them monthly for germination.

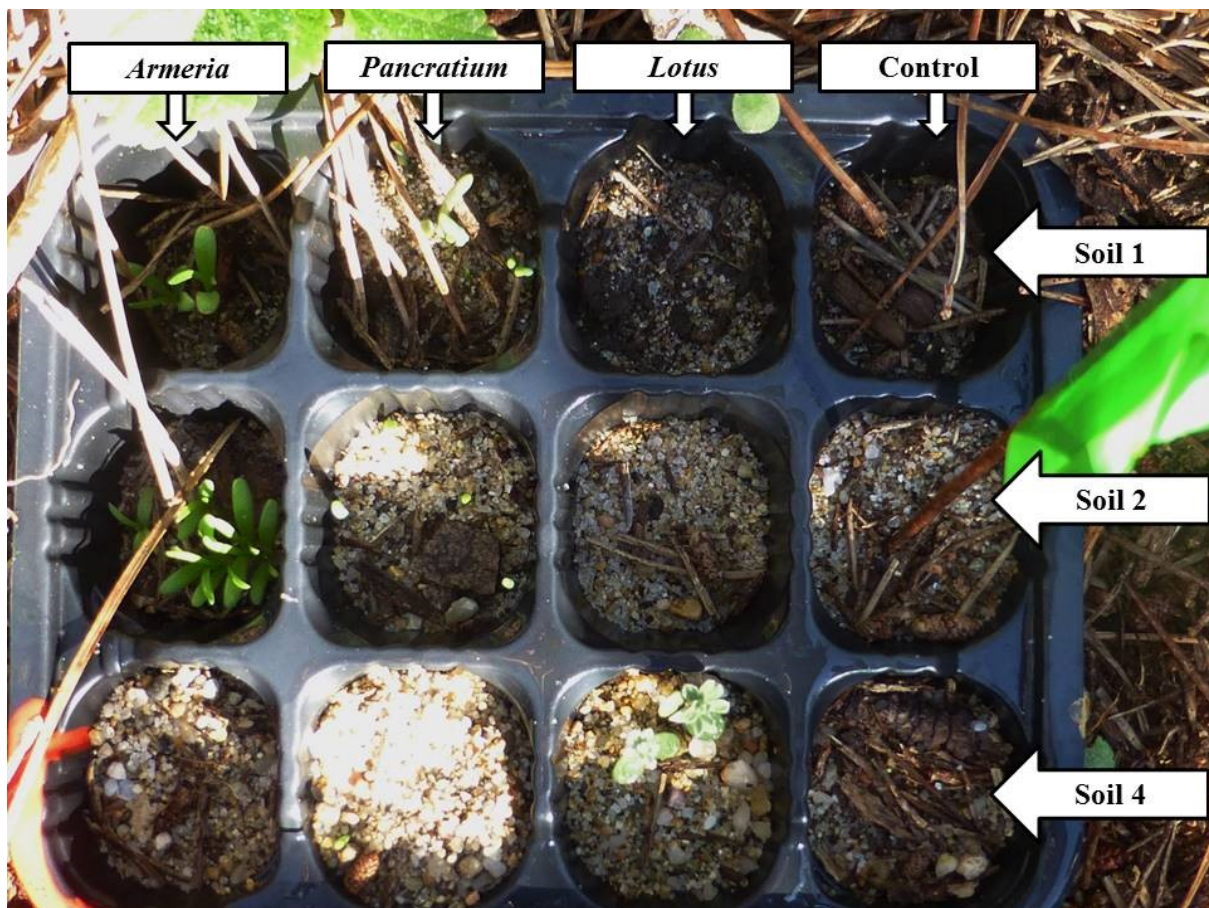


FIG. 3. Plastic greenhouse seedling pot used to test *Pinus* allelopathy. In columns are the seeds as indicated and in rows are three of the different soils as reported in the text: 1) *Pinus* soil, 2) middle dune bare soil and 4) potting soil mixed with sand. Seedlings of all the species are visible in some of the soils.

Seed germination and survivorship data of *Armeria*, and *Lotus* were transformed with Ln (X+1) and all were analyzed with *Pinus* treatment x soil type ANOVA. To further test the possible effect of different soils on seed germination, we performed the same experiment as detailed above in a green house using the same combination soils x seeds. We sowed the

green house pots at the end of December, and kept them with natural light, watered them periodically with very low amounts of water to mimic field conditions. We scored germination and measured seedling growth every 3 weeks, similarly to our field monitoring. Seed germination and seedling survivorship and growth data for each species were sorted with a log rank test, a χ^2 test on log rank data.

3.3 Results

3.3.1 Reciprocal species removal experiment results

Evaluation of interspecific interactions of common plant species in all dune zones with reciprocal species removal experiments initially revealed significant interspecific interactions (Fig. 4 and 5).

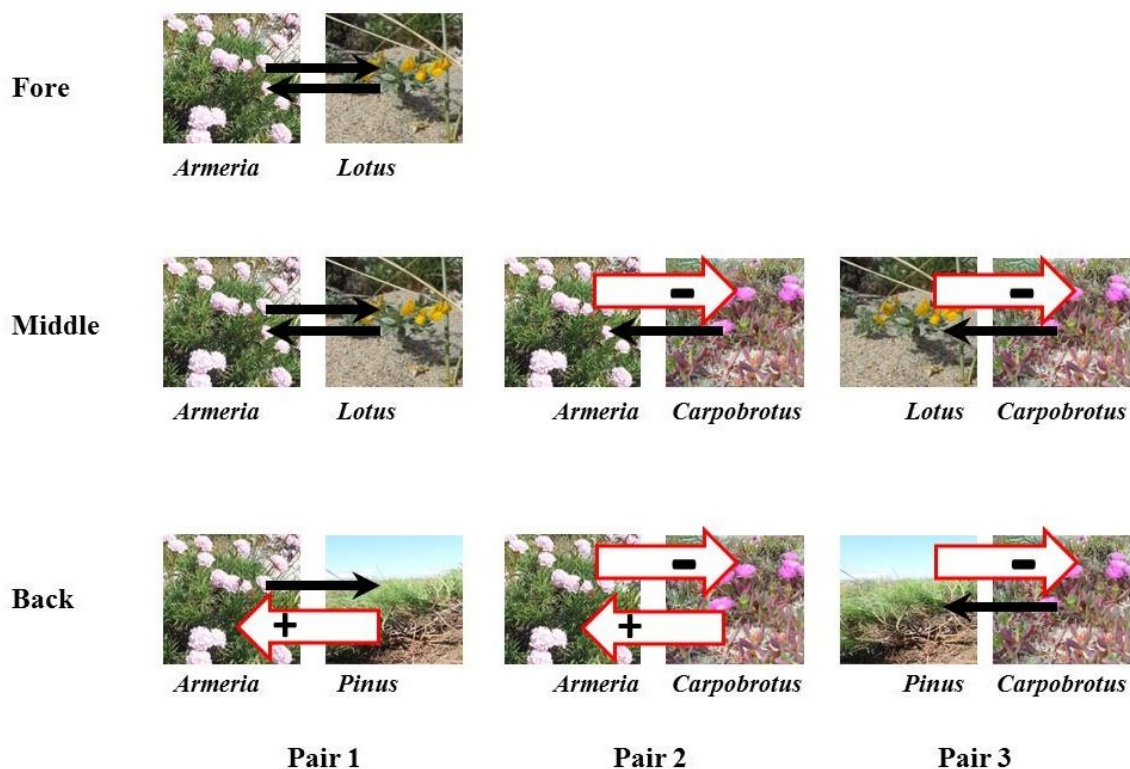


FIG. 4. Reciprocal species removal: a schematic explanation of the results.

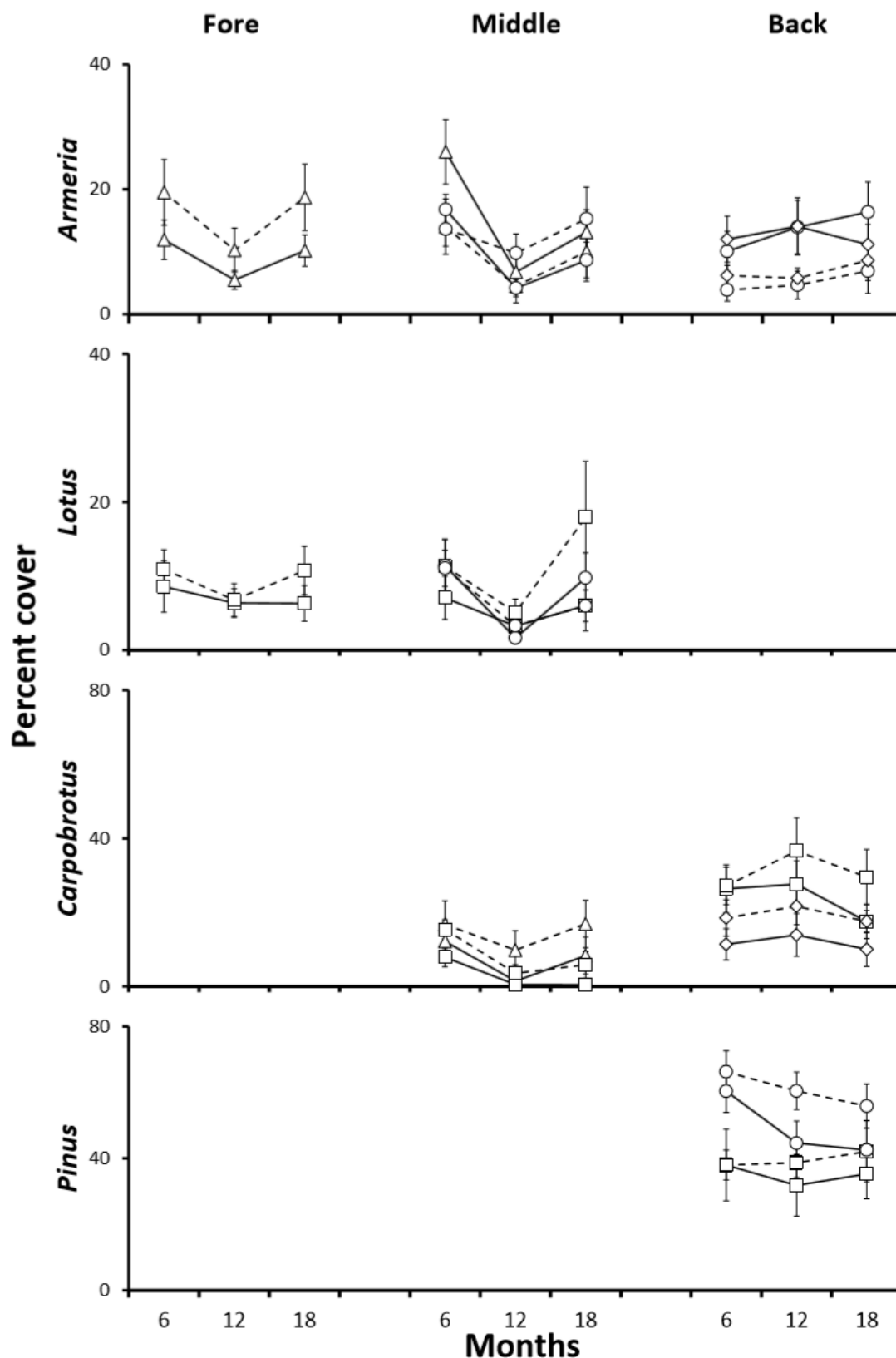


FIG. 5. Reciprocal species removal: *Armeria* (\square), *Lotus* (\triangle), *Carpobrotus* (\circ), *Pinus* (\diamond), Species control (—), Reciprocal species removal (- - -). Results of pair-wise reciprocal species removal experiments after 18 months in the fore, middle and back dune.

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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. 6, 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. 5, 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. 5, treatment $F_{3,56}=12.12$ $p < 0.03$, SNK test 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. 5, 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. 6, $p < 0.001$, t test).

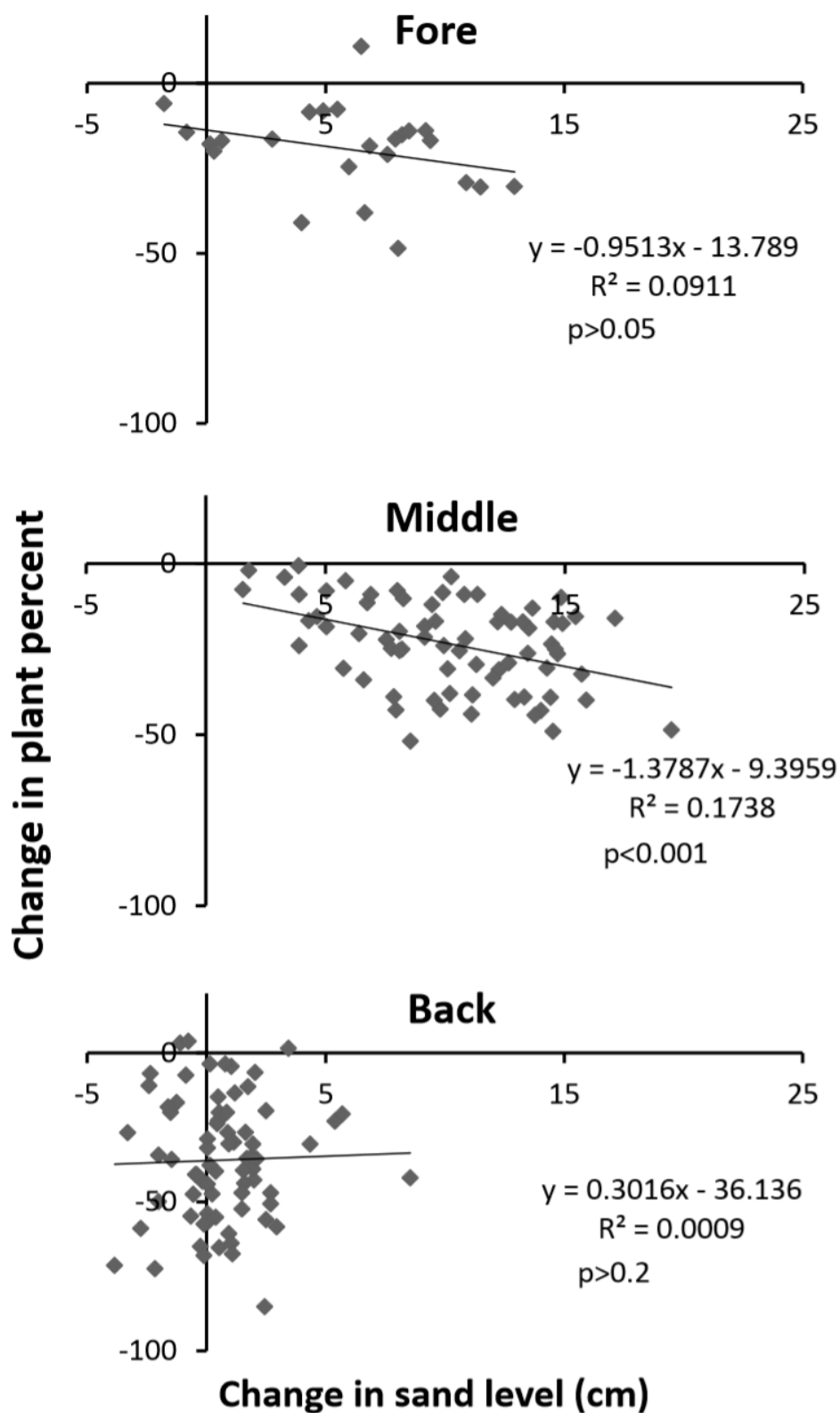


FIG. 6. Relationship between sand burial and percent plant cover in the pair-wise reciprocal species removal experiments after 2 years in the fore, middle and back dune.

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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. 5, 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. 5, treatment $F_{3,56}=12.12$ $p<0.03$, SNK test 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 any significant species interactions (Fig. 5, interaction x treatment $F_{1,28}=0.18$ $p>0.05$). In the back dune species removal plots, one of the most conspicuous trends over time was the decrease in the cover of *Carpobrotus* during summer (Fig. 7). This left large areas of dead, desiccated *Carpobrotus* cover dominating the seaward border of the back dune at the end of the summer (Cussetdu, personal observation).

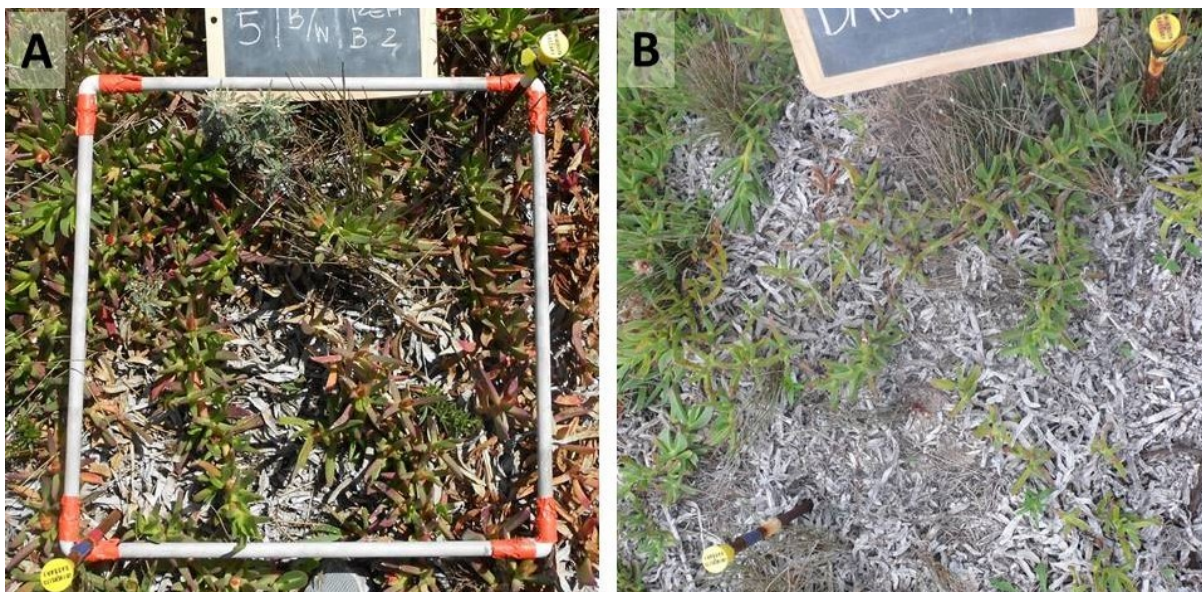


FIG. 7. Decrease in cover of *Carpobrotus* during summer. (A) a back dune plot before summer and (B) after summer, with a large area of dead, desiccated *Carpobrotus*.

3.2.2 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. 8).

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. 8, 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. 8, $F_{6,126}=5.02$ $p=0.0001$ and $F_{6,126}=7.06$ $p<0.0001$, respectively), even if, for both species, there were no significant posthoc 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).

In the green house seed germination experiment, each species was analyzed separately and germination was compared among soil types. *Armeria* and *Lotus* germination was much lower than in the field, while *Pancratium* germination was ~60%, similar to the field (Fig. 9). In spite of the difference in germination rate for *Armeria*, the same effect of soil type was found as in the field, with higher germination in middle dune soil (Fig. 9, $p<0.001$, χ^2). No differences in *Pancratium* and *Lotus* germination were found among soil types (Fig. 9, $p>0.1$ and $p>0.5$ respectively, χ^2).

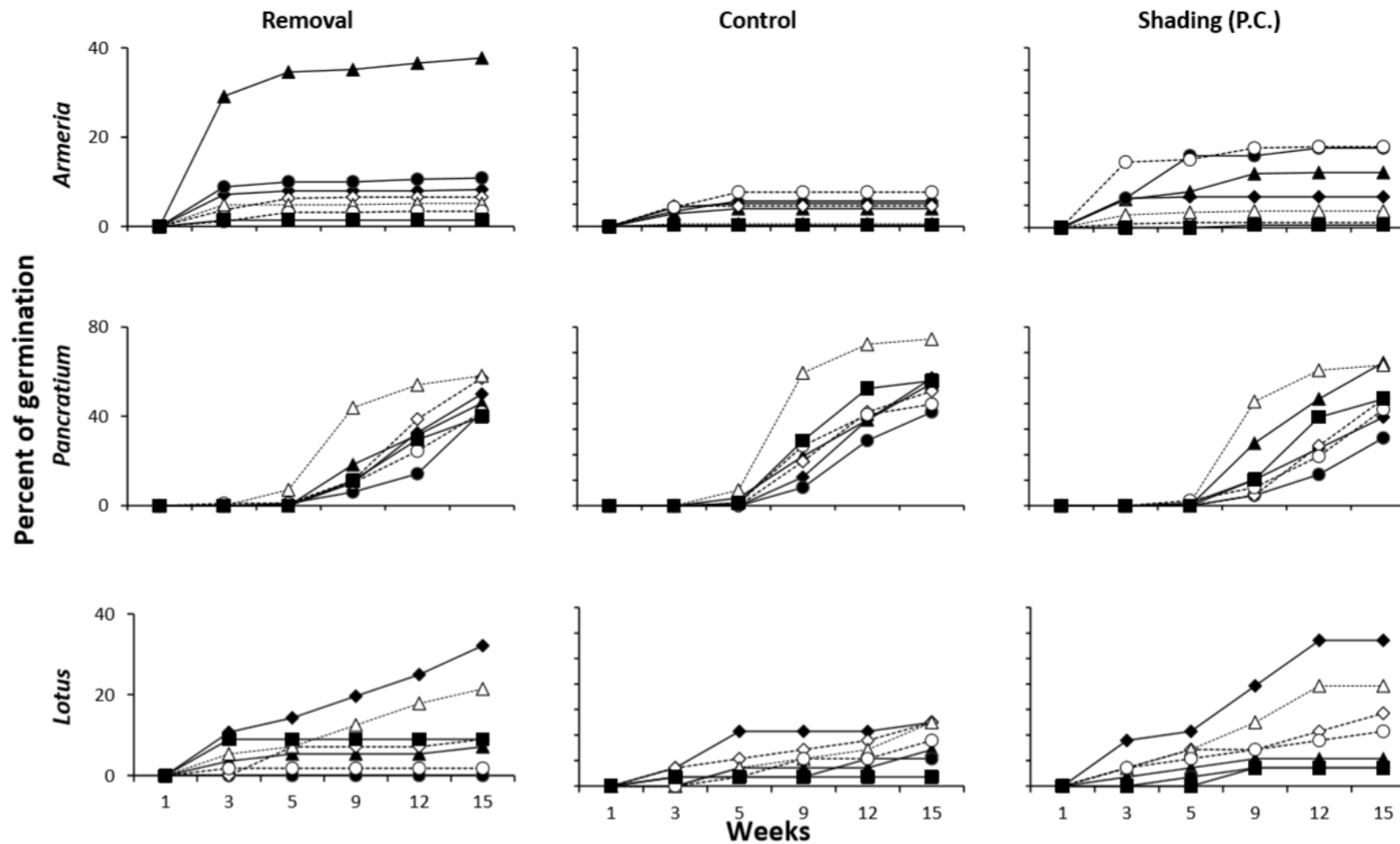


FIG. 8. Back dune competitive release experiment. Germination of *Armeria*, *Pancratium* and *Lotus* seeds sowed in different soils: *Pinus* soil (◆), middle dune soil (▲), *Carpobrotus* soil (●), potting with *Pinus* needles (◇), potting with middle dune soil (△), potting with *Carpobrotus* soil (○) 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.

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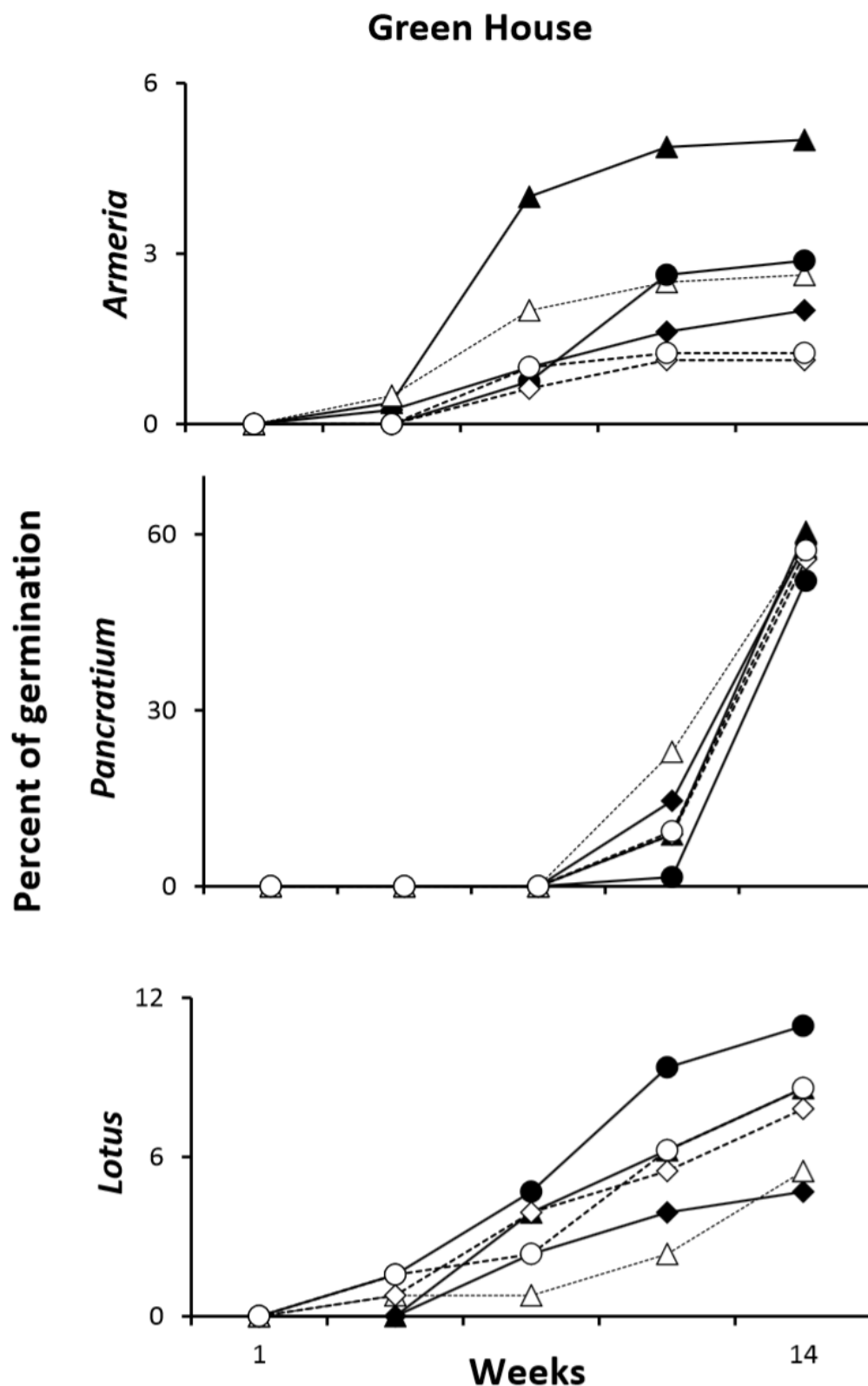


FIG. 9. Competitive release green house experiment. Germination of *Armeria*, *Pancratium* and *Lotus* seeds sowed in different soils: *Pinus* soil (◆), middle dune soil (▲), *Carpobrotus* soil (●), potting with *Pinus* needles (◇), potting with middle dune soil (△), potting with *Carpobrotus* soil (○) in a green house under controlled conditions. Data are percent of seeds germinated out of the total potential.

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3.3 Discussion

3.3.1 *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 and 5). 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. 6). 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 presence of the dense *Pinus* canopy, the germination of other middle dune species is precluded and in some cases even soil type prevents seedling recruitment. In addition, high summer temperatures in the back dune limit competitive dominance by ice plants, because it suffers drying and seasonal die-off and regrowth limit its dominance (see Chapter 4), favoring *Pinus*.

3.3.2 *Sand burial disturbance*

While our interspecific plant interaction experiments initially revealed significant competitive dominance and facilitative relationships (Fig. 5), 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 and maintaining the dune, sand mobility, is responsible for plant mortality and diversity, particularly in the middle dune.

CHAPTER 4: Physical stressors

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4.1 Introduction

Descriptive and correlative studies of sand dune communities in North America and Europe suggest that substrate stabilization, water and nutrient limitation, and plant facilitation and competition are the main drivers of sand dune community structure and organization (e.g. Barbour et al. 1985, Ehrenfeld 1990, Lichter 1998, Isermann 2011). Commonly, distinct plant communities occur in coastal sand dune systems along a coast-to-inland gradient (Hesp 1991, Lortie and Cushman 2007, Acosta 2009). On the seaward border of sand dunes, the fore dune, a limited number of clonally spreading pioneer plant species with deep roots trap and bind sand, initiating dune formation. These pioneer dune plants stabilize the 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 dramatically, but unvegetated free space remains common. At these intermediate elevations, substrate stabilization appears to remain important, but interspecific plant interactions, including facilitation (Franks 2003, Castanho et al. 2015) and competition (Lichter 2000), as well as water and nutrient limitation also appear to mediate plant success. At the even higher elevations of 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 plants, generally woody shrubs and/or trees, appear to competitively displace the plants that dominate intermediate dune elevations (Lichter 2000). This descriptive structure of dune communities is consistent with sand dune plant communities fitting a hierarchical community organization model and supports the stress gradient hypothesis of community assembly.

Here we present a long term manipulative experiment testing the nutrient and water limitation and the substrate stability effect on the recruitment of bare spaces. With the same objective we tested the effect of the longstanding shading operated from the boardwalks on *Carpobrotus*.

4.2 Material and methods

4.2.1 Physical stress alleviation experiment

To test the hypothesis that physical stress limits dune plant growth, expansion, and persistence 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), substrate stability (with and without substrate stabilizing fish net attached flush to the surface with wire staples; Fig. 1) and water limitation (with 2 liters of tap water every 2-3 weeks and without water additions).

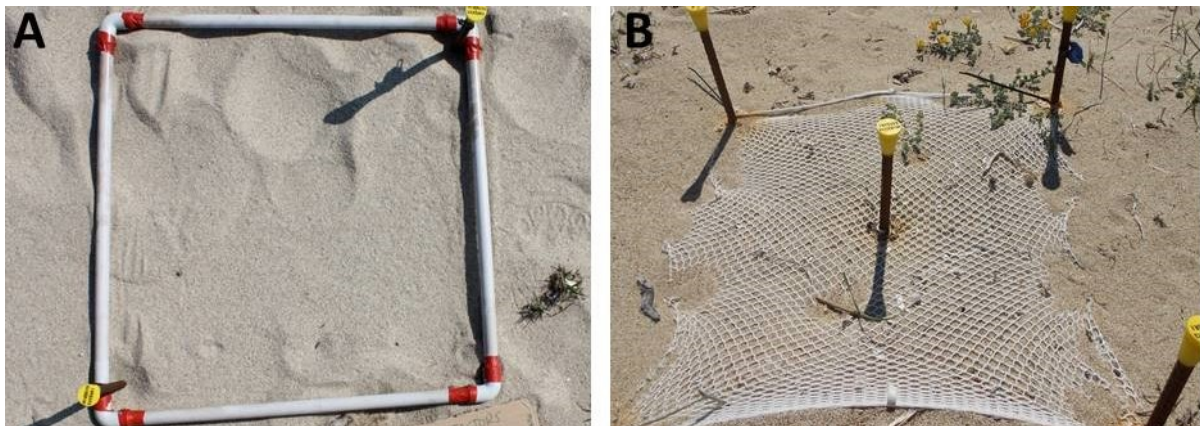


FIG. 1. Substrate stability. (A) bare plots without and (B) with substrate stabilizing fish net.

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; Fig. 2).

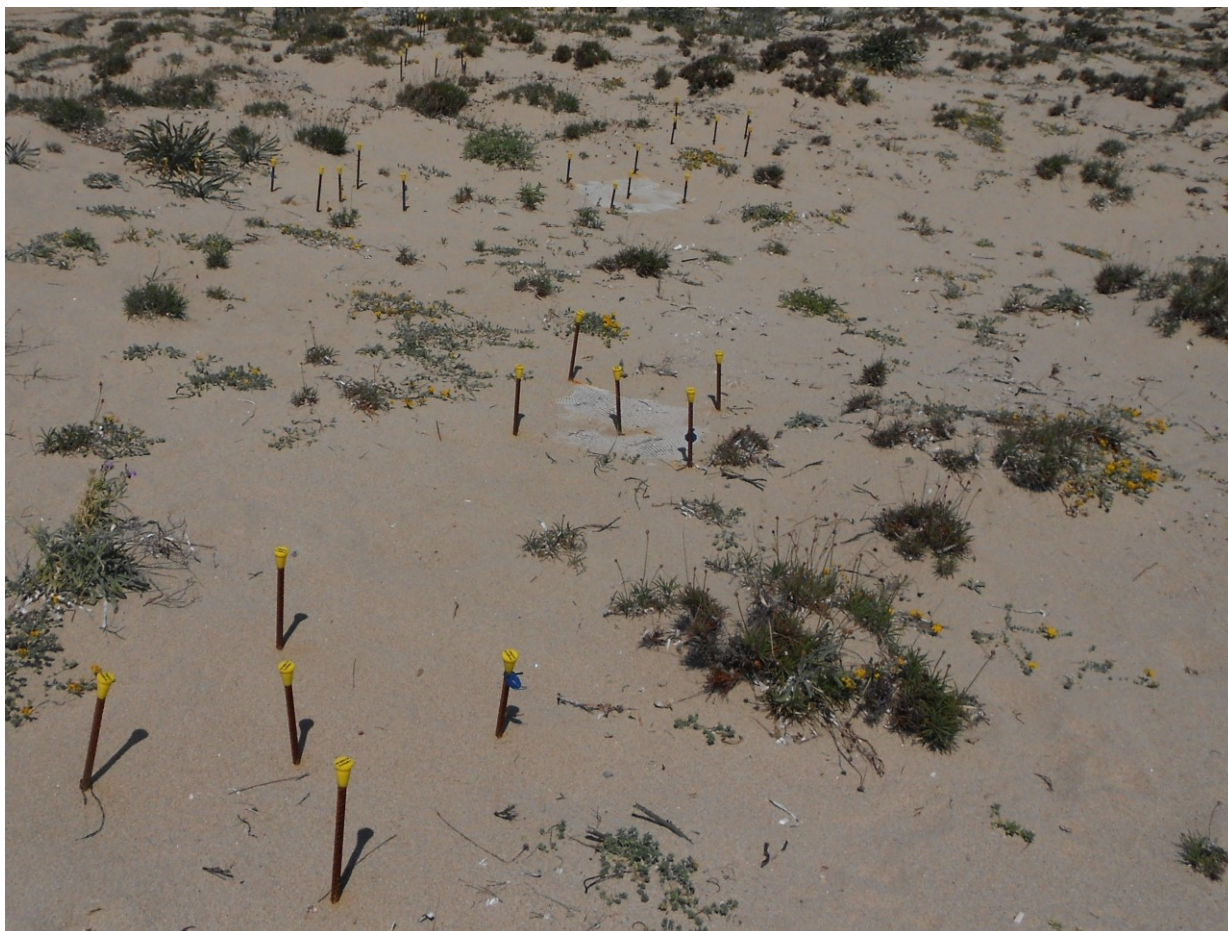


FIG. 2. View of some of the 72 0.5x0.5 m plots with bare sand substrate in the fore dune zone.

Replicate treatments were maintained as necessary for two years and all plots were monitored for percent plant cover initially and then for two years in the spring and fall. Plant cover in the plots was transformed to meet the assumption of parametric statistics and analyzed with an experimental treatment x dune zone ANOVA.

4.2.2 Boardwalk shadow effect sampling

During our first year studying the Badesi dune we noticed that during the summer months, temperatures in the middle and back dune, which were 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) that ran perpendicular to the shoreline and extended from the fore dune to the highest point of the back dune (Fig. 3), and 4 m from both sides of the boardwalks.



FIG. 3. Boardwalk at Badesi dune. (A) the boardwalk runs from the fore dune to the back dune and (B) *Carpobrotus* on the shaded zone on the side of the boardwalk.

We hypothesized that shading provided by the boardwalk would improve the success of ice plants. We sampled 2 boardwalks that had been in place for 5 years. At each boardwalk we quantified live and dead ice plant percent cover from quadrats every 2 m from the beginning of the boardwalk to the back dune habitat dominated by *Pinus*. For analysis we excluded plots without ice plants and pooled the data to obtain an equal number of replicates in the adjacent and control transects. We transformed both the dead/alive ratio of *Carpobrotus* percent cover and dead *Carpobrotus* percent cover to meet the assumptions of parametric statistics and analyzed both with a one-way ANOVA.

4.2 Results

4.2.1 *Physical stress alleviation experiment*

Experimentally manipulating water availability (with and without monthly water additions), nutrient availability (with and without NPK nutrient additions) and substrate stability (with and without 1x1 cm mesh fish net substrate stabilization cloth) across dune zones (fore, middle and back) in a fully factorial 2 year-long experiment at a 0.5x0.5 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. 4, fore $F_{9,70}=1.95$ $p>0.05$, middle $F_{9,70}=2.16$ $p<0.04$ but not significant posthoc SNK test, back $F_{9,70}=0.57$ $p>0.05$).

There were no significant responses in plant biomass or species identity to the treatments within zones, but a two-way ANOVA (considering zone and treatment orthogonal and fixed), showed a difference in the recovery among zones, with recovery increasing from the fore to back dune (Fig. 4, $F_{2,210}=18.30$ $p<0.0001$, SNK test fore<mid<back), although treatment and the zone x treatment interaction were still not significant ($F_{9,210}=1.70$ $p>0.05$ and $F_{18,210}=0.87$ $p>0.05$, respectively). Thus, manipulating the physical factors thought to mediate sand dune community structure at small spatial scales had minimal effects.

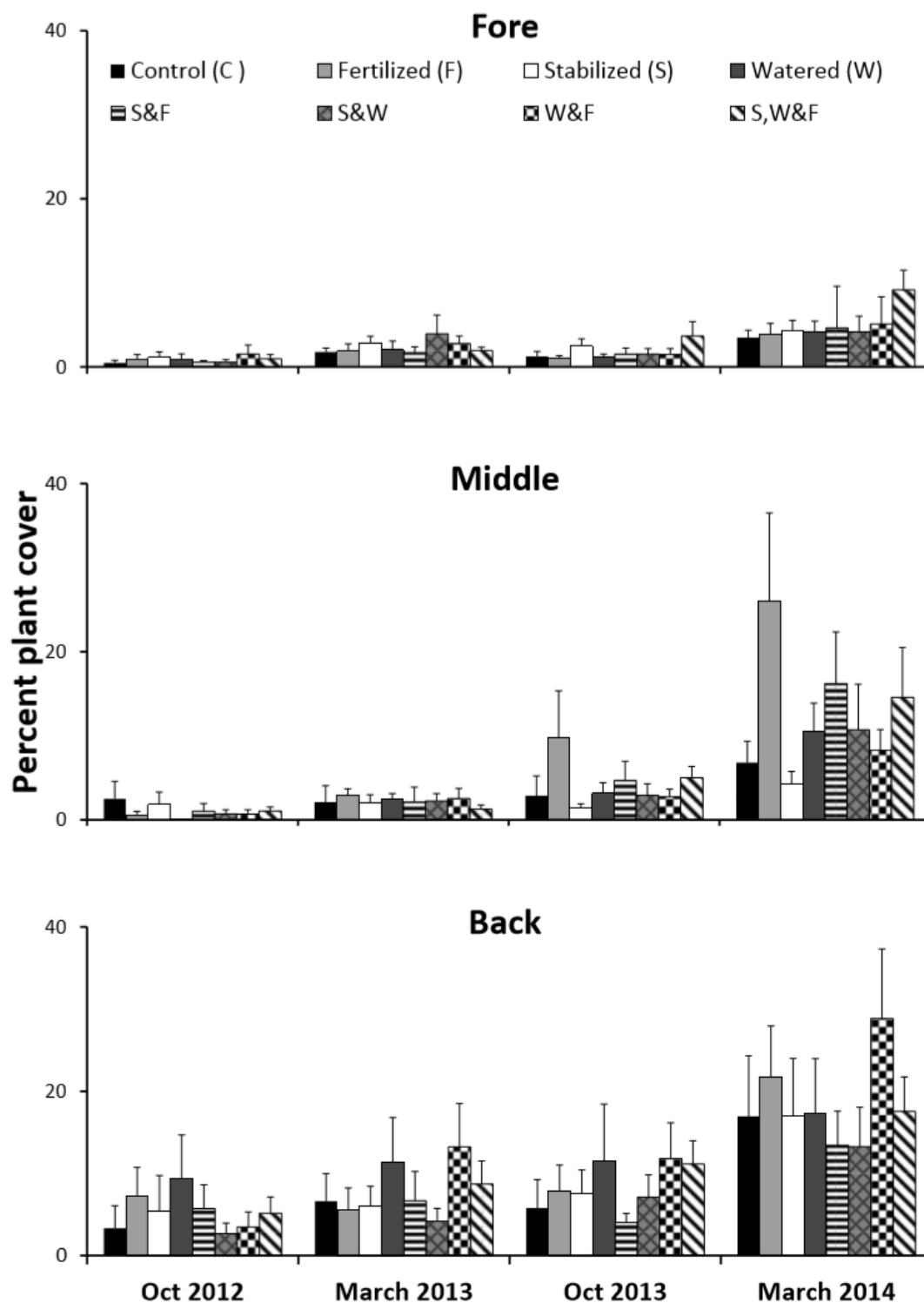


FIG. 4. Physical stress alleviation experiment. Percent plant cover (mean + SE) of the fully factorial manipulation of water availability, nutrient availability and substrate stability during 2 years in the fore, middle and back dune. No single or combination of stress alleviation treatments affected plant cover, but higher recovery was found in back zone.

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4.2.2 Board walk-shadow effect experiment

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 than in adjacent unshaded habitats (Fig. 5, $F_{1,190}=5.58$ $p<0.02$). There was also nearly 10% more dead ice plant in unshaded habitats than under the shade of boardwalks (Fig. 5, $F_{1,190}=18.76$ $p<0.0001$). Since ice plant death occurs almost exclusively in summer (Cusseddu, personal observation), summer ice plant mortality common 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.

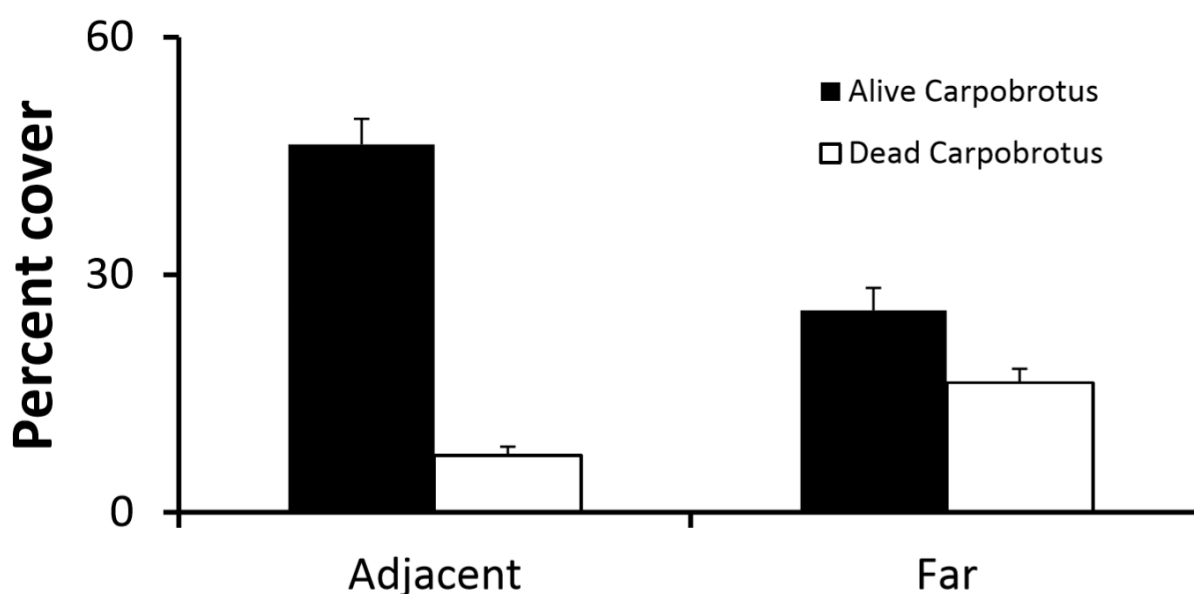


FIG. 5. Board walk-shadow effect experiment. Percent cover (mean + SE) of alive and dead ice plant sampled adjacent to the board-walks and 2m far away.

4.3 Discussion

Since all of these physical and biological factors have been shown through correlations to impact sand dune community organization at regional spatial scales, our results imply that spatial scale is a particularly important consideration 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. 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. However, this result suggests that the physical factors we considered are not dominant, community-shaping stresses for recovery in our environment, even if they are for some species of other dune systems (He and Bertness 2014). In the meantime the highest recovery of the back dune zone suggests that several mechanisms are involved because of the higher presence of adult plants that can produce and trap seeds (see Chapter 2), as well as their capability of invading the bare space from the border through vegetative reproduction.

Variation in local abiotic conditions can determine differences in invasibility within a plant community (Lortie and Cushman 2007), as highlighted by the boardwalk experiment. Boardwalks have been placed in several coastal areas to avoid the trampling effect due to touristic pressure, because they are thought to be an effective device that may help to mediate between the touristic use and a sustainable management of the environment. However, in our system, their presence, that is an easy way to reach the seaside without damaging the vegetation, turned out to be a microhabitat that favours *Carpobrotus* presence. Nevertheless, the hot season temperatures that characterize this environment for at least four months during the year seem to control the ice plant abundance. The finding of our experiment suggests that the effect of direct sun exposure provides a natural regulation to iceplant invasibility preserving Badesi dune system from an uncontrolled spread of this exotic plant. It has been demonstrated for similar systems that climatic disturbances can determine the composition of

species of a community (Miller et al. 2010), consistently to our finding that a physical seasonal feature is responsible for buffering the presence of an alien species.

CONCLUSION

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, Paine 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.

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. Without foundation plant species, mechanisms such as sand binding, erosion, mobility, and burial act as barriers to plant community development 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. 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. Plant species cover and richness increased from 35% in the fore dune to 63% in the middle dune. Like the fore dune, however, recovery from foundation removal was minimal, being less than 1% in 3 years. Moreover, while the middle dune contained initially strong interspecific plant interactions, it was subjected to heavy sand burial that limited the emergence of species interactions, seed germination, and seedling success. Therefore, frequent sand burial disturbances have a large influence over the middle dune zone.

In the back dune we see nearly 100% plant cover and the emergence of competitive dominants. 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, as was the recovery of bare space. 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 as we saw even in the green house experiment. 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 were competitively excluded from the back dune over time because *Pinus* acts as a physical barrier, having a dense prostrate morphology.

These results are summarized in a conceptual model of the assembly rules of the Badesi sand dune plant community (Fig. 1). 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 interaction mechanisms determined among species are transient in nature due to the high instability of the zone 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 that is built and maintained by the 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.

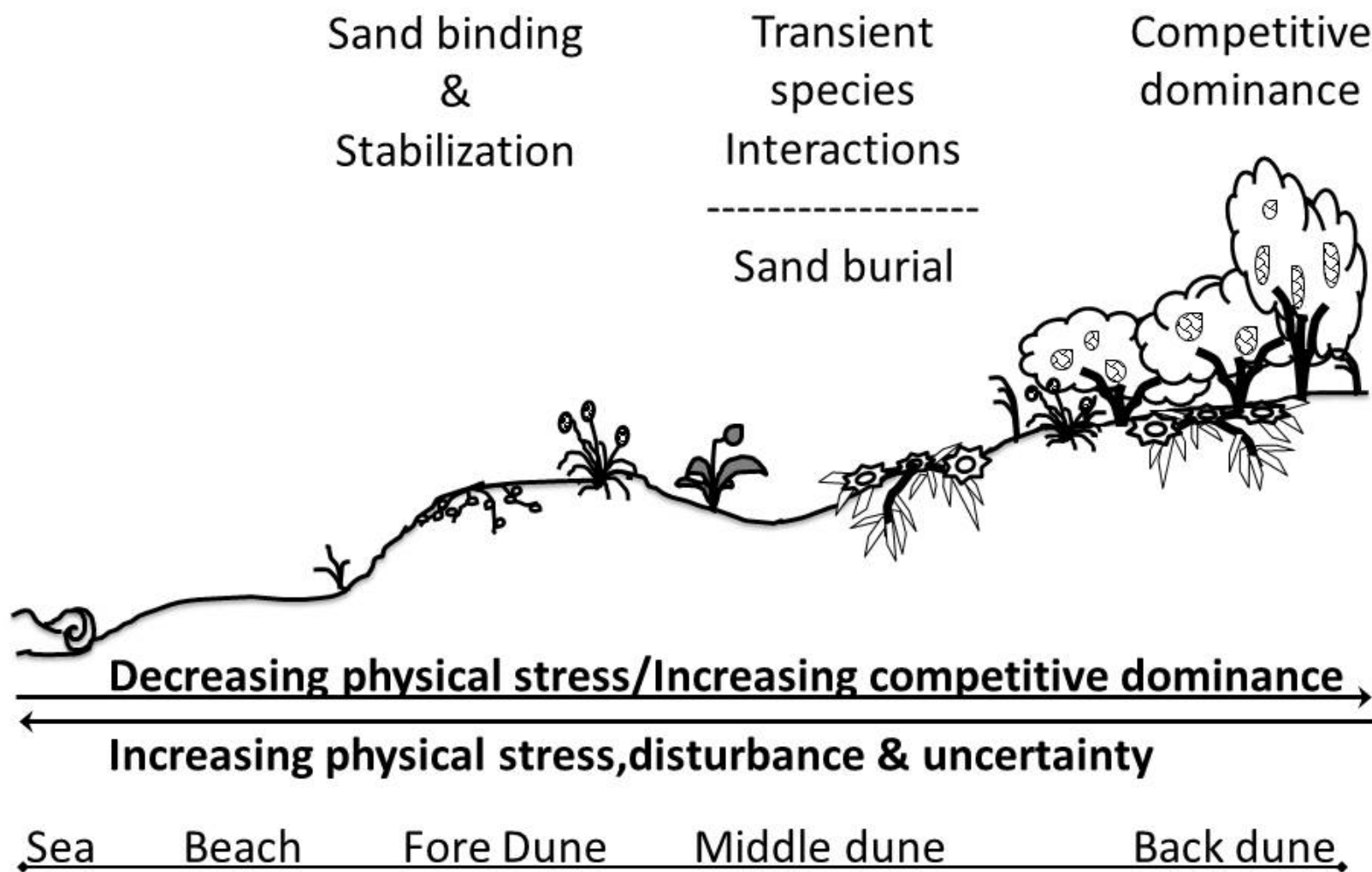


FIG. 1. Conceptual model of the assemble rules of the Badesi sand dune plant community.

Ecosystems, like sand dunes, where the establishment of foundation species ameliorates stresses allow community development that wouldn't occur without foundation species. Coral reefs (Hughes and Jackson 1985), salt marshes (Pennings 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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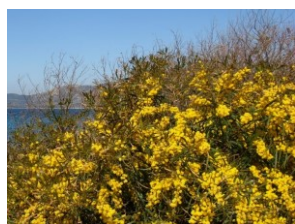
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Appendix A in the Ecological Archive includes 1 figure and 1 table detailing soil edaphic conditions for each zone (fore, middle and back) pH, conductivity, total CaCO₃, organic carbon, soil organic content and N total.

Table 1. Soil edaphic conditions of Badesi dune. Data are mean and standard error (SE) for 8 replicates per each zone (fore, middle and back). ND=not detected.

	Fore		Middle		Back	
	Mean	SE	Mean	SE	Mean	SE
pH (H₂O)	9.30	0.04	9.25	0.05	8.51	0.06
Conductivity 5:1 25°(µS/cm)	78.38	10.63	54.24	3.40	67.03	4.09
Total CaCO₃	36.38	2.61	40.88	2.57	23.63	2.66
Organic Carbon	1.32	0.51	1.58	0.40	6.36	0.88
Soil organic content	1.79	0.50	2.71	0.68	10.97	1.52
N total	ND	-	ND	-	0.27	0.05



Acacia cyanophylla



Armeria pungens



Astragalus thermensis



Cakile maritima



Carpobrotus acinaciformis



Ephedra distachya



Helichrysum microphyllum



Juniperus spp.



Lotus cytisioides



Otanthus maritimus



Pancratium maritimum



Pinus spp.

Figure 1: Badesi dune species named at least once in the text.