# Hierarchical organization of a Sardinian sand dune plant community

Valentina Cusseddu, Giulia Ceccherelli, Mark Bertness

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.

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19  *Abstract* (246 words). Coastal sand dunes have attracted the attention of plant ecologists for

20  over a century, but they have largely relied on correlations to explain dune plant community

21  organization. We experimentally examined longstanding hypotheses that sand binding,

22  interspecific interactions, abiotic factors and seedling recruitment are drivers of sand dune

23  plant community structure in Sardinia, Italy. Removing foundation species from the fore,

24  middle and back dune habitats over 3 years led to erosion and habitat loss on the fore dune

25  and limited plant recovery that increased with dune elevation. Reciprocal species removals in

26  all zones suggested that interspecific competition is common, but that dominance is transient,

27  particularly due to sand burial disturbance in the middle dune. A fully factorial 2-year

28  physical factor manipulation of water, nutrient availability and substrate stability revealed no

29  significant proximate response to these abiotic factors in any dune zone. In the fore and

30  middle dune, plant seeds are trapped under adult plants during seed germination, and seedling

31  survivorship and growth generally increase with dune height in spite of increased herbivory

32  in the back dune. Sand and seed erosion lead to limited seed recruitment on the fore dune

33  while high summer temperatures and allelopathy lead to competitive dominance of woody

34  plants in the back dune. Our results suggest that Sardinian sand dune plant communities are

35  hierarchically organized, structured by sand binding foundation species on the fore dune,

36  sand burial in the middle dune and increasingly successful seedling recruitment, growth and

37  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

67    land/sea interface, like sand dune, salt marsh, sea grass and mangrove communities, provide

68    important ecological services including stabilizing shorelines from erosion and storm

69    damage, harboring animal diversity, providing nursery habitats to threatened avifauna,

70    marine turtles, and shellfish, as well as processing nutrient-rich terrestrial runoff (Barbier et

71    al. 2013). Provisioning of these services relies on informed management facing increasing

72    threats. Sand dunes, however, are generally not conservation priorities and not managed to

73    protect their ecosystem services (Ehrenfeld 1990, Everard et al. 2010).

74    Most research on sand dune communities has been descriptive and reliant on dated

75    correlative literature (e.g. Cowles 1899, Oosting and Billings 1942, Mack and Harper 1977).

76    Experimental work has been restricted to small scale sand burial, seed dispersal and

77    disturbance studies (Maun and Perumal 1999, Franks and Peterson 2003, Miller et al. 2010),

78    field studies of annuals, nurse plant effects and comparisons among chronosequences as a

79    proxy for successional shifts in plant species interactions (Lichter 2000, Franks 2003,

80    Cushman et al. 2010), experimental grazer studies (Huntzinger et al. 2008) and greenhouse

81    studies of the importance of plant/mycorrhizal associations (Gemma et al. 1989).

82    Experimental studies of the roles of the dominant foundation species that have long been

83    hypothesized to build and maintain sand dune plant communities by binding sand and

84    ameliorating potentially limiting physical conditions such as water and nutrient limitation are

85    notably absent. Consequently, the critical interactions between biological and physical

86    processes that have been assumed to generate the organization of sand dune communities

87    have not been rigorously tested.

88    Descriptive and correlative studies of sand dune communities suggest that substrate

89    stabilization, water and nutrient limitation, and plant facilitation and competition are the main

90    drivers of sand dune community structure and organization (Barbour et al. 1985, Ehrenfeld

91    1990, Lichter 1998, Isermann 2011). Typically, distinct plant zonation occurs in coastal sand

92  dune systems (Hesp 1991, Lortie and Cushman 2007, Acosta 2009). On the seaward border

93  of sand dunes, the fore dune, a limited number of clonal pioneer plant species with deep roots

94  trap and bind sand, initiating dune formation. These plants stabilize substrate, trap seeds, and

95  grow vertically and horizontally as sand accumulates, building the seaward border of sand

96  dunes (Cowles 1899, Oosting and Billings 1942). At higher elevations in the middle dune,

97  plant species diversity increases, but unvegetated free space remains common. At these

98  elevations, substrate stabilization remains important, but interspecific plant interactions,

99  including facilitation (Franks 2003, Castanho et al. 2015) and competition (Lichter 2000),

100  and water and nutrient limitation also appear to mediate plant success. In the back dune,

101  furthest from ocean winds and salt spray, the dominant physical stresses of substrate

102  instability and low soil nutrients and moisture are less severe, plant cover typically reaches

103  100%, and numerically dominant woody shrubs or trees, appear to competitively displace

104  plants that dominate lower dune elevations (Lichter 2000).  This descriptive structure of dune

105  communities is consistent with a hierarchical community organization model and the stress

106  gradient hypothesis of community assembly.

107  The hierarchical model of community organization (Bruno and Bertness 2001, Ellison et al.

108  2005, Altieri et al. 2007) hypothesizes that within communities built by biogenic foundation

109  species, dependent species are only able to persist through positive interactions and feedbacks

110  initiated by primary foundation species. The stress gradient hypothesis (Bertness and

111  Callaway 1994, Maestre et al. 2009, He and Bertness 2014) proposes that the biological

112  processes controlling community development shift from positive, facilitative interactions in

113  physically and biologically harsh environments to negative, competitive interactions in

114  benign environments. Many shoreline communities built and maintained by foundation

115  species, such as salt marshes, coral reefs, mangrove forests, mussel reefs, and seagrass beds,

116  have been hypothesized to be hierarchically organized by the general principles of the stress

117  gradient hypothesis (Bruno and Bertness 2001).

118  In this paper we examine long-standing assumptions of sand dune plant community ecology.

119  We hypothesize that sand dunes are hierarchically organized, initially built by facilitation, but

120  ultimately structured by spatially and temporally predictable shifts from facilitative

121  interactions to competitive interactions and seedling recruitment across decreasing stress

122  gradients. Many of these patterns were initially theorized in classic descriptive work (Cowles

123  1899), but have never been tested experimentally. We take a field experimental approach to

124  test these underlying assumptions and elucidate the sand dune community assembly by

125  examining the hypotheses that: 1) foundation species are responsible for stabilizing the

126  seaward border of the dune from erosion and habitat loss, 2) at intermediate dune elevations,

127  interspecific plant interactions, plant resource availability, and seedling recruitment dictate

128  plant abundance and distribution, and 3) at high dune elevations, reduced physical stresses

129  lead to increased plant abundance, interspecific competitive dominance and displacement.

130  Our results support a hierarchical model of sand dune community organization and confirm

131  many, but not all of the assumptions of early work on the organization of these communities.

132                              **MATERIALS AND METHODS**

133  Our study was carried out on the Badesi dunes (40° 56' 45.571'' N, 8° 49' 41.048'' E) on the

134  North coast of Sardinia, in the Mediterranean Sea. It is a wide dune system at the mouth of

135  Coghinas River and has a plant community similar to other dune systems in Sardinia

136  (Cusseddu personal observations), it is within a Site of Community Importance (SIC) and the

137  Town of Badesi gave us the field permit to undertake the field experiments (approval

138  number: 3343 - 23/03/2012). The Coghinas dunes are almost three kilometers long

139  overlooking the Asinara Gulf, with a width of approximately five hundred meters. Sardinia

140    has a warm temperate Mediterranean climate with hot, dry summers and cooler, wet winters.

141    As in other Mediterranean habitats, most plant growth and reproduction occurs during the

142    winter months (Blondel and Aronson 1999). Previous studies of Sardinian sand dune plant

143    communities reveal that they have high species richness, endemism (Bacchetta et al. 2008,

144    Prisco et al. 2012) and adaption to physical stress (Fenu et al. 2013).

145    *Zonation survey*

146    To quantify the plant distribution across the Badesi dune, we surveyed transects in each

147    distinct plant zone. We defined three major dune plant zones: the fore dune, located on the

148    seaward border of the dune and characterized by a steep lower border of stabilized sand and

149    low plant diversity; the middle dune, characterized by high but patchy plant species diversity

150    and 40-60% bare sand cover; and the back dune, characterized by total woody plant cover

151    (Fig. 2). In addition to these zones we will also refer to the front of the fore dune, or the

152    seaward border of the fore dune where embryo dunes (Cowles 1899) form and transition

153    areas between major vegetation zones. Our objective was to develop a site description of the

154    plant community.

155    To assess the plant zonation, we quantified vegetation along 120 m transects parallel to the

156    water in each major dune zone: the embryonic fore dune, the top fore dune on the first ridge

157    of the dune formation, the middle dune with high plant species richness and bare substrate,

158    and the back dune dominated by woody plants. In each zone we ran a transect line, starting

159    from a random point, parallel to the shoreline and every 2 m placed a 0.5x0.5 m sampling

160    quadrat subdivided into a 25 5x5 cm sampling grid to estimate plant species and unvegetated

161    substrate cover. Sixty quadrats were sampled/zone. We characterized species diversity for

162    each zone with the Shannon diversity index (H). All plants, after introduced, will be referred

163    to by genus names.

164  In addition to vertical zonation, a conspicuous feature of plants on the fore and middle dunes

165  were that they generally occurred on elevated sand mounds. Since sand in these zones is

166  mobile and windblown, we hypothesized that these mounds were created by the presence of

167  plants rather than plant establishment on transient dune features (Cowles 1899). We used an

168  auto level and stadia rod to quantify the elevation of plants in each zone (n=20/zone) and bare

169  sand adjacent to (25-cm away) each plant. In each zone we randomly selected 20 adult

170  individuals of the most common plant species, measured their elevation and the elevation of

171  unvegetated sand 10-20 cm away. Plant and adjacent sand elevation differences were

172  calculated, tested for homogeneity of variances with Cochran's test, transformed as necessary

173  (Underwood 1997) and analyzed by species with a one-way ANOVA and by a zone x plant

174  ANOVA for *Armeria*, the only species in all zones.

175                              *Foundation species removal experiment*

176  To test the hypothesis that foundation dune plants bind sand and build the dune habitat, as

177  well as to quantify secondary succession, we performed a foundation species removal

178  experiment. In each of the 3 major dune zones in March 2012, we marked the corners of 24

179  randomly placed 1x1 m plots each separated by at least 10 m. Each plot was individually

180  labeled and then randomly assigned in each zone as controls, total species removals, or

181  procedural controls (n=8/treatment/zone). The 4 corners of each plot were marked with 2 cm

182  diameter rebar driven to refusal into the sand and cut to initially extend 10-15 cm above the

183  sand surface. Control plots were otherwise untouched. In plant removal replicates, plots were

184  perimeter trenched to 30 cm with straight edged shovels and all vegetation in the plots was

185  sprayed weekly for 3 weeks with a systemic herbicide (Roundup, Monsanto) until all

186  vegetation was dead. Dead aboveground vegetation was left to simulate natural death.

187  Roundup is widely used in ecological research, has localized effects if plots are trenched to

188  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

238    coincided with the death of the ice plant *Carpobrotus* at high dune elevations. To examine

239    this hypothesis, we quantified the long-term effect of shading on the Badesi dune as a proxy

240    for high temperature impacts. In February 2015, we quantified shaded and unshaded

241    vegetation adjacent to boardwalks (1.5 m wide, elevated 50 cm over the substrate)

242    perpendicular to the shoreline that extended from the fore dune to the highest point of the

243    back dune, and 4 m on both sides of the boardwalks. We hypothesized that shading by the

244    boardwalk would decrease solar stress on ice plants. We sampled 2 boardwalks that had been

245    in place 5 years. At each boardwalk we quantified live and dead ice plant % cover in 0.5x0.5

246    m quadrats every 2 m from the beginning of the fore dune to the back dune. For analysis we

247    excluded plots levels without ice plants and pooled the data from adjacent and control plots.

248    Dead/alive ratio of *Carpobrotus* % cover and dead *Carpobrotus* % cover were transformed to

249    meet the assumptions of parametric statistics and analyzed with a one-way ANOVA.

250                              *Physical stress alleviation experiment*

251    To test the hypothesis that physical stress limits dune plant recovery across the dune, we ran a

252    fully factorial experiment across all zones manipulating all combinations of nutrient

253    limitation (with 33 ml of slow release Osmocote NPK pellets spread on them every 6 months

254    and without nutrient additions), water limitation (with 2 liters of tap water every 2-3 weeks

255    and without water additions) and substrate stability (with and without substrate stabilizing

256    fish net attached flush to the surface with wire staples). In each zone we located 72 0.5x0.5 m

257    plots with bare sand substrate. Every combination of nutrient, water, substrate limitations and

258    controls were marked and labeled with numbered rebar corner posts (n=8/treatment

259    combination/zone). Replicate treatments were maintained for two years and all plots were

260    monitored for % plant cover initially and then for 2 years in the spring and fall. Plant cover in

261    the plots was transformed to meet parametric statistic assumptions and analyzed with a

262    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 $\chi^2$ 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    *Pancratium maritimum*). For each species, we collected dehiscing seed heads and dissected

287     out and separated the seeds of each species. We then sorted them into aliquots of 4-10 seeds

288     depending on species seed availability and placed them by species into polyester mesh bags

289     that would retain the seeds, but would allow germination and seeding growth. For each

290     species we planted 21 seed bags in each zone (fore, middle and back) under conspecific

291     adults and in unvegetated sand >30 cm from adult vegetation and marked their location with

292     color-coded wire markers. We monitored seed transplants weekly for germination and

293     seedling survivorship for 3 months. Germination and seedling survivorship were analyzed

294     separately by species with non-parametric log rank data, using a $\chi^2$ test.

295     To test the hypothesis that loose seeds were eroded away on the fore dune, we also

296     transplanted seeds of *Pancratium* in mesh bags (n=10), loose in the sand (n=10), and loose in

297     the sand covered on the sand surface with nylon mesh (2 mm mesh, 5x5 cm cover pinned to

298     the sand surface with garden fabric staples; n=10). We only did this experiment in the fore

299     dune since the fore dune was the only zone that showed significant erosion. Seed species

300     were marked and identified by color-coded wires (2 mm). All seeds were planted 3 cm below

301     the sand surface. We monitored these transplants for germination and survivorship weekly for

302     the first 3 months and monthly for one year. Germination and seedling survivorship data were

303     analyzed with a non-parametric log rank data, using a $\chi^2$ test.

304                      *Back dune competitive release experiment*

305     It was not possible to do *Pinus* removal experiments similar to the reciprocal plant species

306     removal experiments or foundation species removals in other zones because of the size of

307     *Pinus* trees. Since the Badesi dune is a protected conservation area, removing entire *Pinus*

308     trees would have been destructive and not permitted. To test the hypothesis that *Pinus*

309     domination of the terrestrial high dune border is due to competitive dominance, we did a

310     competitive release experiment by removing large (~2m long) *Pinus* branches and estimated

311 natural plant recruitment by following seed germination and cover in 1.5x1.5 m plots under

312 *Pinus* canopies (n=10), where *Pinus* shading was alleviated by branch removal (n=10) and in

313 areas where *Pinus* shading was removed but replaced by a similar level of shading by shade

314 cloth (n=10), as procedural controls. A central 0.5x0.5m quadrat in each plot was monitored

315 photographically monthly for a year.

316 To test the hypothesis that allellopathy contributes to the dominance of *Pinus* and ice plant in

317 the back dune, we also manipulated sediment in the *Pinus* plots described above. Allelopathy

318 was suggested because in dense *Pinus* and ice plant stands, natural substrate is covered by

319 *Pinus* needles and ice plant leaves, but seedlings of all species are extremely rare

320 (Ceccherelli, personal observation). In 100 ml plastic greenhouse seedling pots we planted

321 seeds of *Armeria*, *Pancratium*, and *Lotus* (plus no seed controls) with either 1) *Pinus* soil, 2)

322 middle dune bare soil, 3) middle dune ice plant soil, 4) potting soil mixed with sand, 5)

323 potting soil with a 2 cm layer of *Pinus* needles and 6) potting soil with a 2 cm layer of ice

324 plant leaves (n=10/*Pinus* treatment/soil type) and scored them monthly for germination. Seed

325 germination and survivorship of *Armeria*, and *Lotus* were transformed with Ln (X+1) and all

326 were analyzed with *Pinus* treatment x soil type ANOVA.

327 **RESULTS**

328 *Zonation survey*

329 Elevational zonation of plants across the Badesi dune is striking (Fig. 1). The fore dune has

330 low plant richness and cover (35%) and 65% unvegetated sand cover. *Armeria* (sea rose),

331 *Otanthus maritimus* (cotton weed) and *Lotus* (trefoil of the cliffs) are the numerically

332 dominant plant species on the fore dune and all are clonally spreading, deep-rooted

333 perennials. The width of the fore dune varies at Badesi from ~20-25 m. The middle dune has

334 over 28% higher plant cover and 53% higher species richness than the fore dune, but still has

335    considerable bare sand substrate (Fig. 2, 38%). *Armeria*, *Carpobrotus* (ice plant) and

336    *Ephedra distachya* (joint pine) are the most common middle dune plant species and are all

337    clonally spreading perennials. The middle dune is ~30-35m wide.

338    The transition from the middle to back dune is more gradual (Fig. 1). The seaward border of

339    the back dune has the highest plant species richness on the dune, and is dominated by the ice

340    plant, *Carpobrotus*, a perennial succulent, that can be seen overgrowing other back dune

341    plants like *Pinus* on the terrestrial border of the dune (33%). At higher elevations of the back

342    dune, *Pinus* dominates the landscape as a solitary evergreen species that has a prostrate

343    morphology at lower elevations, an arborescent morphology at higher elevations and an

344    understory of bare substrate in the dense *Pinus* canopy that dominates high elevations.

345    Substrate topography in relation to plants varied across the dune. In dune zones closest to the

346    water, plants were found on elevated sand mounds that decreased in elevation with distance

347    from the water, while in the back dune, furthest from the water, plants were not associated

348    with sand mounds. On the front dune, *Armeria* and *Otanthus* were found on sand mounds 53

349    $\pm$ 2.16 cm (mean $\pm$ SE) and 56 $\pm$ 2.51 cm higher than adjacent substrate, respectively. For the

350    front dune, the one-way ANOVA did not reveal any significant difference between the two

351    species ($F_{1,38}$=0.36 p>0.05). On the fore dune, *Lotus* and *Armeria* were similarly found on

352    mounds 17 $\pm$ 4.64 and 24 $\pm$ 1.92 cm high, respectively, and no significant effect was detected

353    with the ANOVA ($F_{1,38}$=2.07 p>0.05). In contrast, in the middle dune, *Armeria*, *Carpobrotus*,

354    *Lotus*, *Helicrysum microphyllum* and *Ephedra* were found on smaller sand mounds 22 $\pm$ 1.47,

355    12 $\pm$ 2.06, 11 $\pm$ 1.06, 16 $\pm$ 1.27, and 12 $\pm$ 2.48 cm high, respectively. In this zone the

356    difference among species was significant ($F_{4,95}$=7.51 p<0.0001, SNK: *Armeria*>all others).

357    These front, fore and middle dune plants occur on elevated sand mounds that appear to be

358    actively trapped by passive deposition of wind-blown sand. *Armeria*, a robust perennial

359    shrub, was found on higher sand mounds than all other species.  One-way ANOVA on

360    *Armeria* revealed differential *Armeria* sand binding by zone ($F_{3,76}$=111.36 p<0.0001, SNK:

361    front>fore top=middle>back). In the back dune, buffered from winds and sand transport by

362    the lower dune zones, *Armeria* = 9 ± 1.65 cm, *Carpobrotus* = 4 ± 1.31 cm, *Pinus* = 4 ± 2.76

363    cm, *Acacia cyanophylla* = 0 ± 3.8 cm, and *Juniperus* spp. (*Juniperus macrocarpa and*

364    *Juniperus phoenicea*) = -2 ± 4.72 cm were not found on elevated locations and there were no

365    significant species effects ($F_{4,95}$=1.93 p>0.05).

366                            *Foundation species removal experiment*

367    Removing foundation species had different effects across zones (Fig. 3). We performed a

368    two-way ANOVA of zone and treatment, both considered as orthogonal fixed factors. The

369    analyses revealed a significant zone x treatment interaction ($F_{4,63}$=2.67 p<0.05). In the fore

370    dune, foundation species removal led to sand erosion of >2 cm/year, in contrast to control and

371    procedural control plots that had annual sand accretion rates of >4 cm/year (Fig. 3, p<0.05,

372    SNK test fore: removal<procedural control=control). Sand erosion in the fore dune

373    foundation species removal plots was dramatic and led to the collapse of the fore dune edge

374    in just two years. This was reflected in the erosion of the seaward corner posts in the fore

375    dune, where, while the seaward edge corner post of procedural control (5.29 ± 1.3 cm/year)

376    and control plots (3.66 ± 1.77 cm/year) showed net sand accretion, when foundation species

377    were removed, erosion on the seaward edge corner posts increased over 60% leading to an

378    amount of sand dispersion of -2.8 ± 1.6 cm/year (Fig. 3).

379    In the middle dune, sediment variation resulted in net sand accretion that occurred in all

380    treatments (Fig. 3, p<0.05, SNK test: fore=back<middle), but was nearly twice as high in

381    control and procedural control plots with live vegetation to bind and trap sand than in

382    removal plots (SNK middle: removal<procedural control=control).

383    In the back dune, annual sediment accretion was more than an order of magnitude less than

384    middle dune plots with foundation species (Fig. 3). Sand accretion in the back dune was also

385    similar among foundation removal, control and procedural control treatments (Fig. 3, p<0.05,

386    SNK back: removal=procedural control=control). In all treatments, annual accretion was <1

387    cm/year and when foundation species were removed there was no sand accretion.

388    Mean minimum temperature (Min) did not differ among treatments or zones (fore 18.73 ±

389    0.45° C and 18.50 ± 0.4° C, middle 19.11 ± 0.28° C and 19.12 ± 0.18° C, back 16.59 ± 0.77°

390    C and 17.07 ± 0.87° C for removal and control, respectively; $F_{2,228}$=1.77 p>0.05 for the

391    interaction zone x temperature range x treatment). In contrast, mean maximum temperature

392    (Max) differed among zones (fore 33.73 ± 1.40° C and 32.83 ± 2.46° C, middle 41.86 ± 2.74°

393    C and 38.31 ± 1.34° C, back 44.89 ± 3.61° C and 40.26 ± 3.45° C; $F_{2,114}$=39.85 p<0.0001,

394    SNK: fore<middle=back) peaking in back zone removal treatments ($F_{1,114}$=10.72 p<0.002,

395    SNK: Removal>Control). Maximum temperatures peaked around 70° C, and averaged 45° C

396    in the removal plots, 5° C lower than when vegetation was present.

397                *Reciprocal species removal experiments (Interspecific plant interactions)*

398    Evaluation of interspecific interactions of common plant species in all dune zones with

399    reciprocal species removal experiments initially revealed significant interspecific interactions

400    (Fig. 4). Over time, however, these interactions were lost due to sand burial and erosion

401    disturbance, particularly in the fore and middle dune zones as evidenced by a decrease in

402    plant cover over time in the species interaction plots (Fig. 5, middle p<0.001, t test). After 18

403    months, 22% of the middle dune reciprocal species interaction plots had been completely lost

404    due to sand burial, and an additional 10% of the plots were still recognizable but almost

405    totally buried, while no plots were lost in the fore or back dune. To analyze this experiment,

406    we ran separate two-way ANOVAs for each species after 12 months and 18 months of

407    treatments, taking into account species interaction (comparison of each pair in which the

408    species is involved) and treatment (reciprocal species removal vs control) as orthogonal and

409    fixed factors. Insignificant interaction p values will always refer to the last sampling date (18

410    months). In the fore dune after 6, 12, and 18 months, there was no evidence of reciprocal

411    effects between *Armeria* and *Lotus* (Fig. 4, interaction x treatment $F_{4,70}=1.96$ p>0.05 and

412    $F_{2,42}=2.27$ p>0.05, respectively).

413    In the middle dune, after one year of treatment, there was evidence of *Armeria* and *Lotus*

414    competitively depressing *Carpobrotus*, but after two seasons this competitive effect was no

415    longer apparent (Fig. 4, treatment $F_{3,56}=12.12$ p<0.03, SNK reciprocal species

416    removal>control at 12 months, interaction x treatment $F_{3,56}=1.23$ p>0.05 at 18 months). At

417    the same time, there was no evidence of interactions affecting *Armeria* or *Lotus* (Fig. 4,

418    interaction x treatment $F_{4,70}=1.96$ p>0.05 and $F_{2,42}=2.27$ p>0.05, respectively). In all the

419    interspecific interaction plots in the middle dune there was, over time, a general decrease in

420    plant cover associated with sand burial disturbance (Fig. 5, p<0.001, t test).

421    In the back dune sand burial was not prevalent, but a summer die off of *Carpobrotus* was

422    seen every year (see below). *Carpobrotus* and *Pinus* had a facilitating effect on *Armeria* after

423    12 months but not at 18 months (Fig. 4, interaction x treatment $F_{4,70}=3.31$ p<0.02, SNK test

424    for both Back pairs: reciprocal species removal<control at 12 months, $F_{4,70}=1.96$ p>0.05 at 18

425    months). While, after one year of removal, the opposite was found for *Carpobrotus*, which

426    was competitively depressed by both *Armeria* and *Pinus*, but after another two seasons of

427    observation these interactions were no longer detectable (Fig. 4, treatment $F_{3,56}=12.12$

428    p<0.03, SNK reciprocal species removal>control at 12 months, interaction x treatment

429    $F_{3,56}=1.23$ p>0.05 at 18 months). Reciprocal removal of *Pinus* in the back dune did not reveal

430    significant species interactions (Fig. 4, interaction x treatment $F_{1,28}=0.18$ p>0.05). In the back

431    dune species removal plots, the most conspicuous trend over time was the decrease in the

432    cover of *Carpobrotus* during summer. This left large areas of dead, desiccated *Carpobrotus*

433    dominating the seaward border of the back dune at the end of the summer (Cusseddu,

434    personal observation).

### *Boardwalk shadow effect sampling*

436    Analysis of *Carpobrotus* cover data (dead/alive ratio) adjacent to and 2 m away from the

437    shade of boardwalks revealed almost twice as much live ice plant cover under the shade of

438    the boardwalks ($46 \pm 3.24\%$) than in adjacent unshaded habitats ($25 \pm 2.84\%$; $F_{1,190}=5.58$

439    $p<0.02$).  There was also nearly 10% more dead ice plant in unshaded habitats ($16 \pm 1.76\%$)

440    than under the shade of boardwalks ($7 \pm 1.10\%$, $F_{1,190}=18.76$ $p<0.0001$). Since ice plant death

441    occurs almost exclusively in summer (Cusseddu, personal observation), ice plant mortality at

442    high dune elevations appears to be driven by heat and desiccation from direct sun exposure

443    leaving desiccated ice plant skeletons marking their initial distribution.

### *Physical stress alleviation experiment*

445    Experimentally manipulating water availability, nutrient availability and substrate stability

446    across dune zones (fore, middle and back) in a fully factorial 2 year experiment at a 0.5x0.5

447    m spatial scale revealed that these factors, long thought to be critical to sand dune plant

448    communities, had no effect on plant recovery, as evidenced for each zone using ANOVA

449    with treatment as orthogonal and fixed factor (Fig. 6, fore $F_{9,70}=1.95$ $p>0.05$, middle

450    $F_{9,70}=2.16$ $p<0.04$ but not significant post hoc SNK, back $F_{9,70}=0.57$ $p>0.05$). There were no

451    significant responses in plant biomass or species identity to treatments within zones, but a

452    two-way ANOVA (zone and treatment orthogonal and fixed), showed that recovery increased

453    from the fore to back dune (Fig. 6, $F_{2,210}=18.30$ $p<0.0001$, SNK fore<mid<back), although

454    treatment and the zone x treatment interaction were not significant ($F_{9,210}=1.70$ $p>0.05$ and

455    $F_{18,210}=0.87$ $p>0.05$, respectively).  No effect of the environmental manipulations was found

456     at these levels, but this does not mean that other levels would not have an effect, or that water

457     and nutrient availability are unimportant for the structure of dune communities.

458                         *Seedling recruitment: seed and seedling quantification*

459     Seeds of the most common dune plants were most abundant under adult plants independent of

460     species identity and dune zone (Fig. 7). For each zone, we performed a three-way ANOVA

461     considering the species, the substrate (under adult vs bare sand) and the seeds category

462     (conspecific vs other seeds) all as orthogonal and fixed factors. We will present only the

463     factors and/or the interactions that were significant.

464     On the front of the fore dune, seeds of *Armeria* and *Otanthus* were almost exclusively found

465     under conspecific adults, whereas the seeds of other species were rare and not found

466     associated with adult plants (Fig. 7, substrate x seeds interaction $F_{1,72}=0.11$ p<0.02). On the

467     top of the fore dune, seeds of *Armeria* were more common under *Armeria* adults than in

468     nearby bare sand, but for *Lotus* and *Pancratium*, seeds of conspecifics were just as common

469     in bare sand than under adults (Fig. 7). For *Lotus* and *Pancratium,* conspecific seeds were

470     equally distributed under and away from adults, but seeds of other species were significantly

471     more common under *Lotus* and *Pancratium* than in adjacent bare sand (Fig. 7, species x seed

472     category interaction, $F_{2,108}=4.43$ p<0.02 and species x substrate x seeds category $F_{2,108}=3.45$

473     p<0.04, respectively).

474     In the middle dune, *Armeria* seeds were more common under *Armeria* adults than in adjacent

475     bare sand, but seeds of other species were not (Fig. 7), while for *Carpobrotus*, conspecific

476     seeds were rare, but seeds of other species were more than an order of magnitude more

477     abundant under *Carpobrotus* than in bare sand (Fig. 7, species x substrate x seeds interaction,

478     $F_{1,72}=12.59$ p=0.0007). In the back dune, *Armeria* seeds were 20 times more common under

479     conspecifics than in adjacent bare sand, while for *Carpobrotus*, seeds of other species were

480    significantly more abundant under *Carpobrotus* than in adjacent bare substrate (Fig. 7,

481    species x substrate x seeds interaction $F_{1,72}=8.34$ p<0.006).

482    The survivorship of marked seedlings was analyzed with a log rank test comparing the

483    survival of the seedlings of each species comparing the proximity with adult (next to adult vs

484    adjacent bare sand) and of the seedlings in general pooled in three groups corresponding to

485    the three zones (fore vs. middle vs. back seedlings), on the times (weeks) of survival events.

486    The percent of seedlings survivorship increased dramatically with dune zone elevation but

487    was not affected by neighboring plants (Fig. 8, p<0.05, $\chi^2$). In the fore dune, no *Lotus*

488    seedlings survived (independent of the proximity to adult neighbors), no *Pancratium*

489    seedlings survived on bare sand, and less than 5% of *Pancratium* seedlings survived next to

490    adults, but this result was not significant (p>0.5, $\chi^2$). In the middle zone only 5% of *Lotus*

491    seedlings survived with or without adult neighbors (p>0.5, $\chi^2$), while for *Armeria* 25% of

492    marked seedlings in bare sand survived, nearly three times (10%) the number of seedlings

493    that survived next to adults (p<0.025, $\chi^2$). No *Pancratium* seedlings survived. In the back

494    dune survivorship of *Armeria* and *Carpobrotus* seedlings was over 80%, far higher than any

495    other zone, and was not influenced by the presence or absence of neighbors (Fig. 8).

496                    *Seedling recruitment: seed transplant experiments*

497    Of the six dune plant species seeds used in transplant studies, three species, *Pancratium*,

498    *Lotus*, and *Cakile* germinated. Only 2 and 3 *Armeria* and *Otanthus* germinated from back and

499    fore dune planted seeds respectively, while *Astragalus thermensis* and *Carpobrotus* did not

500    germinate in any location or treatment. For each of the three species that had sufficient

501    germination, we compared the germination and survival in each of the three zones. 30-40% of

502    *Pancratium* seed transplants germinated, but germination was similar among zones (p>0.1,

503    $\chi^2$). Survivorship of seedlings from the seed transplant experiment showed that all species had

504  the highest survivorship in the back dune, but this pattern was only significant for

505  *Pancratium*, which had the highest sample size (Fig. 9, p<0.005, $\chi^2$). Less than 10% of the

506  transplanted *Lotus* seeds germinated and *Lotus*, found ubiquitously across the dune, had

507  higher germination in the back dune, and lowest germination in the middle dune (Fig. 9,

508  p<0.025, $\chi^2$), while survivorship did not differ among zones (p>0.5, $\chi^2$). For *Cakile*, a pioneer

509  species found naturally in the fore and middle dune, 12-18% of transplanted seeds germinated

510  and among zones had higher germination in the middle than in fore and back dune (Fig. 9,

511  p<0.05, $\chi^2$).  Survivorship, however, did not different among zones (p>0.5, $\chi^2$).

512  In the fore dune seed stabilization experiment with *Pancratium*, germination and survivorship

513  were analyzed comparing germination and survival among the three treatments (seeds in bags

514  vs loose seeds vs. loose seeds with net covers). Due to erosion, germination was highest in

515  the bagged seed treatment, second highest in the seed treatment with stabilized substrate (net

516  cover), and lowest in the loose seed treatments (Fig. 10, p<0.005, $\chi^2$). The difference in

517  germination between loose seeds and the secured seed treatments was >10%, suggesting that

518  sand erosion in the fore dune can lead to seed loss. Seedling survivorship in this experiment

519  was similar among stabilization treatments (p>0.25, $\chi^2$).

520  *Back dune competitive release experiment*

521  Natural plant recruitment was not observed under *Pinus*, irrespectively of canopy removal

522  treatments. However, germination of experimentally planted seeds under *Pinus* occurred,

523  although it was generally lower in control plots, especially for *Armeria* and *Lotus* (Fig. 11).

524  We ran a two-way ANOVA by species with canopy treatment and soil type as fixed and

525  orthogonal factors. Under the *Pinus* canopy, *Armeria* seeds attracted small animals as they

526  were often removed or eaten. *Armeria* germination, however, was significantly higher in the

527  *Pinus* removal treatment in middle dune soil, and very little germination occurred in any soil

528    type under *Pinus* canopy and in procedural control plots (Fig. 11, canopy treatment x soil

529    type interaction, $F_{12,126}=2.54$ p<0.005). *Pancratium* and *Lotus* germination was not

530    influenced by the *Pinus* treatment ($F_{2,126}=2.11$ p>0.05 and $F_{2,126}=0.79$ p>0.05, respectively),

531    but they were significantly affected by soil type (Fig. 11, $F_{6,126}=5.02$ p=0.0001 and

532    $F_{6,126}=7.06$ p<0.0001, respectively), even if, for both species, there were no significant post

533    hocs comparisons. The interaction *Pinus* treatment x soil type was not significant for both

534    species ($F_{12,126}=0.96$ p>0.05 and $F_{12,126}=0.84$ p>0.05, respectively).

535                                              **DISCUSSION**

536    Our experiments testing the basic assumptions of sand dune community organization

537    generally support earlier descriptive and correlative studies. This is unusual. Experimental

538    examination of community assemblages typically has not upheld the findings of earlier

539    correlative conclusions. For example, rocky intertidal, salt marsh, kelp forest, and coral reef

540    community dynamics once thought to be driven by physical forces (e.g. Odum 1953, Lewis

541    1964, Mann 1982), were later shown to be strongly regulated by interactions between

542    physical and biological factors once community level experiments were carried out (Dayton

543    1971, Estes and Palmisano 1974, Bertness and Ellison 1987). Our results suggest that fore

544    dune foundation species are responsible for binding, stabilizing and maintaining sand dune

545    communities, and that interspecific plant interactions occur across the dune, but are generally

546    overshadowed by physical stresses, particularly sand burial in the middle dune. Additionally,

547    they indicate that while physical stresses limit plant distributions across the dune, their

548    impacts are largely stochastic, except in the back dune where competitively dominant woody

549    plants competitively displace other sand dune plants.

550              *Biogenic Communities, Foundation species and Hierarchical organization*

551   Like most communities in physically and/or biologically stressful habitats, the Badesi sand

552   dune plant community is dependent on foundation species that ameliorate stress and facilitate

553   community development (Angelini et al. 2011). Our foundation plant species removals reveal

554   that plant species on the fore dune bind and stabilize sand, building the habitat that supports

555   all the dune plants living at higher elevations, making them foundation species-dependent

556   (Fig. 3). Without habitat-modifying foundation species that initially colonize potential fore

557   dune habitats, sand dune plant communities would not develop or be maintained. Biogenic

558   communities that are dependent on foundation species often display this type of hierarchical

559   organization (Bruno and Bertness 2001, Angelini et al. 2011) and include salt marshes

560   (Angelini et al. 2011), terrestrial forests (Ellison et al. 2005), seagrass meadows (Duarte et al.

561   2000), as well as smaller scale associations within communities (Angelini and Silliman

562   2014). This common dependency of communities on foundation species habitat modification

563   needs to be incorporated into ecological theory (Bruno et al. 2003). It is a major organizing

564   force in community assembly, confirmed by the overwhelming evidence that it is the

565   template for communities in physically and biologically stressful habitats due to habitat

566   amelioration and associational defenses, respectively (Bertness and Callaway 1994, Ellison et

567   al. 2005, Crotty and Bertness 2015).

568                                   *Competitive dominance*

569   Pairwise quantification of the interaction of the numerically common plants in each dune

570   zone revealed that interspecific plant competition was common across the dune landscape

571   (Fig. 4). In the middle dune, however, burial disturbance over the 18-months time course of

572   our experiments revealed that, while interspecific plant interactions occurred, their

573   importance was transient and not of long term consequence in generating species distribution

574   patterns. Rather, sand burial disturbance limited the role of interspecific competition and

575   competitive displacement, but promoted plant species diversity and coexistence in the middle

576    dune (Fig. 4). Similar results have been found on rocky shores, intertidal boulder fields,

577    mussel beds, salt marshes, and grasslands (see Dayton 1971, Sousa 1979, Paine and Levin

578    1981, Bertness and Ellison 1987, and Platt 1975, respectively). In the back dune, where sand

579    burial is less common, competitive dominance by woody plants emerges as a major factor

580    leading to competitive dynamics that determine the prevalence of shrub and woody species

581    like *Pinus* and *Armeria* and exclusion of other dune species. Due to the dense *Pinus* canopy,

582    germination of other middle dune species is precluded and in some cases soil type prevents

583    seedling recruitment. Moreover, high summer temperatures in the back dune limit ice plant

584    competitive dominance, because it suffers drying and summer die-off limiting its dominance,

585    favoring *Pinus*.

586                                    *Sand burial disturbance*

587    While our interspecific plant interaction experiments initially revealed significant interactions

588    (Fig. 4), sand burial, particularly in the middle dune, was ultimately the most pervasive and

589    powerful driver of plant community patterns at lower dune elevations, rendering competitive

590    dominance and facilitative interactions inconsequential. Thus, the same physical force that is

591    responsible for building the dune, sand mobility, is responsible for plant mortality and

592    diversity, particularly in the middle dune. Burial in sand is recognized as a major aspect

593    shaping the arrangement and composition of vegetation in coastal sand dune communities

594    (Ranwell 1958, van der Valk 1974, Maun and Lapierre 1986). Sand deposition has been

595    renowned as a main selective force in the evolution of seeds, in survivorship of seedlings and

596    adult plants and, to a larger scale, in zonation and succession of vegetation (Maun 1994 and

597    1998).

598                                    *Seedling Recruitment*

599    Sexual recruitment is generally problematic in physically and biologically stressful

600    communities like salt marshes (Pennings and Callaway 1996) and coral reefs (Hughes and

601    Jackson 1985), respectively. As a result, asexual reproduction and clonal growth play a

602    leading role in the community dynamics of communities in physically or biologically

603    stressful habitats. Sexual recruitment is limited in Sardinian sand dune plant communities

604    exposed to severe substrate mobility and strong wind and salt spray exposure. Dispersed

605    seeds of dune plants are trapped near substrate stabilizing adult plants and are uncommon in

606    unvegetated substrate (Fig. 7). The survivorship of marked seedlings was low, but generally

607    increased from the fore to the back dune and was higher when seedlings were associated with

608    adult plants than when on bare sand substrate without neighbors.

609    Field germination experiments with 3 common species had low germination rates and poor

610    survivorship in all zones except the back dune. Seedlings of back dune grew the best, but

611    suffered from desiccation, due to high temperature and herbivory (Fig. 9). Like in other

612    stressful environments, the low success of seed reproduction appears to lead to nurse plant

613    effects (Franks 2003) and strong reliance of dune plants on clonal reproduction, particularly

614    at low elevations (Maun 1998). Nurse plant effects have been identified as a strong generator

615    of pattern in sand dune plant communities in general (Martinez 2003).

616                                  **CONCLUSIONS**

617                        *Lessons from Sardinian sand dunes*

618    Our results reveal that sand dune plant communities are hierarchically organized and

619    dependent on the establishment of fore dune foundation plants that bind sand above and

620    belowground, leading to sand dune initiation, development, and maintenance (Fig. 3).

621    Without foundation plant species, mechanisms such as sand binding, erosion, mobility, and

622    burial act as barriers to plant community development (Fig. 3) and are more pronounced than

623    at higher elevations that are less exposed to sand mobility. On the fore dune, removing the

624    foundation species led to habitat loss, while competitive and facilitative plant species

625    interactions were not detectable due to sand erosion and burial disturbance (Fig. 4 and 5).

626    Manipulating physical factors thought to be critical in sand dune communities (i.e. water

627    additions, nutrient additions, substrate stabilizations) in well replicated plots of all these

628    factors alone and in combination also did not affect plant colonization at the small $0.25m^2$

629    spatial scale manipulated. Seed and seedling success were also rare on the fore dune

630    suggesting that asexual clonal expansion and colonization was more common in the highly

631    disturbed fore dune habitat. This also suggests that disturbance on the fore dune trumps all

632    other biological and physical factors. Since all of these physical and biological factors have

633    been shown to correlatively impact sand dune communities at regional spatial scales, our

634    results imply that spatial scale is important in understanding process and pattern in sand dune

635    ecosystems since they are so fundamentally shaped by disturbance processes and their

636    interaction with foundation plant species that stabilize the substrate.

637    Plant species cover and richness increased from 35% in the fore dune to 63% in the middle

638    dune (Fig. 1). Like the fore dune, however, recovery from foundation removal was minimal,

639    being less than 1% in 3 years. Moreover, while the middle dune initially revealed

640    interspecific plant interactions, it is subjected to heavy sand burial that limited the role of

641    species interactions, seed germination (Fig. 9), and seedling success (Fig. 8). Therefore,

642    frequent sand burial disturbances have a large influence over the middle dune zone (Fig. 5).

643    In the back dune we see nearly 100% plant cover and the emergence of competitive

644    dominants (Fig. 1 and 2c). On the seaward border of the high dune the ice plant is common

645    and appears to be competitively dominant to middle dune plant species, but, at higher dune

646    elevations, the evergreen *Pinus* competitively displaces most other dune plants, forming a

647    back dune *Pinus* monoculture. Seedling germination and survivorship were higher in the back

648     dune than at lower dune elevations (Fig. 9), as was the recovery of bare space (Fig. 6).  The

649     competitive dominance of *Pinus* in the back dune was shown by a competitive release

650     experiment in which removing *Pinus* canopy in the back dune lead to the success of

651     important foundation species such as *Armeria*, especially favored by middle dune soil (Fig.

652     11). In general, however, seeds of plants characteristic of the middle and fore dune planted in

653     *Pinus* zone showed a quite good capability of germination.  This suggests that they are

654     excluded from the back dune because *Pinus* acts as a physical barrier, having a dense

655     prostrate morphology (Fig. 11).

656      These results are summarized in a conceptual model of the assembly rules of the Badesi sand

657     dune plant community (Fig. 12). The gradient of stress in this dune system acts crosswise,

658     from land to sea and back again, creating an area of aggregation of the various factors in the

659     middle of the dune, in which the living conditions are particularly difficult. It is for this

660     reason that facilitation mechanisms are determined among species, however they are transient

661     in nature due to the high instability of the conditions. Physical stress on the vascular plants of

662     the dune, including wind exposition, sand scouring, and other stressors of terrestrial origins,

663     decreases with increasing dune elevation exposure. Conversely, the role of biological factors

664     in shaping the dune plant community increases with increasing dune elevation. These factors

665     include interspecific plant competition and facilitation, increasing seedling recruitment and

666     survival, and increasing herbivory. The Badesi dune plant community is a biogenic

667     community built and maintained by foundation plant species that stabilize sand on the fore

668     dune. On the middle dune, sand burial disturbance is the dominant structuring force that

669     limits the role of sexual recruitment and interspecific competition in structuring the middle

670     dune zone. In the back dune, ice plant and *Pinus* competitively dominate all other dune

671     plants, but high summer temperatures limit ice plant dominance in the back dune, resulting in

672     *Pinus* competitively dominating the back dune terrestrial border.

673    Ecosystems, like sand dunes, where the establishment of foundation species ameliorates

674    stresses allow community development that would not occur without foundation species.

675    Coral reefs (Hughes and Jackson 1985), salt marshes (Bruno and Bertness 2001), mangroves

676    (Ellison and Farnsworth 2001), seagrass (Duarte et al. 2000), and forest ecosystems (Ellison

677    et al. 2005) have all been explicitly or implicitly described as hierarchically organized

678    systems. In all of these ecosystems, once foundation species enabled community

679    establishment, while other biotic factors like competition, recruitment, and trophic dynamics

680    became important community structuring forces (Bruno and Bertness 2001). These findings

681    and their implications are important for understanding community organization well enough

682    to make it a more predictive science and for conservation since they stress that managing,

683    protecting, and restoring foundation species should often be the first step in many

684    conservation efforts, rather than focusing on charismatic species.

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Figure 1

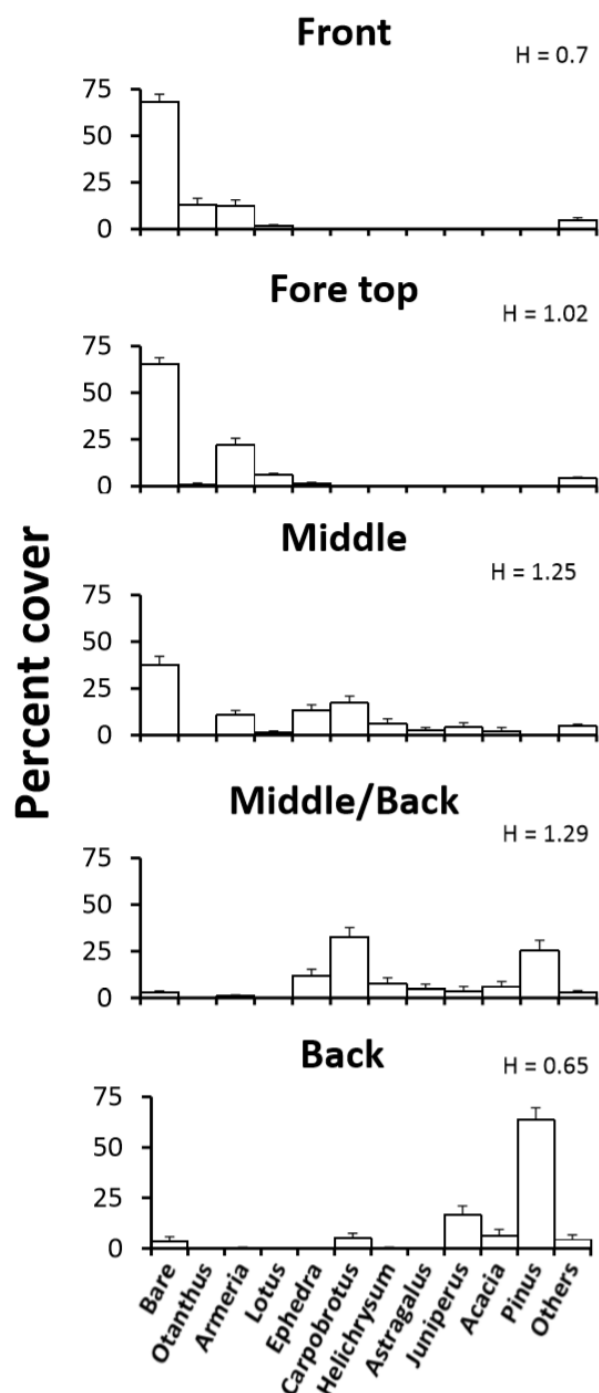



FIG. 1. Plant zonation (mean percent cover + SE) at the Badesi dune. Transects parallel to the

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.

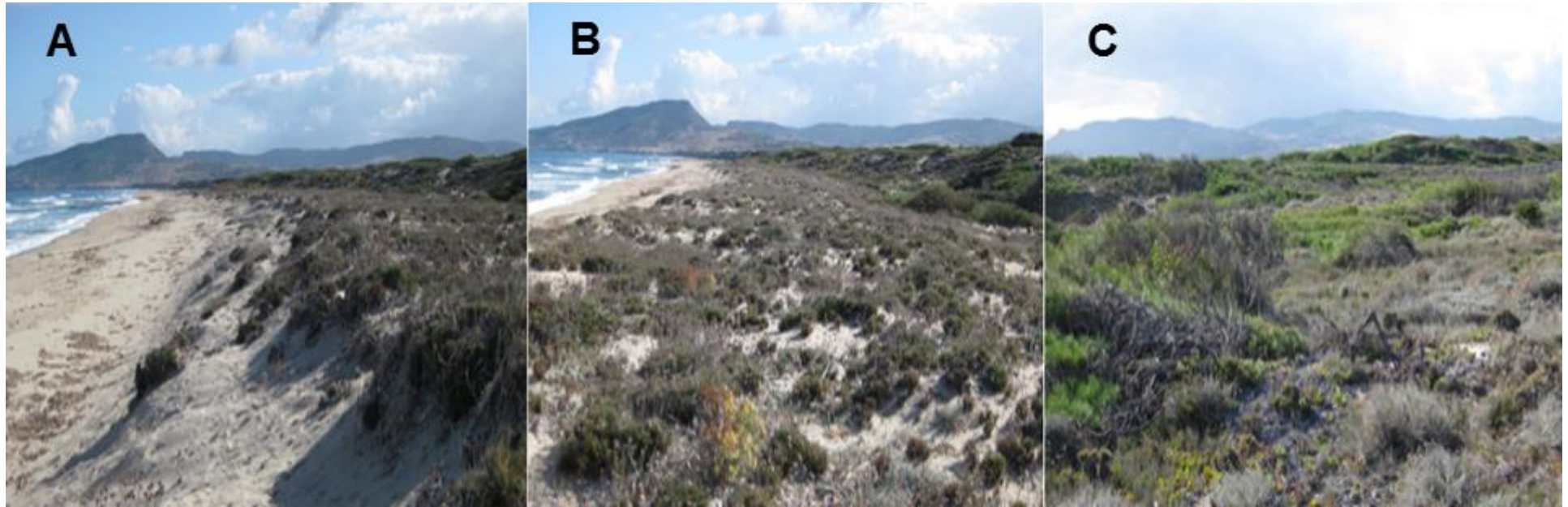
Figure 2

F<small>IG</small>. 2. Photograph of the dune zones at Badesi (Sardinia, Italy). (A) fore dune, (B) middle dune and (C) back dune.

Figure 3

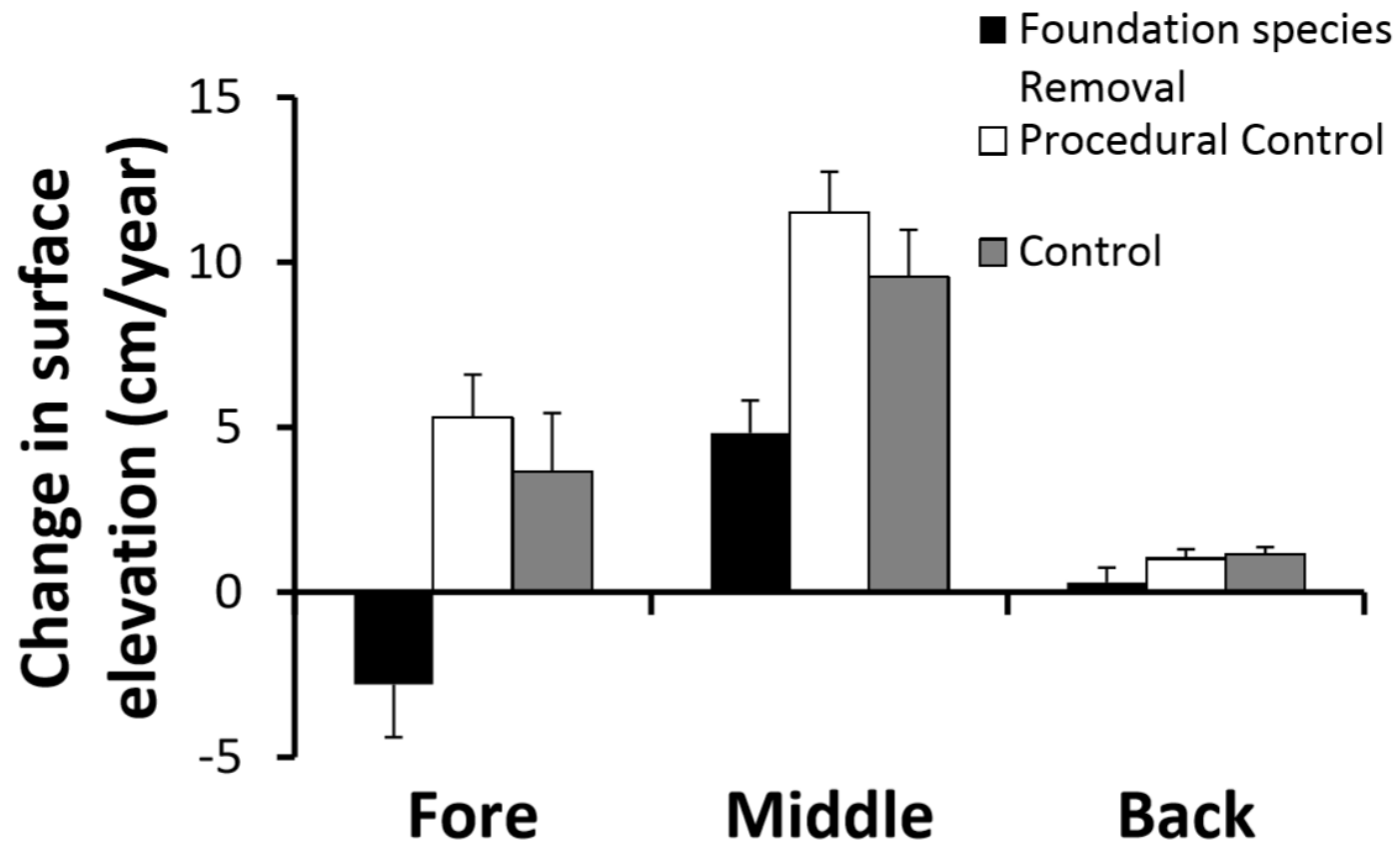



FIG. 3. Surface elevation or sand erosion/deposition balance (mean + SE) of the foundation plant species removal experiment in the three dune zones.

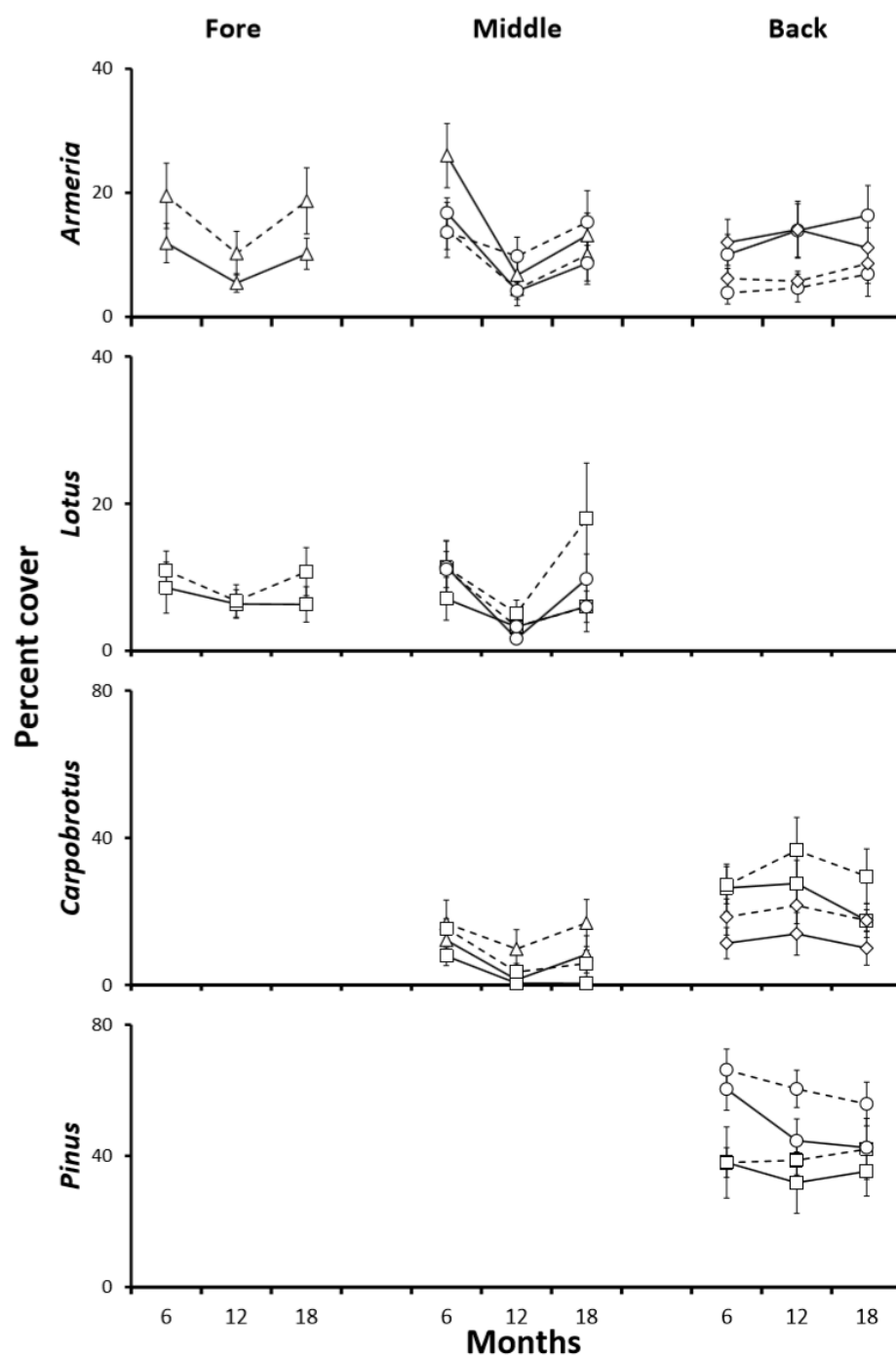



FIG. 4. Reciprocal species removal: *Armeria* (□), *Lotus* (△), *Carpobrotus* (○), *Pinus* (◇),

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

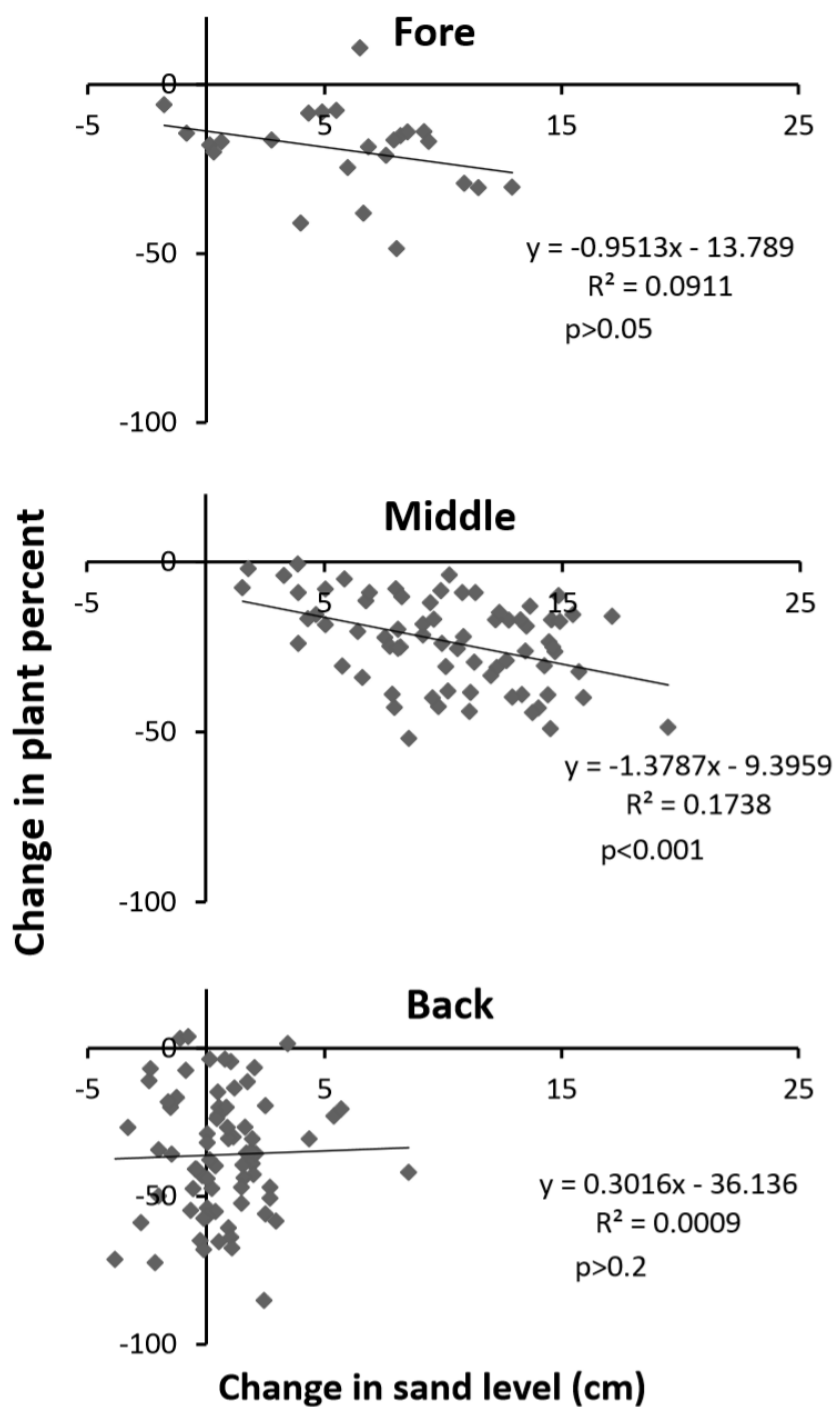



FIG. 5. Relationship between sand burial and percent plant cover in the pair-wise reciprocal

species removal experiments after 12 months in the fore, middle and back dune.

Figure 6

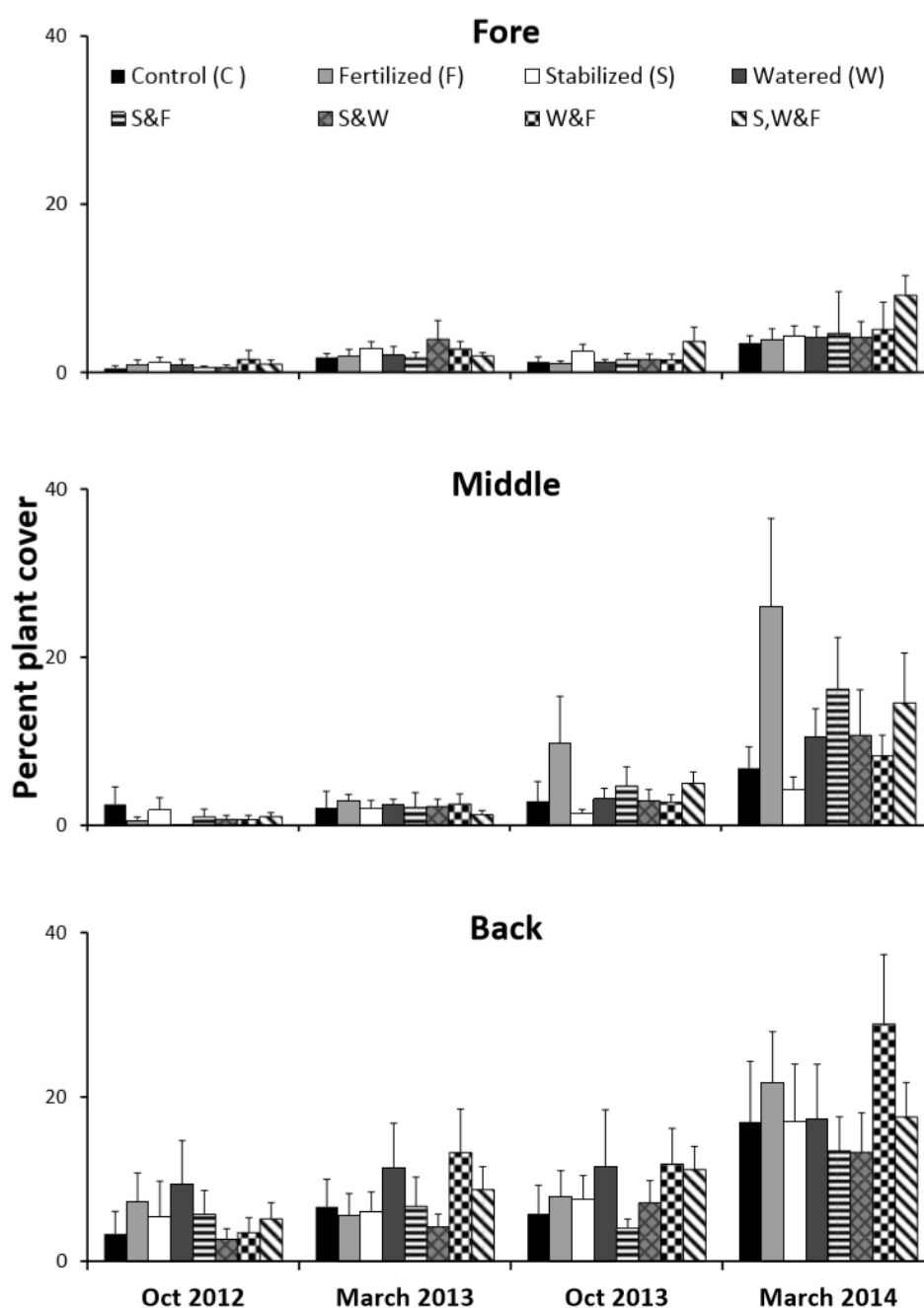



FIG. 6. Physical stress alleviation experiment. Percent plant cover (mean + SE) of the fully factorial manipulation of water availability, nutrient availability and substrate stability during 2 years in the fore, middle and back dune. No single or combination of stress alleviation treatments affected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

Figure 7

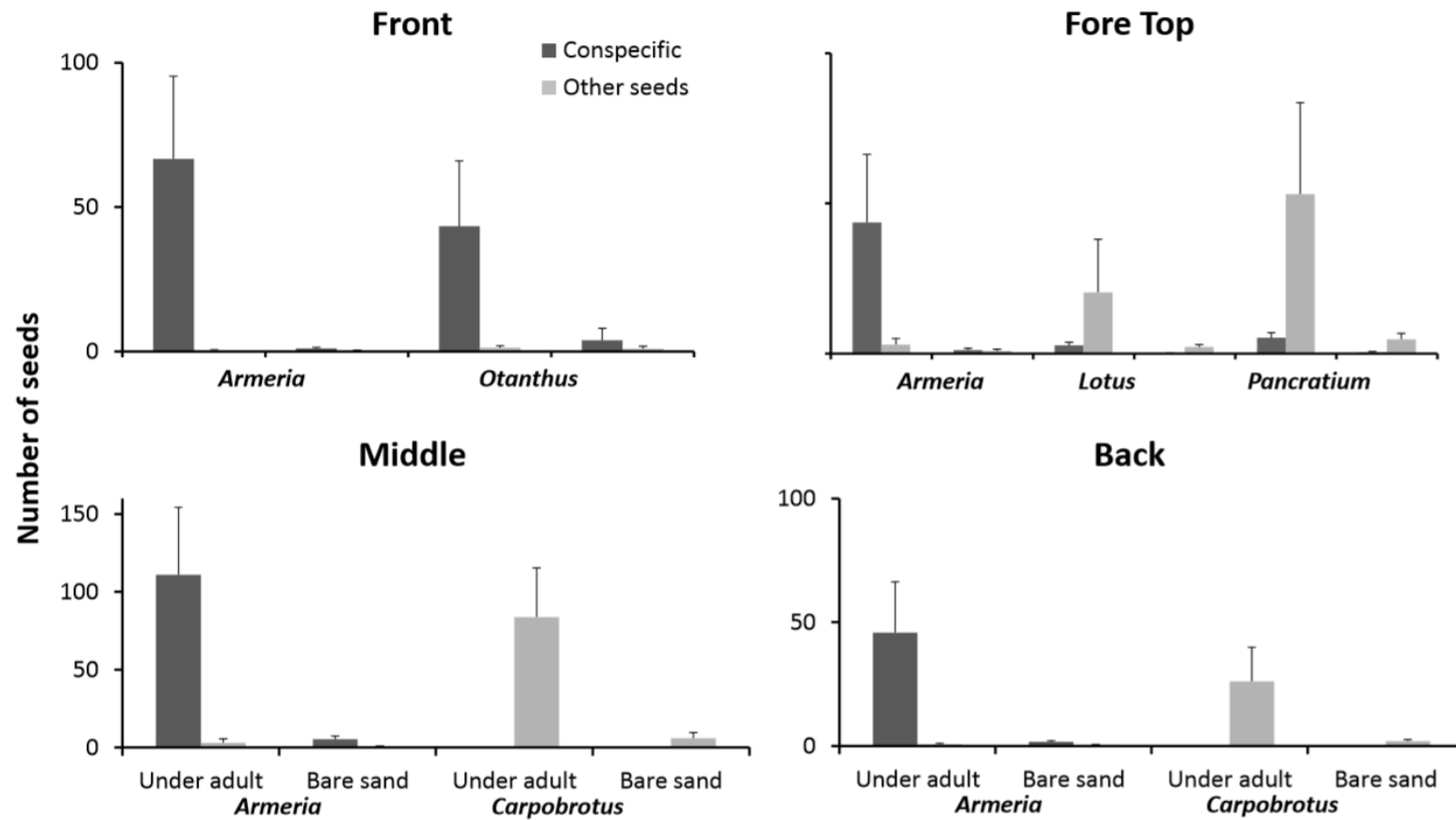



FIG. 7. Seeds distribution (mean + SE) of common dune plants across zones under adult plants and in adjacent unvegetated sand. Data are given separately for conspecific and non-conspecific seeds.

Figure 8

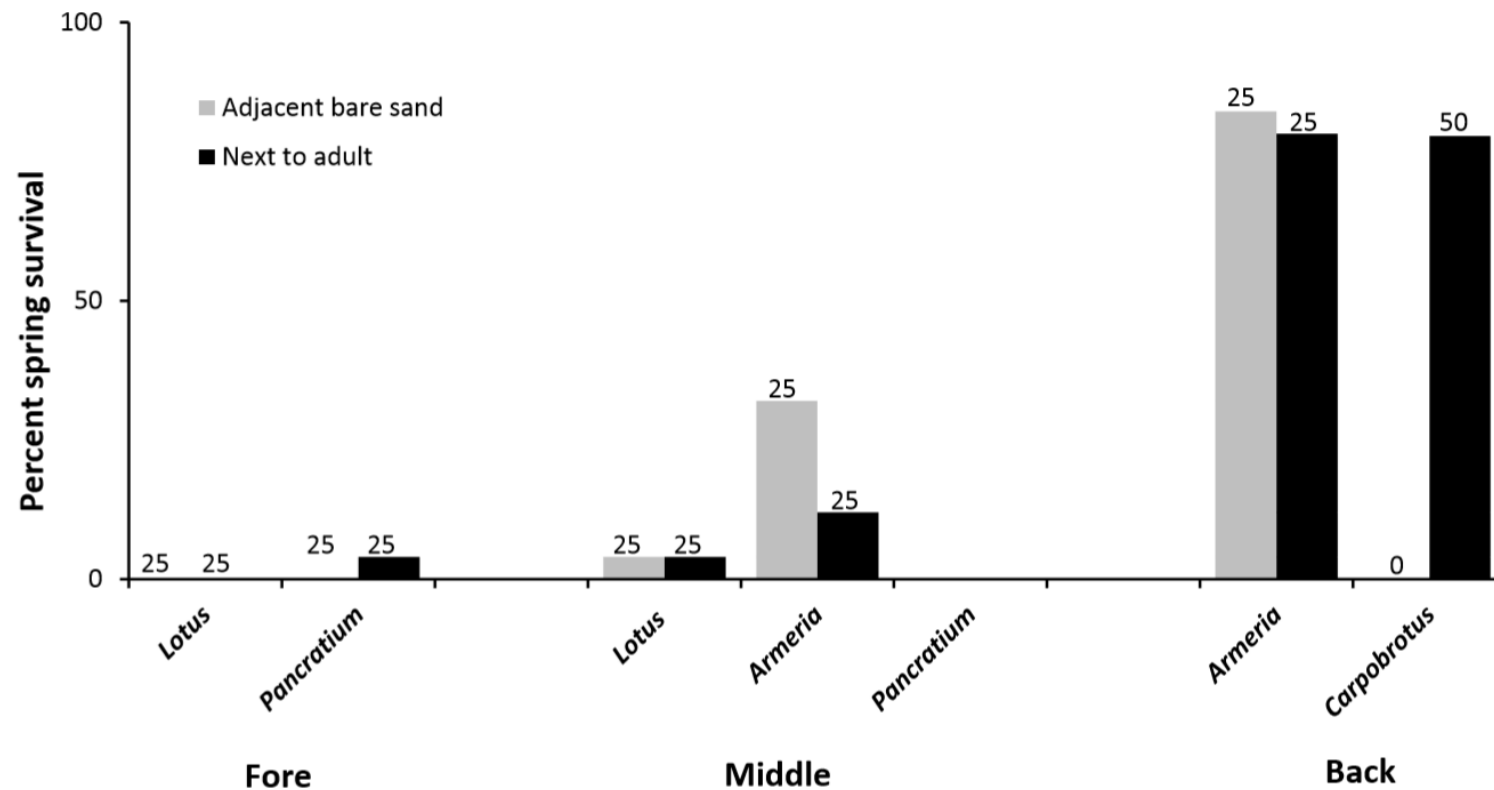



FIG. 8. Survivorship of natural seedlings marked in October 2013. For each zone and species, 25 seedlings were marked associated with conspecific adults and 25 seedlings were marked in bare substrate >30 cm from adult vegetation. Ice plant seedlings were only found under adult ice plants, so all 50 marked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

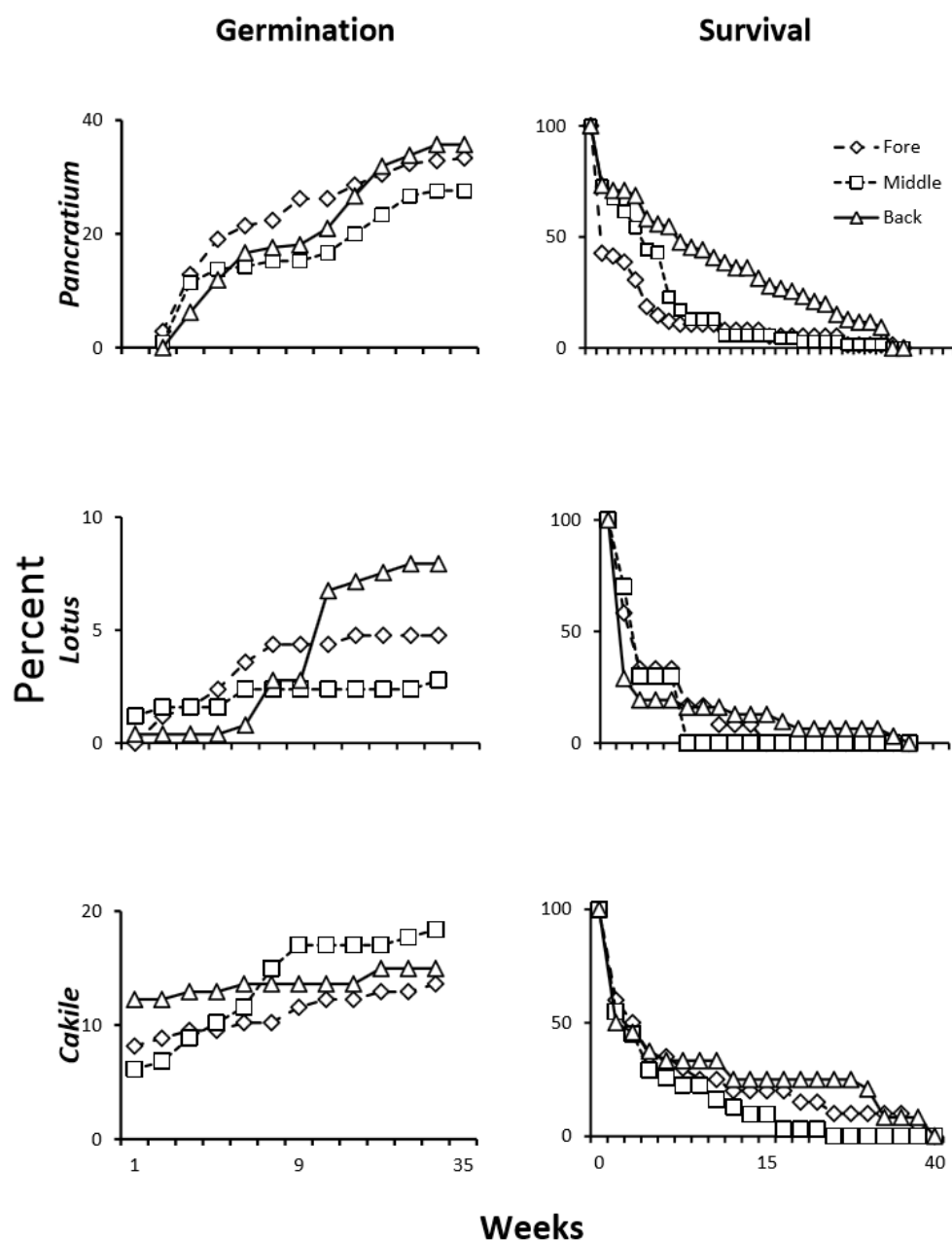



FIG. 9. Results of the seed transplant experiment where germination permeable net bags of

locally collected seeds (n=21/zone/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

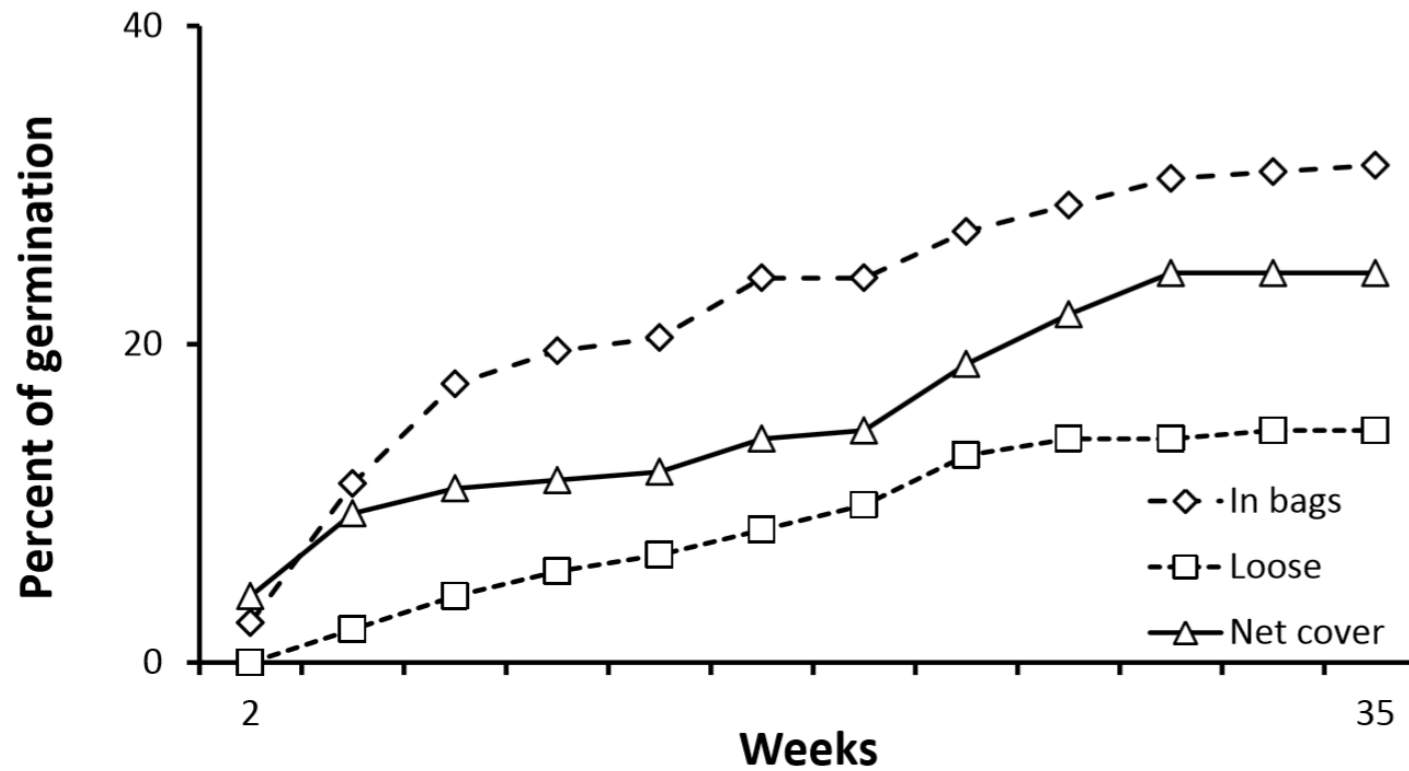



FIG. 10. Seeds erosion experiment. Germination in the fore dune where *Pancratium* seeds were transplanted in germination permeable net bags,

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

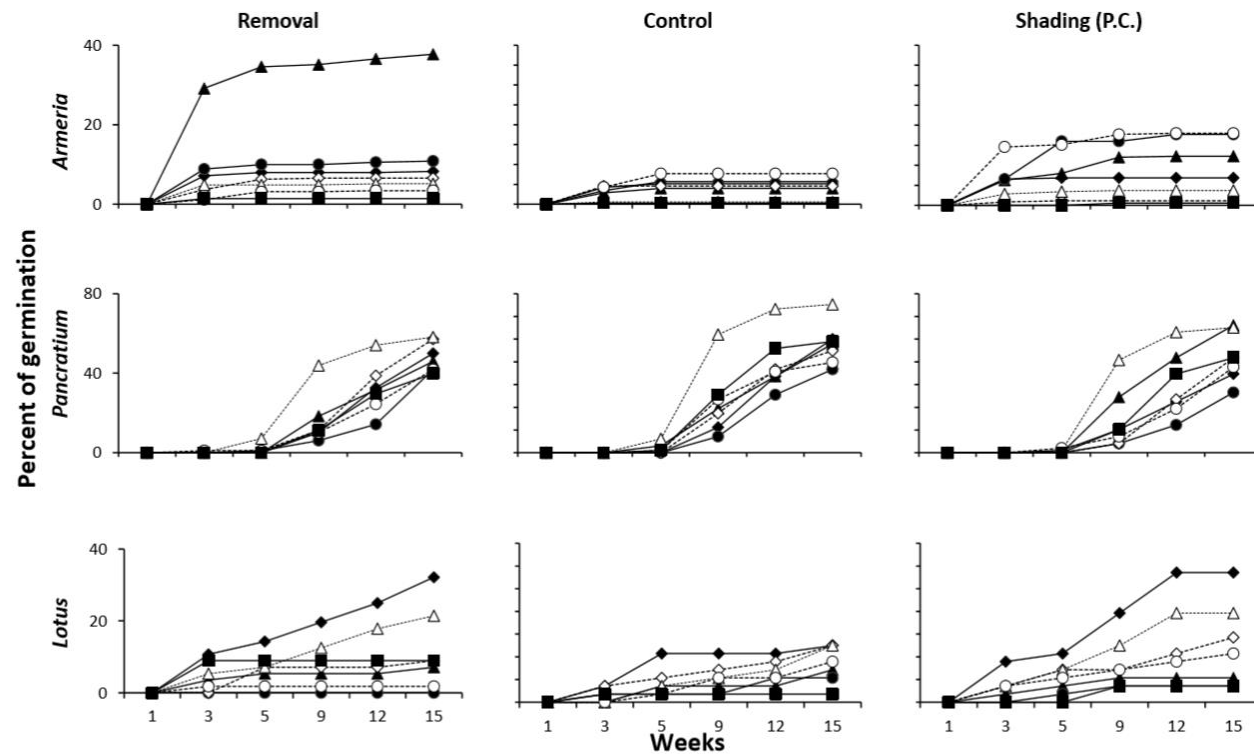



FIG. 11. Back dune competitive release experiment. Germination of *Armeria*, *Pancratium* and *Lotus* seeds sowed in different soils: *Pinus* soil

(◆), 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

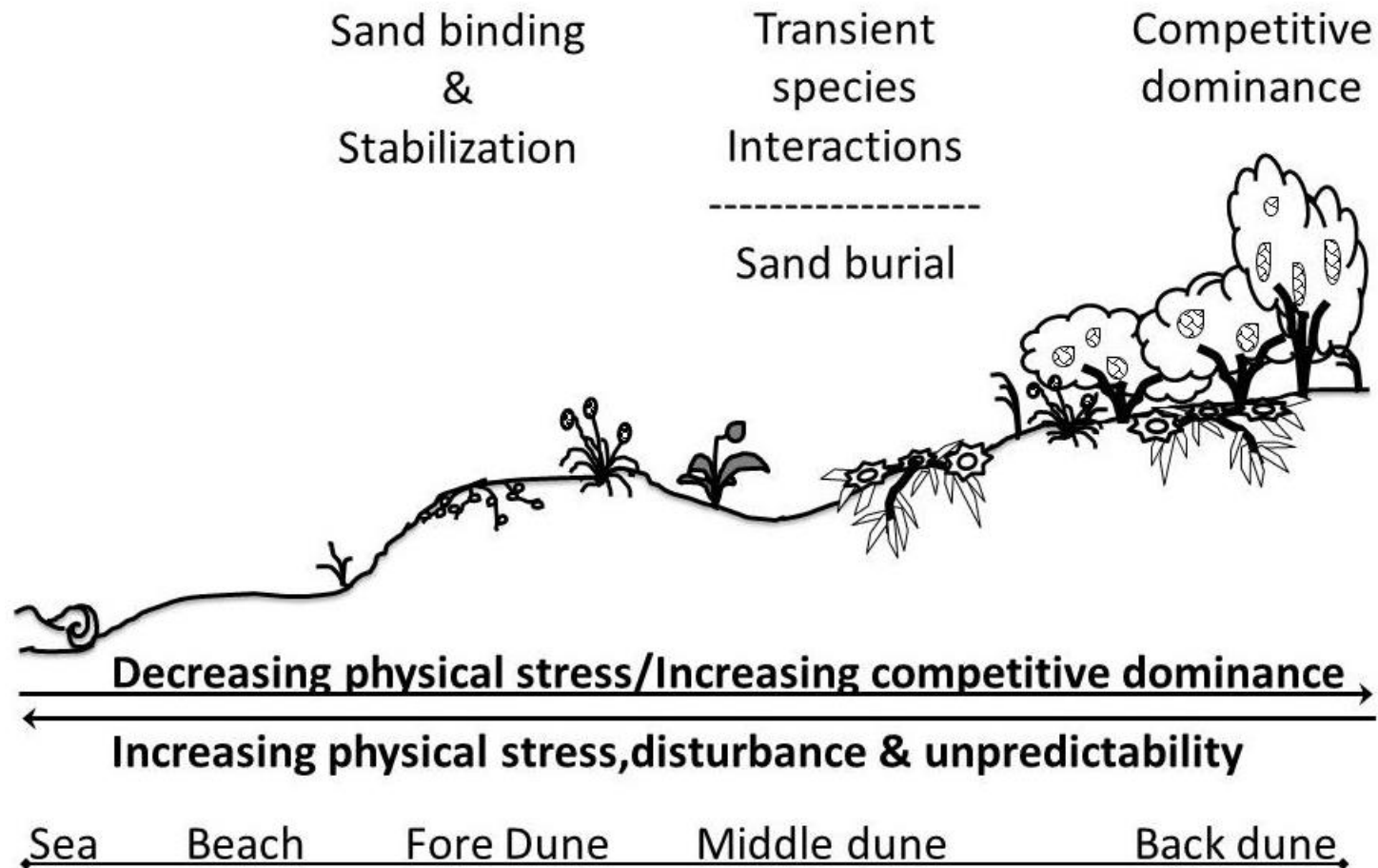



FIG. 12. Conceptual model of the assemble rules of the Badesi sand dune plant community.