

# Germination responses to abiotic stress shape species distributions on coastal dunes

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**Abstract** Plant species occupy distinct zones on coastal dunes, but the mechanisms limiting their distributions have not been fully explained. We combined field surveys of plant distributions and abiotic conditions with controlled germination experiments to assess the contribution of germination requirements to plant zonation. Species presence and abiotic conditions were measured in ten transects across the barrier dune at Waquoit Bay, Massachusetts. Germinating seeds of six species were exposed to four fully crossed treatments: pre-treatment (soaked in fresh water, soaked in salt water, or not soaked), temperature (low, moderate, or high), soil salinity (none, moderate, or high), and light (full light, shade, dark). Species distributions in the field were affected by both distance from the shore and presence of dominant shrubs. Germination tolerance of soil salinity reflected species zonation: species found on the front slope of the dune tolerated salinity, while germination of other species was limited by salinity alone or by salinity in combination with high temperature. Shrubs reduced soil surface temperature and decreased light, but these conditions had limited

effects on germination. These results indicate that limitations to germination can contribute to explaining species distributions on coastal dunes.

**Keywords** Coastal dunes · Germination · Shade · Soil salinity · Species distributions

## Introduction

Plant communities on coastal sand dunes exhibit striking zonation patterns which have intrigued ecologists since Cowles (1899) first described the vegetation of Lake Michigan dunes. Despite a long history of investigations (Cowles 1899; Oosting and Billings 1942; van der Valk 1974; Pemadasa and Lovell 1975; Sykes and Wilson 1983; Ishikawa et al. 1995), the processes that lead to vegetation zonation on dunes are still not fully understood (Maun and Perumal 1999; Wilson and Sykes 1999). Understanding the limitations on species distributions within the dune system is becoming more urgent as dunes are subjected to changes in temperature and storm regimes due to climate change (Seabloom et al. 2013; Prisco et al. 2013; Mendoza-González et al. 2013), invasion by non-native species (Magnoli et al. 2013; Novoa and González 2014; Vecchio et al. 2015), and human disturbances related to recreational use (Santoro et al. 2012; Ciccarelli 2014).

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Plants that germinate, establish and grow on coastal dunes experience a unique set of stresses, including resource limitation, high substrate temperature, exposure to salinity, and burial by shifting sand (Oosting and Billings 1942). These stresses vary with distance from the shoreline, geography of the dune, and presence of foundation species. Conditions are harshest on the ocean-facing front slope of the dune, where levels of salt spray and soil salinity are highest (Rajaniemi and Allison 2009) and the movement of sand is greatest (Rajaniemi and Allison unpublished data). Stresses are ameliorated under shrubs, where soil temperatures and salt spray are reduced (Shumway 2000; Rajaniemi and Allison 2009). Previous research has focused on understanding how tolerance of seedlings and adult plants to abiotic stress correlates with observed distributions, while the role of regeneration from seed has received less attention.

Various studies have found that growth of seedlings and of established plants on coastal dunes may be limited by salt spray (Barbour and DeJong 1977; Sykes and Wilson 1989) and burial (Maun and Lapierre 1986; Sykes and Wilson 1990; Brown 1997). In addition, growth is enhanced under shrubs due to increased nutrients and decreased water stress (Shumway 2000). Growth responses to these physical stresses can contribute to patterns of vegetation zonation (Oosting and Billings 1942; Barbour and DeJong 1977; Maun and Perumal 1999; Wilson and Sykes 1999), but do not fully explain species distributions (Maun and Perumal 1999; Wilson and Sykes 1999).

Germination requirements may also shape species distributions across the dune environment. Like growth rates, germination rates of dune species are influenced by salinity (Mariko et al. 1992; Martinez et al. 1992) and burial (Pemadasa and Lovell 1975; Maun and Lapierre 1986). Light and temperature, which are reduced under shrubs, also affect germination of dune species (Pemadasa and Lovell 1975; Martinez et al. 1992). Previous studies have either investigated germination requirements of species from similar micro-environments within the dune (Pemadasa and Lovell 1975; Maun and Lapierre 1986), or compared dune species to species from other habitats (Mariko et al. 1992; Martinez et al. 1992). The contribution of germination to zonation of species within a dune system has not been assessed. Germination requirements may help to further explain

species distributions if germination is more sensitive than growth to environmental stress, or if germination is limited by different stressors than growth.

Here, we first summarize the results of a four-year survey to measure abiotic conditions and establish the positions occupied by species on the dune system at Waquoit Bay Estuarine Research Reserve. We then describe an experiment testing the germination tolerances of six species. We hypothesize that the realized distributions of species are limited by the ability of their seeds to germinate under physical stress. Specifically, we predict that (1) species found at the dune front are able to germinate when exposed to high temperatures and soil salinity, and require light to germinate and (2) that species found in association with shrubs are less tolerant of high temperature and salinity, and are able to germinate in shade.

## Methods

### Study site and species

Field work was performed at Waquoit Bay National Research Reserve in Falmouth, MA, 41°34'53.24"N, 70°31'30.22"W. This system consists of a barrier beach separating Waquoit Bay and salt ponds east of the bay from the Atlantic Ocean at Vineyard Sound. The barrier beach is widest (175 m) at its western end, where it ends at Waquoit Bay, and narrowest (65 m) at its eastern end, where it transitions to salt ponds. A single, low dune crest, generally less than 2 m high, extends along the southern edge of the beach.

*Ammophila breviligulata* Fernald (American beachgrass) dominates the front slope of the dune, facing the ocean. *Rosa rugosa* Thunb. (rugosa rose) is abundant on the back slope of the dune. The flat expanse behind the dune is sparsely vegetated except for scattered shrubs including *Rosa rugosa* and *Morella pensylvanica* (Mirb.) Kartesz (northern bayberry).

Germination was studied for six dune species. Two species were annual forbs: *Cakile edentula* (Bigelow) Hook. (American searocket) and *Arenaria serpyllifolia* L. (thyme-leaf sandwort, non-native). Three species were biennial or perennial forbs: *Solidago sempervirens* L. (seaside goldenrod, perennial), *Oenothera parviflora* L. (northern evening primrose, biennial), and *Artemisia campestris* L. (field sagewort,

biennial or perennial). The final species was *Rosa rugosa*, a non-native shrub.

Nomenclature follows the USDA Plants Database (USDA, NRCS 2018). We refer to species henceforth by genus.

### Field survey

In 2005, ten transects were established at 200-m intervals across the beach, perpendicular to the shoreline. Each transect had its starting point within one meter of the dune crest. Plots measuring 1 m × 1 m were placed along each transect every 10 m, in both directions. Plots were added towards the shore until the transect reached the high tide line and no vegetation was present (never more than one additional plot per transect), and away from the shore until the transect reached the salt pond or the bay, or reached a maximum of 200 m from the starting point. Each plot was visited once in June or July in 2006, 2007, 2008, and 2009. Percent cover of each plant species in each plot was estimated visually.

To determine species distributions, plots were categorized as belonging to one of three zones: the dune front (at the dune crest, or on the beach side of the dune), the dune back (1–21 m from the crest, on the salt pond/bay side of the dune), or the dune flat (29 m or more from the crest). Each plot was further designated as having high or low vegetation cover (greater than or less than 30% cover of *Ammophila*, *Rosa*, or *Morella*).

Soil salinity, soil temperature, and light in transect plots were measured in July 2007, with each variable measured on a single day to avoid variation due to weather conditions. A single soil core measuring 1.7 cm in diameter and 30 cm deep was collected from the center of each plot. Soil salinity was quantified by adding 100 ml of deionized water to 20 mg of field-moist soil, shaking for 30 min, and measuring conductivity of the solution after allowing the soil to settle (Rowell 1994). Soil temperature was measured at the soil surface and with a 30 cm probe within 2 h of solar noon. Photosynthetically active radiation was measured with a Decagon Sunfleck Ceptometer (Decagon, Pullman, Washington, USA), which integrates PAR at 1-cm intervals along a 40 cm wand. PAR was measured within two hours of solar noon. Measurements were taken just above the soil surface and again above all vegetation, and the

percentage of light reaching the soil surface was calculated.

To gauge the potential for burial of seeds, sand movement was quantified by driving a bamboo pole into the soil in each plot. The length of the exposed portion of the pole was measured in September 2006 and again in September 2007. A decrease in length indicated sand accumulation, while an increase indicated sand removal. The absolute value of the change was calculated as a measure of sand movement.

### Germination experiment

Mature seeds were collected in late October 2009 and October and November 2010 at Waquoit Bay and stored at 4 °C for at least three months. All seeds were surface-sterilized in 8% bleach solution for 10 min and sown in 2 oz, lidded dishes containing 15 mL of sterilized sand. In each dish, ten seeds of a single species were pressed into the sand till just buried. Sets of 10 dishes of the same species were placed in plastic zipper-sealed bags to reduce evaporation. Each bag was assigned to a combination of pre-treatment, soil salinity, temperature, and light treatments.

Before planting, one third of the seeds were soaked in artificial seawater (Instant Ocean, Spectrum Brands, Blacksburg, VA, USA) for 18 h. Another third of the seeds were soaked in fresh water for 18 h. After 18 h, seeds were rinsed with de-ionized water and immediately planted in dishes. Soaking was intended to mimic the effect of inundation of the soil with either ocean water or rainwater during storms. The remaining seeds were not soaked and acted as a control.

Soil salinity treatments were applied at the time of planting by adding 5 ml of deionized water (no salinity), 5 ml of artificial sea water (high salinity), or 2.5 ml of artificial sea water mixed with 2.5 ml of deionized water (moderate salinity) to each dish. Soil salinity, when measured by the method applied to field samples, was 0.79 ppt in the moderate salinity treatment and 1.58 ppt in the high salinity treatment. The artificial seawater (Instant Ocean) contained no nitrogen or phosphorus and only low concentrations of potassium and calcium (400 and 420 ppm respectively) and so did not supply additional macronutrients to the dishes. Dishes remained moist and were not watered again for the duration of the experiment.

Dishes were maintained at one of three temperatures: a low temperature of 10 °C, a moderate

temperature of 20–26 °C and a high temperature of 32 °C. The low and high temperatures were achieved by incubating dishes in growth chambers. Because space in growth chambers was limited, the moderate temperature treatment was accomplished by placing bagged dishes in a germination room at ambient temperature. The room temperature was constantly monitored, and use of the room was discontinued once the temperature rose above the target range.

Finally, dishes were exposed to one of three light treatments. All dishes were placed approximately 25 cm from fluorescent lighting. Dishes in the full light treatment were exposed to light on a 12/12 h light/dark cycle. Dishes in the shade treatment were covered with 40% shade cloth to mimic the light conditions under shrubs in the field. Dishes in the dark treatment were wrapped in aluminum foil to simulate the effects of seed burial.

Dishes were incubated for 15 days. Dishes were checked every other day for germination, which was defined as the emergence of a radicle, hypocotyl, or cotyledons. Germinated seeds were counted and removed. All bags were rotated approximately every four days.

Treatments were applied in a fully crossed design with two bags of ten dishes per treatment combination per species, for a total of 1620 dishes per species, except that *Arenaria* was not grown at the low temperature because there were not enough seeds available. Due to limited incubator space, it was necessary to perform the experiment in batches. *Oenothera* was planted in January 2011, *Arenaria* and *Cakile* in March, and *Solidago* in May. *Artemisia* in the low temperature treatment was planted in March, and in the moderate and high temperatures in August. At that time, the temperature of the germination room exceeded 26°, so the seeds in the moderate temperature treatment were kept in incubators at 23 °C.

#### Data analysis

All statistical analyses were conducted using R statistical software, version 3.4.3 (R Core Team 2017).

The objective for use of the cover data was to determine which microenvironments each species is able to establish and grow in. Therefore, species distributions were characterized based on presence or absence over all four survey years. A species was

considered present in a plot if it was observed in the plot at least once. Frequency of presence of each species in each zone, with high and low vegetation cover, was calculated. For species with sufficient data, logistic regression was used to test whether presence/absence was dependent on zone or vegetation cover. No dune front plots had high cover, so interactions were excluded from the model. For *Rosa*, which made up the vegetation cover on the dune back, only zone was tested.

Two-way ANOVA was used to test for effects of zone and vegetation cover on each of the abiotic variables. Tukey post hoc tests were applied when the effect of zone was significant. Soil salinity, soil temperature, and sand movement were natural-log transformed to improve normality and homogeneity of variance, and percent light at the soil surface was arcsine-square root transformed. Mean values of abiotic conditions in the three dune zones were compared to treatment levels in the germination experiment.

In the analysis of the germination experiment, individual dishes, rather than bags of dishes, were treated as replicates, despite not being fully randomized. Conditions within the bags were expected to be identical, and we saw no evidence of differences such as drying or fungal growth affecting individual bags. Per-seed germination rates were low. Of the dishes with germination, 58% had only one germinating seed and only 6% had four or more germinating seeds. We therefore consider whether germination was observed in a dish of ten seeds rather than analyzing germination percentage within a dish.

The effects of treatments and their two-way interactions were tested with logistic regression, with the binary outcome of germination or no germination for each dish. Each species was analyzed separately. Treatment levels were excluded from analysis if two or fewer dishes (of 360 for *Arenaria* or 540 for other species) had germinating seeds.

Our interpretation of results focuses on observing the treatments or treatment combinations under which each species is able to germinate. Therefore, we categorize treatments as resulting in no germination, minimal germination (two or fewer dishes, of 360 for *Arenaria* or 540 for other species, had germinating seeds), or some germination. Treatment levels with no germination or minimal germination are discussed but were excluded from regression models.

**Results**

Field survey

Transects included a total of 100 plots. *Cakile* did not occur in transect plots, but was observed occasionally near the high tide line, in plots that were otherwise unvegetated. The placement of plots along our transects usually missed sampling in the narrow zone where *Cakile* occurred. *Solidago* was seen in at least one year in a total of 42 plots, in all three zones (Fig. 1). It occurred most frequently at the dune front (effect of zone on presence/absence,  $\chi^2_2 = 11.93$ ,  $P = 0.003$ ), and was found more often in plots with high shrub cover (effect of vegetation,  $\chi^2_1 = 3.88$ ,

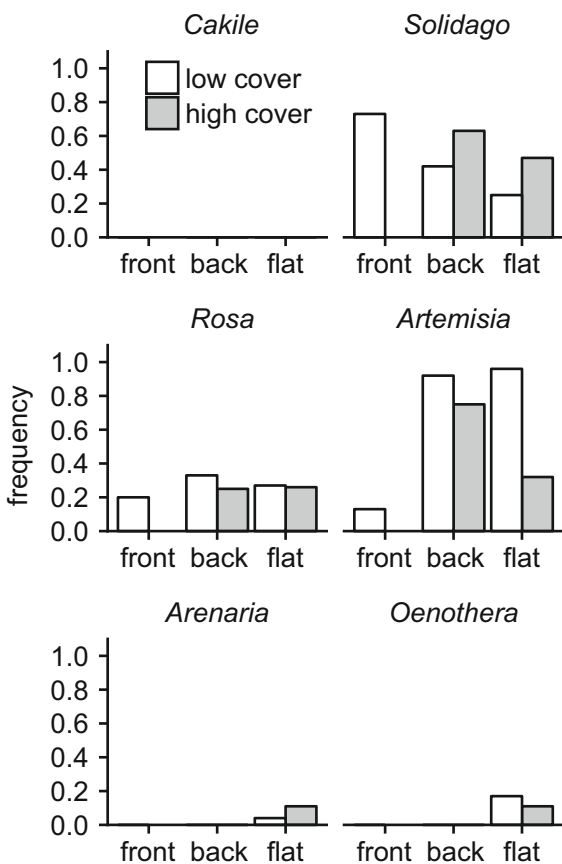
$P = 0.049$ ). *Artemisia* was found in 71 plots and in all three zones (Fig. 1). It was least common at the dune front ( $\chi^2_2 = 44.54$ ,  $P < 0.001$ ) and was found more often in plots with low shrub cover ( $\chi^2_1 = 29.64$ ,  $P < 0.001$ ). *Rosa* occurred in 17 plots in all three zones ( $\chi^2_2 = 0.47$ ,  $P = 0.790$ ; Fig. 1). *Oenothera* and *Arenaria* were found only in the dune flat (Fig. 1), and both occurred infrequently (10 and 4 plots, respectively). Both occurred in plots with low and high shrub cover.

Soil salinity was greater in dune front plots than in dune back or flat ( $F_{2,96} = 17.74$ ,  $P < 0.001$ ; Fig. 2a) and was not affected by vegetation cover ( $F_{1,96} = 0.42$ ,  $P = 0.508$ ). The moderate salinity treatment in the germination experiment was close to the mean value for dune front soil, while the high salinity treatment was about 0.5 standard deviations above the maximum observed salinity (Fig. 2a).

Soil temperature was reduced in plots with high vegetation cover (for surface temperature  $F_{1,97} = 36.44$ ,  $P < 0.001$ ; for temperature to 30 cm depth,  $F_{1,97} = 19.41$ ,  $P < 0.001$ ; Fig. 2b) but did not differ significantly among zones ( $F_{2,97} = 0.47$ ,  $P = 0.627$  and  $F_{1,97} = 0.01$ ,  $P = 0.987$ ). In the germination experiment, the high temperature treatment of 32 °C was similar to mean surface temperature in plots with high vegetation cover, while moderate temperature treatment of 20–26 °C was typical of soil temperature below the surface (Fig. 2b).

Light at the soil surface was reduced by high vegetation cover ( $F_{1,97} = 111.57$ ,  $P < 0.001$ ; Fig. 2c). The effect of zone on light was also significant ( $F_{2,97} = 3.23$ ,  $P = 0.0413$ ), but was due to variation in plant cover among zones. Zone was not significant when total cover was added to the model. The shaded treatment of 40% shade cloth in the germination experiment was typical of the level of shade found under vegetation in the field (Fig. 2c).

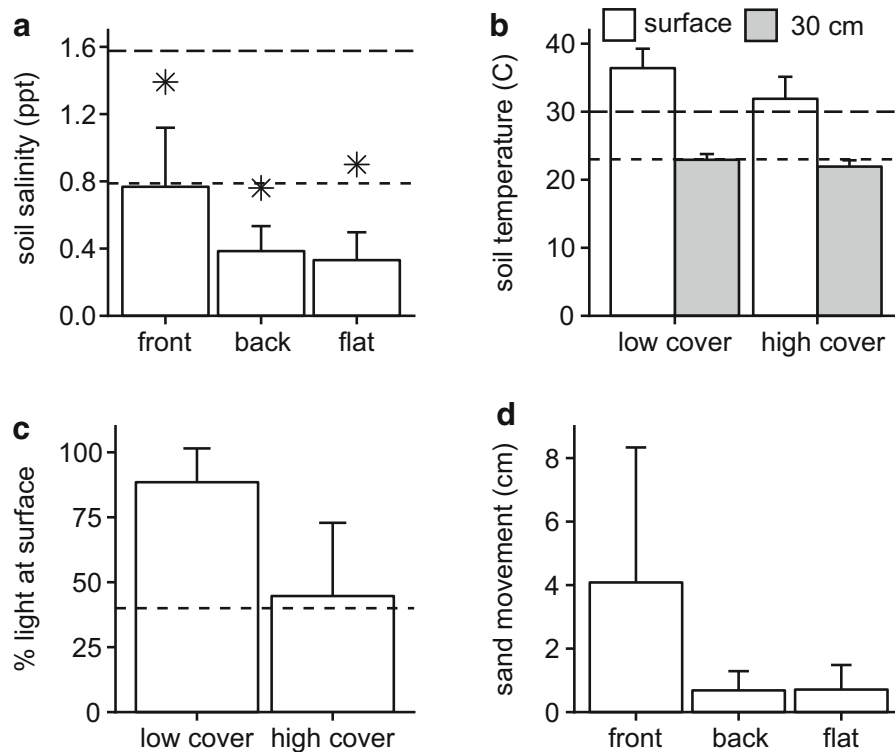
Sand movement was greater at the dune front than in the dune back or flat ( $F_{2,97} = 22.53$ ,  $P < 0.001$ ; Fig. 2d) and was not affected by vegetation cover ( $F_{1,97} = 1.88$ ,  $P = 0.174$ ).



**Fig. 1** Frequency of occurrence of species in zones. Transect plots were categorized into zones based on position relative to the dune crest, and dune back and dune flat plots were further characterized as having low (< 30%) or high (≥ 30%) cover of the dominant shrub. A species was considered present in a plot if it was observed at least once in four years. See methods for details

Germination experiment

*Cakile* germination occurred in all three soil salinity treatments, although germination was reduced by moderate salinity and further reduced by high salinity



**Fig. 2** Abiotic conditions in field plots (mean + SEM) and in experimental treatments (dashed lines). **a** Soil salinity differed significantly among dune zones. Stars in A indicate maximum observed values in the field. Upper and lower dashed lines are values for high and moderate salinity treatments in the germination experiment. **b** Soil temperature differed significantly between plots with low and high shrub cover. Upper and

lower dashed lines are values for high and moderate temperature treatments in the germination experiment. **c** Light at the soil surface differed significantly between plots with low and high shrub cover. The dashed line is the value for the shade treatment. **d** Sand movement differed significantly among dune zones

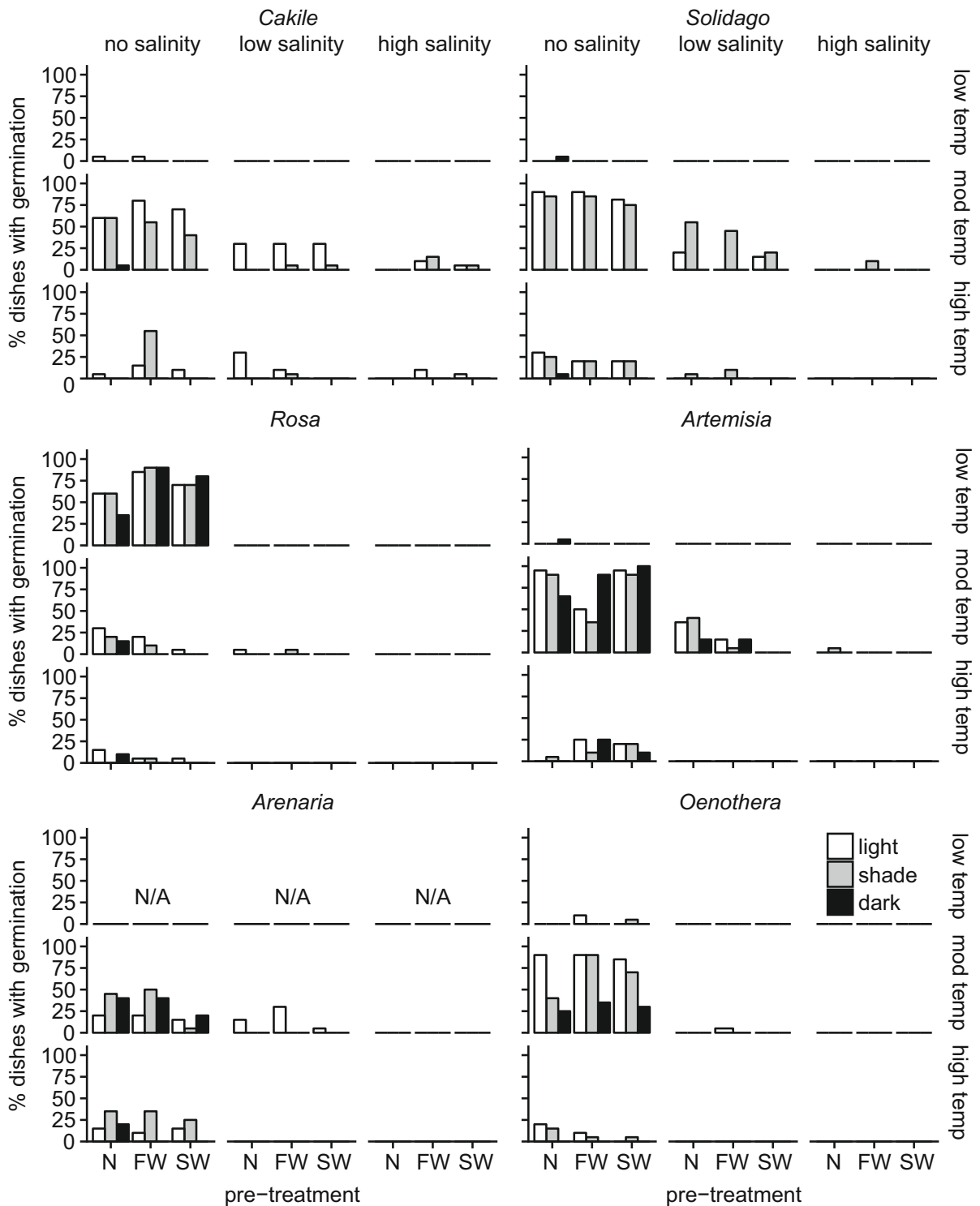
(Fig. 2). Only one seed germinated at low temperature, so this treatment was not included in analysis. Germination was best at moderate temperature, and reduced at high temperature, particularly in combination with soil salinity (salinity  $\times$  temperature interaction,  $\chi^2_2 = 7.72$ ,  $P = 0.021$ ). Germination was light-dependent, with only one seed germinating in the dark (treatment not analyzed). Shade reduced germination when combined with soil salinity (salinity  $\times$  light interaction,  $\chi^2_2 = 12.38$ ,  $P = 0.002$ ). Pre-treatment by soaking seeds in fresh water or salt water had no significant effects (pre-treatment effect and interactions,  $P > 0.05$ ).

*Solidago* had only two germinants at high salinity and one at low temperature (Fig. 2); these treatment levels were not included in the analysis. Germination was reduced in moderate salinity and at high temperature (temperature effect,  $\chi^2_1 = 32.24$ ,  $P < 0.001$ ).

Germination was light-dependent, with only one seed germinating in the dark (not included in analysis). Shade had no effect in the absence of soil salinity, but improved germination with moderate salinity (salinity  $\times$  light interaction,  $\chi^2_1 = 12.14$ ,  $P < 0.001$ ). Pre-treatment had no effects on germination ( $P > 0.05$ ).

*Rosa* never germinated with high soil salinity and rarely germinated with moderate soil salinity (2 dishes, 1 seed each; Fig. 3). The effect of salinity was therefore not analyzed. *Rosa* germination was best at low temperature and reduced at moderate and high temperature, especially after pre-treatment by soaking in salt water (temperature  $\times$  pre-treatment interaction,  $\chi^2_4 = 29.91$ ,  $P < 0.001$ ). Germination was not affected by light level ( $P > 0.05$ ).

*Artemisia* had only one seed germinate at high salinity and one at low temperature (Fig. 3; treatment levels not analyzed). The effects of salinity,



**Fig. 3** Percent of dishes in the germination experiment with at least one germinating seed, in response to pre-treatment (x axis, N none, FW soaking in fresh water, SW soaking in salt water), salinity (columns), temperature (rows), and light (bar color)

temperature, and light were all dependent on pre-treatment. Germination at moderate salinity was reduced, especially after soaking in salt water (salinity  $\times$  pre-treatment interaction,  $\chi^2 = 28.80$ ,  $df = 2$ ,  $P < 0.001$ ). Seeds germinated at high temperature only after soaking in either fresh water or salt water (temperature  $\times$  pre-treatment interaction,  $\chi^2 = 25.69$ ,  $P < 0.001$ ). Seeds that were not soaked had reduced germination in the dark, while seeds soaked in fresh water had reduced germination in full sun or shade (light  $\times$  pre-treatment interaction,  $\chi^2 = 23.42$ ,  $P < 0.001$ ).

*Oenothera* germination was rare with any amount of soil salinity (1 seed; Fig. 3), so the effect of salinity was not analyzed. Its germination was very low at low temperature and reduced at high temperature, especially following pre-treatment by soaking in salt water (temperature  $\times$  pre-treatment interaction,  $\chi^2 = 11.33$ ,  $P = 0.023$ ). Germination was reduced in the dark, but the effect was not significant ( $P > 0.05$ ).

*Arenaria* was not tested at low temperature and did not germinate with high soil salinity (Fig. 3; treatment levels not analyzed). It germinated with moderate soil salinity only at moderate temperature (salinity  $\times$  temperature interaction,  $\chi^2 = 7.02$ ,  $P = 0.008$ ) and with full light (salinity  $\times$  light interaction,  $\chi^2 = 29.78$ ,  $P < 0.001$ ). Without soil salinity, temperature and light had minimal effects on germination, although pre-treated seeds did not germinate in the combination of high temperature and dark ( $\chi^2 = 9.09$ ,  $P = 0.011$ ).

## Discussion

Consistent with our first prediction, the species that occur at the dune front, *Cakile* and *Solidago*, are able to germinate when exposed to high levels of soil salinity, while the combined stresses of soaking in salt water, soil salinity, and high temperatures exclude other species from the dune front. We found less support for our second prediction. The conditions associated with shrubs did not have differential effects on germination.

### Salinity

Seeds at the dune front are exposed to high soil salinity and may experience inundation with salt water during

storms. Species found at the dune front were expected to germinate with soil salinity and after soaking in salt water, while exposure to salinity was expected to exclude other species from that area.

Two species, *Rosa* and *Oenothera*, were unable to germinate with soil salinity typical of the dune front (moderate salinity treatment). These species did not occur at the dune front. *Artemisia*, which was found infrequently on the dune front, and *Arenaria*, which was not observed on the dune front, also germinated in moderate soil salinity, but these species were unable to tolerate the combination of soaking in salt water and soil salinity. The dune front species, *Cakile* and *Solidago*, germinated with soil salinity and were unaffected by soaking in saltwater. *Cakile* also germinated at low rates in the high soil salinity treatment, which was higher than salinity observed in field plots. Therefore, germination responses to salinity appear to allow *Cakile* and *Solidago* to inhabit the dune front and limit *Artemisia* and *Arenaria* in that environment, while excluding *Rosa* and *Oenothera*.

Previous studies have also shown that germination of *Cakile* and *Solidago* is tolerant of salinity (Barbour 1970; Orava and Drake 1997; Debez et al. 2004). In a comparison of species from a range of habitats from salt marshes and dunes to inland sites, germination tolerance to salinity correlated with salinity of the habitat where a species occurred (Mariko et al. 1992).

The ability of seedlings to tolerate salinity is also important in determining species distributions within coastal dunes. Growth of *Cakile* is unaffected by salt spray and soil salinity (Barbour 1970), and may be enhanced by low levels of soil salinity (Debez et al. 2004). Seedlings of *Solidago* are also tolerant of salt spray (van der Valk 1974). In one study of 12 dune species, tolerance of seedlings to both inundation of the soil and aboveground salt spray was correlated with species' typical position on the dune (Barbour and DeJong 1977). In another study of 29 species, responses of shoot growth to aboveground salt spray did not correlate with root growth responses to soil salinity, and seedling salinity tolerance only partially explained species distributions (Sykes and Wilson 1988).

### Temperature

All species were able to germinate at the high temperatures found at the soil surface in unshaded



areas, although with reduced success. However, the combination of high temperature and salinity greatly reduced germination of *Cakile* and *Solidago* and prevented germination of the remaining species. High temperature following soaking in salt water also reduced germination in *Oenothera* and *Rosa*. Soil temperature may further contribute to the exclusion of the less salt-tolerant species from the dune front, and even tolerant species may rely on some amelioration of stress, such as shading of the soil by vegetation, cooler spring temperatures, or cooling on a cloudy day, to germinate.

*Rosa* germinated most successfully at the low temperature, and was the only species to germinate well at that temperature. This species may be able to germinate earlier in spring than the other species.

Temperature effects on dune species have not been extensively studied. A set of eight dune winter annuals was tested for the ability to germinate at temperatures ranging from 5 to 25 °C. These species germinated in the fall in the field. All the species germinated at all temperatures, although germination for some species was delayed by high temperatures (Pemadasa and Lovell 1975). In another study, four out of ten dune species required diurnal temperature fluctuations to germinate (Martinez et al. 1992).

## Light

We expected germination of the dune front species to be light-sensitive. These species are exposed to shifting sands and are likely to experience burial. In our experiment, neither *Cakile* nor *Solidago* germinated in the dark. Previous studies, on the other hand, show that *Cakile* is able to germinate following burial and even that light inhibits germination (Barbour 1970). *Cakile* seeds planted at depths up to 12 cm successfully germinated and emerged (Maun and Lapierre 1986). The average emergence depth of *Cakile* in the field was 3.84 cm, and a few seedlings were observed to have emerged from depths greater than 7 cm (Maun and Lapierre 1986). Germination may have been delayed in the dark, as was observed for other dune annuals (Pemadasa and Lovell 1975), such that our experiment was not continued long enough to observe germination.

Germination was not light-dependent for the other species. Germination was reduced in the dark for *Oenothera*, enhanced for *Arenaria*, and unaffected for

*Rosa* and *Artemisia*. Lack of light may not be a sufficient cue to signal burial and prevent germination following burial at the dune front. Low oxygen or reduced gas exchange may be important for inhibiting germination at depth (Benvenuti and Macchia 2006, 2010). Alternatively, seedlings may germinate at depth but fail to emerge (van der Valk 1974).

Variation in tolerance of seedlings to burial is an important contributor to zonation on lacustrine dunes (Maun and Perumal 1999), although its importance on coastal dunes varies (Sykes and Wilson 1990; Wilson and Sykes 1999). Van der Valk (1974) concluded that burial was an important limitation to both germination and seedling survival among six dune annuals, including *Solidago*.

Most of the species tested can be found growing under shrubs (Fig. 1), and their germination was shade-tolerant. Seedlings of *Solidago* and *Ammophila* growing beneath Northern bayberry shrubs grew larger and produced more flowers, due to the combination of shade, which lowers temperature and reduces water stress, and increased nutrient availability (Shumway 2000). We expected that *Rosa* might require full light to germinate, to avoid germination of seeds under already-established adults, but *Rosa* seeds were also tolerant of shade. However, our shadecloth treatment did not produce a shift in red:far-red ratio, which is an important signal for germination in low light (Pons 2000). Seeds of *Arenaria* have been shown to germinate in the dark but not under the shade of green leaves (King 1975).

## Soaking of seeds

Soaking the seeds before germination did not have strong main effects, but sometimes helped or hindered germination when other stresses were present. For example, soaking in salt water prevented germination at moderate salinity in *Artemisia*. This species appeared to have limited tolerance for osmotic stress, which was exceeded by the combination of the two treatments.

Surprisingly, soaking in either seawater or fresh water allowed *Artemisia* to germinate at high temperature, but reduced germination at high temperature for *Rosa* and *Oenothera*. These combinations of conditions are unlikely to be relevant in the field, as saturated soils would generally be cooler. Overall, exposure of seeds to substrates saturated either by sea

water or by rain probably has little direct effect on germination. One study of overwash due to storms found the effect on the plant community varied with timing of the storm and life history of the plants affected. Three annuals and the perennial *Solidago* were able to produce seeds following an August storm and germinate in spring, whereas no spring germination was observed for two of the annuals following an October storm (Cheplick 2017). In that study, storms did not directly interfere with germination but disrupted seed production and buried or removed seeds.

## Conclusion

The distribution of species across the dune gradient may be partially determined by germination responses to physical conditions. In particular, high salinity in combination with high temperature and burial by shifting sand limits which species are able to germinate at the dune front and which are restricted to the dune back and flat. Previous studies indicate that these same conditions also generally determine growth and survival of any seedlings that do germinate. Growth and survival of *Cakile* and *Solidago* have been shown to tolerate salt spray (Barbour 1970; Cartica and Quinn 1980; Orava and Drake 1997; Lonard et al. 2015). Several of our own experiments have shown that high levels of salt spray greatly reduce survival of *Rosa* seedlings and survival and growth of *Oenothera* seedlings, but that survival and growth of *Artemisia* seedlings are surprisingly unaffected (unpublished data). Given that *Artemisia* seeds are abundant in the seedbank at the dune front (Messina and Rajaniemi 2011), germination requirements may be an important limitation for the distribution of this species.

Germination responses to burial and salinity may become more important limitations as climate change produces stronger storms, greater overwashing by waves, and more sand deposition (Schlacher et al. 2008; Frosini et al. 2012; Seabloom et al. 2013; Cheplick 2017).

The presence of shrubs, which alter physical conditions, had modest effects on relative frequencies of some species. We found no evidence that the conditions associated with shrubs had differential effects on germination, although our experiment did not consider cues such as red:far-red ratio. Shrubs acting as nurse plants facilitative effects on growth and

survival in stressful environments (Filazzola and Lortie 2014), including coastal dunes (Shumway 2000; de Castanho and Prado 2014). Dune shrubs also support a different soil microbial community than is found in open areas of the dune (Rajaniemi and Allison 2009), which may influence plant growth and establishment.

Invasive species similarly have the potential to alter environmental conditions in ways that might enhance or hinder germination. For example, the succulent *Carpobrotus edulis* is a frequent invader of Mediterranean dunes, where it alters soil pH and organic matter content and negatively affects germination, growth, and survival of native species (Conser and Connor 2009; Novoa et al. 2013; Novoa and González 2014). The influence of dominant vegetation, both native and non-native, on the distribution of herbaceous dune species deserves further investigation.

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