WETLAND ECOLOGY

Seed Viability, Spikelet Dispersal, Seed Banks and Seed Storage Requirements for Native and Invasive Cordgrasses (Genus *Spartina*) in Southwest Iberian Peninsula

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Abstract

Seed banks play an important role in determining the spatial and temporal distribution of halophytes in salt marshes. We tested the ability of native *Spartina maritima* and invasive *S. densiflora* spikelets to disperse by flotation on water with different salinity concentrations, and the longevity of spikelet viability relative to environmental conditions including dry or wet storage at low or moderate temperatures, and aqueous salinity concentrations from fresh to sea water. We quantified pre-dispersal seed production and pre- and post-dispersal focal *Spartina* spp. presence in seed banks along intertidal gradients within salt marshes at the Gulf of Cádiz (Southwest Iberian Peninsula). *Spartina densiflora* spikelets, especially from middle and high elevation marshes, showed greater ability than *S. maritima* to remain afloat, which suggests this species could be dispersed over longer distances. Wet-stored *Spartina* seeds were able to maintain viability for months, while seeds under dry storage rapidly lost viability. This decline was most significant for *S. maritima*, and for seeds stored at moderate temperatures. Storage of spikelets under wet and cold conditions optimized spikelet viability. Native *S. maritima* did not establish transient or persistent seed banks, while invasive *S. densiflora* established transient seed banks mainly at higher marsh elevations. Our results on the dynamics of seed dispersal and seed banks and seedling recruitment provide fundamental knowledge that can be applied for conservation of native *S. maritima*, management of invasive *S. densiflora*, and ecological restoration of tidal salt marshes.

Introduction

Soil seed banks play an important role in the establishment, maintenance, regeneration and restoration of vegetation in many plant communities (Bao et al. 2021; An et al. 2022). In salt marshes, seed dispersal and soil seed banks play an important role in determining the spatial and temporal distribution of halophytes (Egan and Ungar 2000; Rand 2000; Crain et al. 2008), and the successional assembly of halophytic communities following disturbance or restoration actions (Dausse et al. 2007; Kottler and Gedan 2019).

The presence and abundance of a species in the soil seed bank depends on its seed production, seed dispersal and its longevity in the soil. Regarding longevity in the soil, there are two general types of seed banks: transient and persistent (Fenner and Thompson 2005; Thompson and Grime 1979). This temporal classification of seed banks is useful in the context of exotic plant invasions, because it provides a predictive model for how long a species may persist in soil as a latent source of invasive propagules (Gioria and Pyšek 2016). In the transient seed bank, seeds are renewed annually, with accumulation of viable seeds for only up to one year. Persistent seed banks include live dormant seeds that are one or more years old. One way for species to survive in stressful environments with high mortality risk is to

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establish persistent seed banks (Baskin and Baskin 2001). Most perennial halophyte species form predominantly transient soil seed banks (Hutchings and Russell 1989; Ungar 2001; Wolters and Bakker 2002; Polo-Ávila et al. 2019).

Species with a transient seed bank depend on seed dispersal to maintain diversity and colonize new areas (van den Broek et al. 2005; Polo-Ávila et al. 2019). Since long-term persistent seed banks usually do not exist for perennials in salt marshes, seed dispersal by seawater (hydrochory) also plays an important role in the preservation and ecological restoration of salt-marsh communities (Wolters and Bakker 2002). In this sense, seed buoyancy enhances seed dispersal in aquatic environments, as salt marshes (van den Broek et al. 2005; Elsey-Quirk et al. 2009). Thus, sexual propagules of many salt marsh species are able to float in seawater (Huiskes et al. 1995; Wolters and Bakker 2002). Seeds of some halophytes can remain afloat for hours, while others may float during months, increasing the chance to disperse further away from their sources (Huiskes et al. 1995). In addition, seeds of halophytes may be influenced by salinity during their dispersal phase, affecting the seed flotation time (Elsey-Quirk et al. 2009). In salt marshes, tidal flooding can disperse and redistribute the seeds locally, affecting to the distributions of local species, or can disperse the seeds over long distances, favouring the colonization of new habitats (Huiskes et al. 1995; Xiao et al. 2016). In addition, seed dispersal seems to be an important factor that affects the spatial distribution of seed banks in salt marshes (Wang et al. 2009).

The spread of exotic species is an important threat to salt marsh conservation (Adam 2002). In this context, the study of soil seed banks and seed dispersal of invasive species is a crucial aspect since our knowledge on seed dynamics in the soil plays an important role in the management of salt marshes, and also may help to improve conservation and restoration actions (Wang et al. 2009; Xiao et al. 2009; Hazelton et al. 2014). Evaluation of soil seed banks can also improve our understanding of factors contributing to the invasiveness of species (Gioria et al. 2012).

The genus *Spartina* (cordgrasses, a monophyletic clade of perennial grasses) is abundant in salt marshes and is distributed worldwide in every continent except Antartica (Bortolus et al. 2019). Frequently, exotic *Spartina* species become invasive when introduced to new geographical areas (Ainouche and Gray 2016). In this context, *Spartina maritima* (Curtis) Fernald is a primary colonizer in salt marshes and the only European native cordgrass (Marchant and Goodman 1969). In Southwest Iberian Peninsula, this native cordgrass co-occurs with exotic and invasive *Spartina densiflora* Brongn. introduced from South America, which colonizes very contracted habitats along the intertidal gradient (Nieva et al. 2001). In general, *Spartina* species disperse

spikelets that are well adapted to float in sea water, allowing them to be transported over long distances (Morgan and Sytsma 2013; McDonald 2014; Xiao et al. 2016). Moreover, *Spartina* species can establish transient seed banks that may vary in seed density and characteristics among different salt marsh habitats (Ungar 2001; Wang et al. 2009; Xiao et al. 2009, 2016).

Until very recently, seed production in *S. maritima* had been described as very low or non-existent (Marchant and Goodman 1969; Castellanos et al. 1994; Castillo et al. 2010), but Infante-Izquierdo et al. (2019a) observed in the Southwest Iberian Peninsula that this species produces a moderate number of caryopses with high seed viability. For this reason, to our knowledge, this is the first work that evaluates seed bank dynamics in *S. maritima*. Only one work has studied the seed bank of *S. densiflora* showing that this invasive species forms transient or persistent seed banks depending on environmental conditions (Abbas et al. 2021). In this work, we tested the ability of *S. maritima* and *S. densiflora* spikelets to disperse in water, the spikelet longevity relative to different environmental conditions, and we quantified the pre-dispersal seed production and the seed banks of both cordgrasses along the intertidal gradient. Differences in the ecology behaviour of these species, *S. maritima* as a pioneer species of mudflat soils, and *S. densiflora* as an invader of all habitats along the intertidal gradient, could be affected to a great extend by their seed dispersal and soil seed bank dynamics. In this sense, we hypothesized that both *Spartina* species would form transient seed banks in salt marsh habitats. We also postulated that daily submersion of inflorescences in tidal waters would decrease seed buoyancy, especially in more frequently inundated low elevation marshes. Our aim was to evaluate the dynamics of seed dispersal and seed banks to provide fundamental knowledge critical for science-based conservation of native *S. maritima*, and for restoration and management of invasive *S. densiflora*-invaded tidal marshes. With this aim, we studied seed viability, spikelet dispersal, seed banks and seed storage requirements for both native *S. maritima* and alien *S. densiflora* in three estuaries located in Southwest Iberian Peninsula.

Methods

Study Sites

This work was carried out in tidal salt marshes in the estuaries of Odiel, Tinto and Piedras Rivers located along the Gulf of Cádiz (Southwest Iberian Peninsula) (Fig. 1). This area is under a Mediterranean climate with Atlantic influence. The coast of the Gulf of Cádiz is mesotidal and the mean
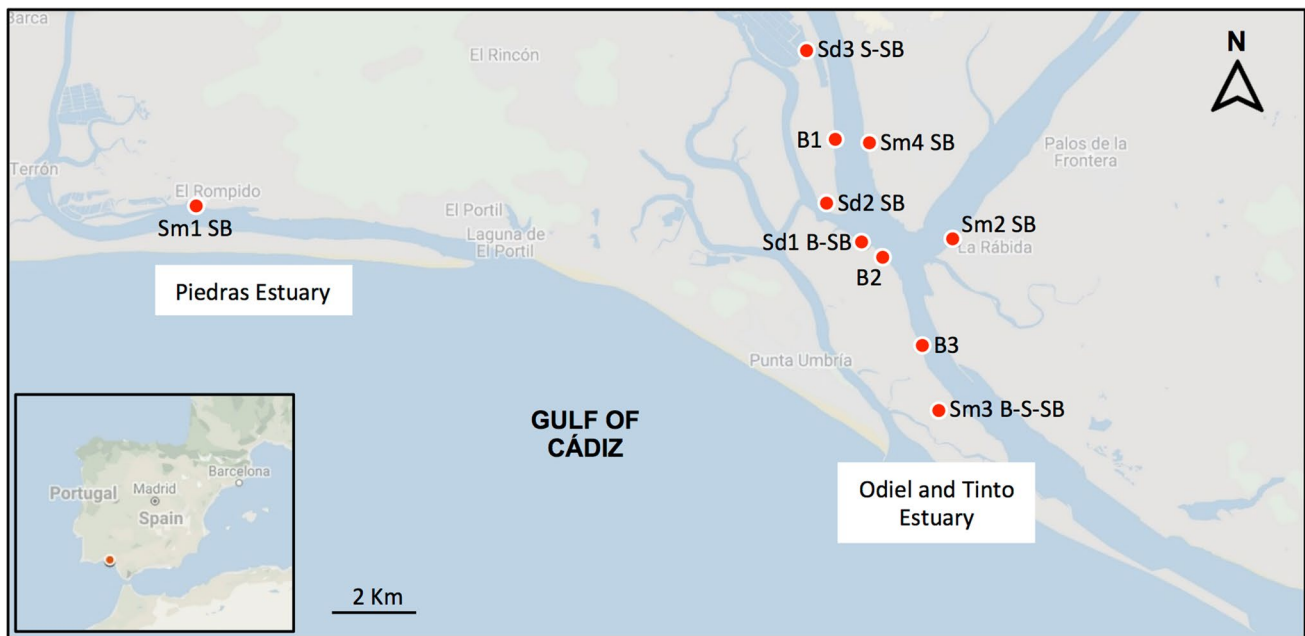
Fig. 1 Sampled points for native *Spartina maritima* (Sm) and invasive *S. densiflora* (Sd) in the Gulf of Cádiz (Southwest Iberian Peninsula). The assays for which locations were sampled (B: buoyancy; S: storage; SB: seed bank) are indicated after the species. The three beaches sampled (B) to study the spikelet dispersion are also indicated (source: Google Maps, data from ©2019 Instituto Geográfico Nacional Spain)

sea level in this area is $+1.85$ m relative to Spanish Hydrographic Zero (SHZ). The tides are semidiurnal and have a mean range of 2.10 m and a mean spring tidal range of 2.97 m, representing 0.40–3.37 m above SHZ (Castellanos et al. 1994). Native vegetation in salt marshes along the Gulf of Cádiz has been described in previous works (Castellanos et al. 1994; Fernández-Illescas et al. 2010). Different vegetation zones can be distinguished based on tidal influence and soil characteristics (Contreras-Cruzado et al. 2017). Low elevation intertidal marshes (hereafter LM) occur between Mean High Water Neap and Mean High Water, and they are dominated by *Sarcocornia perennis* (Mill.) A.J. Scott and *Spartina maritima*; middle marshes (MM) are located between Mean High Water to Mean High Water Spring, and are dominated by *Sarcocornia fruticosa* (L.) A.J. Scott, *Sarcocornia* hybrids (Figueroa et al. 2003) and *Halimione portulacoides* Aellen; high marshes (HM) are at Mean High Water Spring to Highest Astronomical Tide, and they are colonized by halophytes such as *Arthrocnemum macrostachyum* (Moric.) K. Koch, *Suaeda vera* Forssk. ex J. F. Gmel. and *Limoniastrum monopetalum* (L.) Boiss. (Long and Mason 1983; Fernández-Illescas et al. 2010). Native *S. maritima* inhabits LM, and is considered a primary colonizer of bare intertidal mudflats (Castellanos et al. 1994; Infante-Izquierdo et al. 2019a), while exotic and invasive *S. densiflora* invades LM, MM and HM, and with invasive spread, displaces native vegetation (Nieva et al. 2001).

Spikelet Buoyancy

Mature spikelets were randomly collected from one *S. maritima* population at LM in August 2017, and from one *S. densiflora* population at LM, MM and HM in November 2017 (Fig. 1). Then, spikelets containing caryopses were randomly selected and stored in paper bags in dark and dry conditions at $+5$ ºC. Four sets of 25 spikelets from each sampled location and habitat were placed in 500 ml beakers (8 cm diameter), each containing 100 ml of one of three different salt solutions (0.0, 0.3 and 0.6 M NaCl) at $+20$–25 ºC and a 12 h light/dark photoperiod. Radiation was provided by fluorescent lamps that produced a photosynthetic photon flux density of 60 μmol m^{-2} s^{-1}. Floating and sunken spikelets were counted seven times on the first day and twice on the following days during 5 days until practically no spikelets were floating. Beakers were agitated before counting floating and sunken spikelets to simulate wave action that can affect buoyancy (Van den Broek et al. 2005). The percentage of spikelets that remained afloat was calculated over time for each beaker. Sinking rate was calculated as the number of sinking spikelets per hour.

Spikelet Storage

Mature spikelets were randomly collected from one *S. maritima* population at LM in August 2017 and from one *S. densiflora* population at HM in November 2016

(Fig. 1). Immediately following collection, spikelets containing caryopses were randomly selected and sowed to establish a control germination before storage. Spikelets were surface-sterilized before sowing in 5% (v/v) sodium hypochlorite for 10 min to prevent fungal contamination, and then rinsed with distilled water (Muñoz-Rodríguez et al. 2012; Infante-Izquierdo et al. 2019a). Four replicates, each with 25 spikelets, were sown in Petri dishes (9 cm diameter) on two layers of autoclaved filter paper, dampened with distilled water, and sealed with adhesive tape (Parafilm™) to avoid desiccation. Germination was carried at $+20$–25 ºC and a 12 h light (60 µmol m^{-2} s^{-1})/ dark photoperiod. Germination was recorded every 2 or 3 days for 2 months. Spikelets were considered germinated when the coleoptile emerged. The rest of spikelets were stored for 1, 3, 6 and 12 months in one of five storage treatment conditions: (1) dry at $+20$ °C); (2) dry at $+5$ ºC; (3) immersed in distilled water (0 M NaCl) at $+5$ ºC; (4) immersed in 0.3 M NaCl solution at $+5$ ºC; and (5) immersed in 0.6 M NaCl at $+5$ºC. Then, these pretreated spikelets were sown as reported above. Viability of the caryopses from spikelets that did not germinate was tested using the tetrazolium test (Mackay 1972). For this purpose, the embryo was incised with a scalpel and submerged in a 1% aqueous solution of 2,3,5 triphenyl tetrazolium chloride at $+25$ ºC in darkness for 24 h. Then, red stained viable embryos were counted through a magnifying glass. The percentage of spikelets with viable caryopses (germinated plus dormant), the germination percentage (based on spikelets with viable caryopses) and the days necessary to reach 50% of the final germination percentage (T_{50}) were calculated for each Petri dish (Muñoz-Rodríguez et al. 2012; Infante-Izquierdo et al. 2019a).

Spikelet Production and Soil Seed Banks

Both cordgrass species produce spikelets which break off from the mother plant and become a dispersal unit, in which glumes, palea and lemma cover the caryopsis. In our study we quantified the production and the density in the soil of total spikelets, including empty ones, spikelets containing caryopses and spikelets containing viable caryopses. In the soil we have also quantified the density of spikelets with germinating caryopses. We have used the term 'seed bank' as a generic term applied to the densities of all these types of spikelets in the soil, in concordance with seed bank literature. To study the dynamics of the seed banks of *S. maritima* and *S. densiflora*, the different phases in spikelet production and dispersal were followed and compared (Fig. 2). Annual spikelet production (ASP) and soil seed bank were studied at LM in four locations for *S. maritima* (Sm1, Sm2, Sm3 and Sm4; Fig. 1) and at LM, MM and HM, and adjacent bare

mudflats (BM) in three locations (Sd1, Sd2 and Sd3; Fig. 1) for *S. densiflora*.

Annual Spikelet Production

The production of total spikelets (ASP) and of spikelets with caryopses per m^2 of habitat were calculated for four populations of *S. maritima* in low marshes, and the production of spikelets with viable caryopses per m^2 was calculated for two of those populations, using data obtained from Infante-Izquierdo et al. (2019a). For *S. densiflora*, spikelet production was calculated by counting the number of inflorescences on live plants prior to the onset of primary dispersal. Counts were made in 10 randomly distributed squared plots (50×50 cm) in patches of *S. densiflora* formed by coalescent tussocks in MM and HM, while in LM, in which *S. densiflora* grows in discrete tussocks, density of inflorescences was recorded by counting the number of inflorescences in 10 tussocks and measuring each tussock diameter to calculate its area. We randomly collected 20 inflorescences from each *S. densiflora* population and counted the number of total spikelets per inflorescence and the number of spikelets with caryopses, calculating then the density of spikelets with caryopses for each population. Density of spikelets with viable caryopses was calculated as the product of the mean percentage of viability by the density of spikelets with caryopses per population. These data were converted in production per m^2 of habitat by using *S. densiflora* coverage proportion for each habitat and location for the same year, recorded along three 50 m transects in each habitat and location (LM: 0.10 ± 0.02; MM: 0.56 ± 0.09; HM: 0.51 ± 0.08).

Soil Seed Bank Sampling and Analysis

Spartina maritima seed bank was studied in LM at the four studied locations (Fig. 1) in October 2017, just after spikelet dispersal (initial seed bank). *Spartina densiflora* seed bank was sampled in LM, MM, HM and BM and at three locations (Fig. 1) in July 2017 (remnant seed bank) and in January 2018 (initial seed bank). To evaluate the presence of *Spartina* spp. in soil seed banks, we randomly collected ten sediment samples at each designated study location and habitat using stainless steel cores (50 mm diameter, 50 mm height) during low tides. We used this model of stainless steel core since previous seed bank studies in salt marshes have reported that most of the seeds accumulates in the first 50 mm of sediments (Coteff and Van Auken 2006; Zepeda et al. 2014).

To analyze spikelet dispersal away from source populations, we have studied the soil seed banks at three sandy beaches located more than 500 m from the nearest *Spartina* population (B1, B2 and B3 in Fig. 1). These beaches were sampled in February 2018, when both species had finished
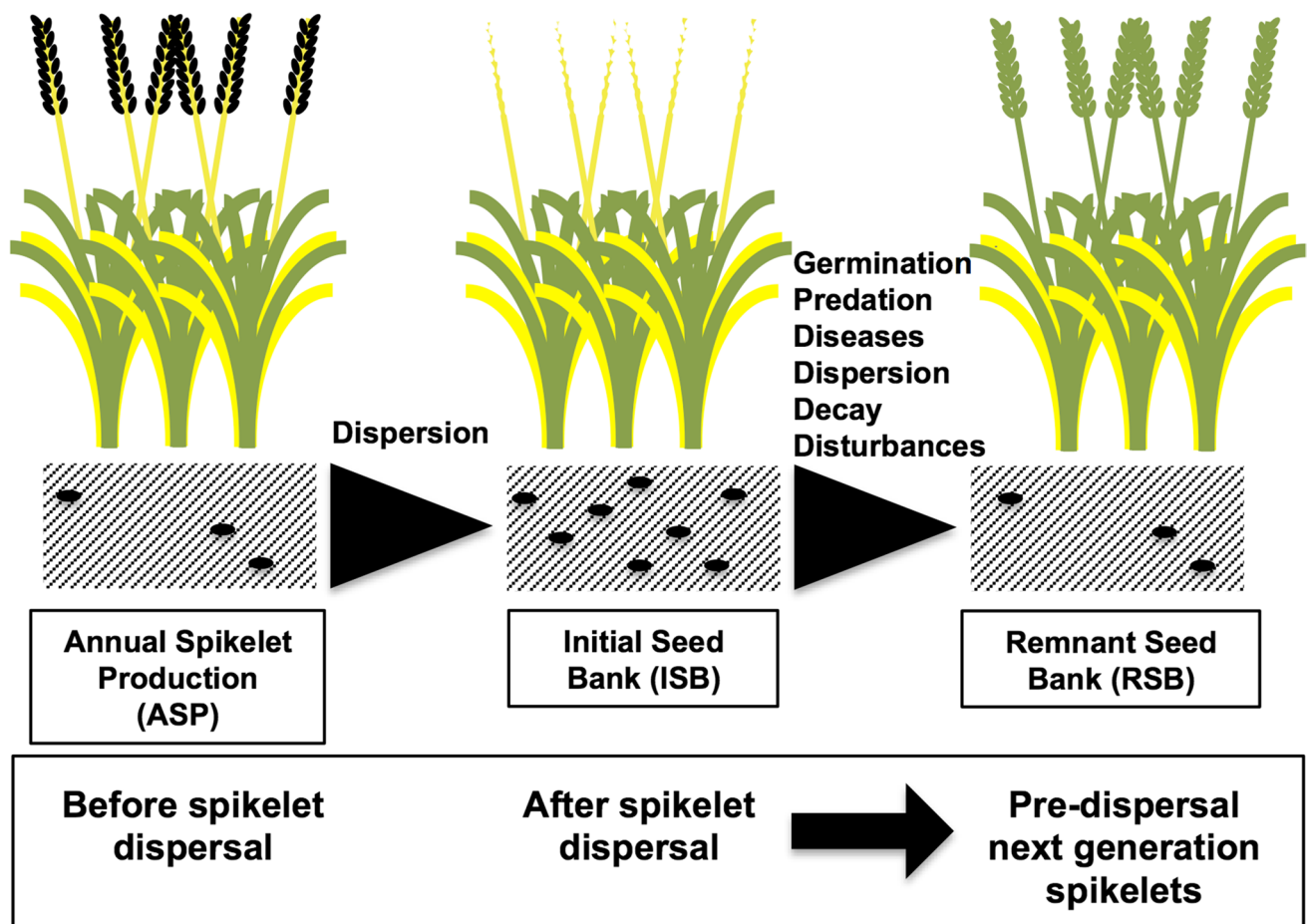
Fig. 2 Scheme showing the different phases in spikelet production and dispersal for *Spartina* species

their spikelet dispersal periods. Three zones (bare mudflats, sand beach and high tide line) were sampled in each beach ($n = 10–20$ sediment samples per zone and beach).

Sediment samples were analysed in the laboratory just after sampling. Spikelets were directly extracted from sediment samples, since methods based on germination are less precise because many seeds may remain dormant and, therefore, the seed bank could be underestimated (Gross 1990; Brown 1992). Thus, each soil sample was washed to reduce the amount of sediment (Wolters and Bakker 2002) over a 1-mm sieve that retained all *Spartina* spikelets (Infante-Izquierdo et al. 2019b). The material that remained in the sieve (sand, gravel, shells, plant wrack and propagules) was placed on a filter paper and, spikelets were counted and extracted under a magnifying glass. Then, glumes, palea and lemma were removed from each spikelet in order to examine the presence or absence of caryopsis. Viability of caryopses was tested using the tetrazolium test as described above. Density of the total spikelets (total spikelets m^{-2}), density of spikelets with caryopses (caryopses m^{-2}), density of spikelets with viable caryopses (viable caryopses m^{-2}) and density of

spikelets in which caryopses were germinating in the field at sampling moment (germinated caryopses m^{-2}) was calculated for each habitat for initial and remnant seed banks.

Statistical Analysis

Statistical analyses were carried out with STATISTICA 8.0 (StatSoft Inc., USA). Deviation from the mean was calculated as Standard Error (SE) for all variables. Test results were considered significant when $p \leq 0.05$. Kolmogorov-Smirnov test and Levene test were used to evaluate the normality and homogeneity of all data series, respectively. Data series were transformed using $\sqrt{x}$, $1/(x+1)$, $\ln(x+1)$ or $\arcsin(x)$ functions trying to achieve normality and homogeneity of variance normality and homogeneity of variance, but they were not achieved in any case. Seed viability and germinability responses of *S. maritima* and *S. densiflora* to storage period treatments (0, 1, 3, 6, 12 months), spikelet production, and seed bank characteristics were all analysed using one-way ANOVAs with Tukey's Honest Significant Difference (HSD) as post hoc tests. In cases where

data transformations did not support use of the parametric ANOVA, non-parametric Kruskal-Wallis H test with Mann-Whitney U as the post-hoc test were used. Spearman's correlation coefficient (ρ) was used to correlate the percentage of spikelets of *S. maritima* and *S. densiflora* that remained afloat over time.

Results

Spikelet Buoyancy

In *S. maritima*, there was a significant and linear reduction of floating spikelets over time in the three saline solutions, from $100 \pm 0\%$ to $9 \pm 1\%$ during the first 24 h, reaching $2 \pm 1\%$ at 48 h (Spearman correlations coefficient, 0 M NaCl: ρ = -0.9591; 0.3 M NaCl: ρ = -0.9359; 0.6 M NaCl: ρ = -0.9518; $p < 0.05$) (Fig. 3a). Sinking rate was 2.50 seeds h^{-1} in 0.0 M and 0.6 M NaCl, and 2.44 seeds h^{-1} in 0.3 M NaCl.

The percentage of floating *S. densiflora* spikelets after the first 24 h was $70 \pm 3\%$. After 70 h, *S. densiflora* maintained $8 \pm 2\%$ of floating spikelets, with $1 \pm 0\%$ reduction in buoyancy recorded on the fourth day (94 h). A linear and significant decrease of floating *S. densiflora* spikelets over time was observed under the three saline solution treatments (Spearman correlations coefficient, 0 M NaCl: ρ = -0.9022; 0.3 M NaCl: ρ = -0.9145; 0.6 M NaCl: ρ = -0.8911; $p < 0.05$) (Fig. 3b), with a sinking rate of 1.25 seeds h^{-1} in 0.0 M and 0.3 M NaCl, and 1.17 seeds h^{-1} in 0.6 M NaCl. *Spartina densiflora* spikelets from LM, MM and HM showed a significant decrease of floating spikelets over time (Fig. 3c) (Spearman correlations coefficient, LM: ρ = -0.9748; MM: ρ = -0.9206; HM: ρ = -0.9281; $p < 0.05$) with similar sinking rates: 1.27 seeds h^{-1} at HM, 1.22 seeds h^{-1} at MM and 1.18 seeds h^{-1} at LM. However, while the first sunken spikelets from MM and HM were observed at 8.5 h, those from LM started to sink immediately. No *S. maritima* or *S. densiflora* spikelets germinated during the flotation experiment.
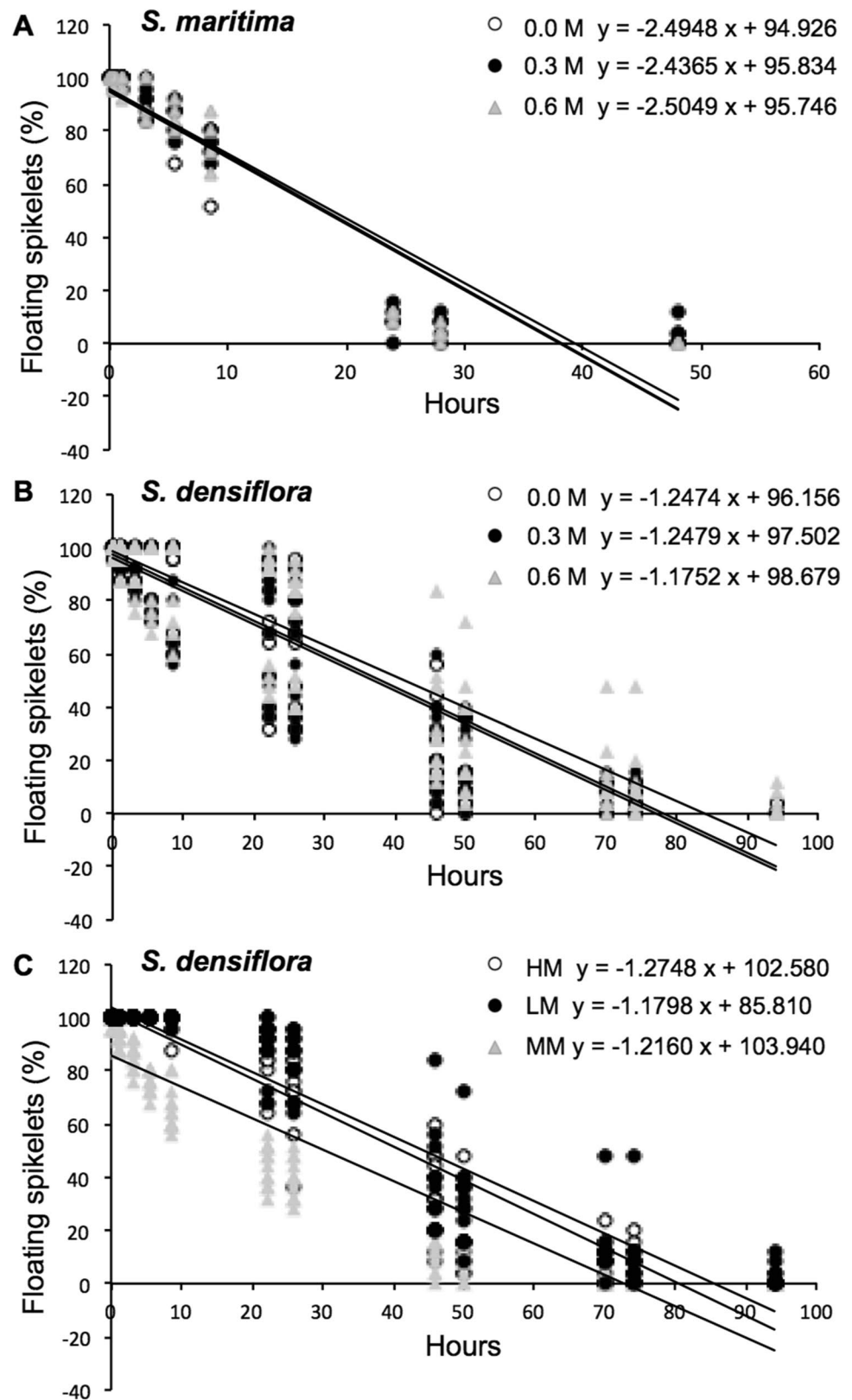
Spikelet Storage

In *S. maritima*, spikelet viability was $80 \pm 4\%$ for the control treatment. Spikelets stored dry at $+20$–25 ºC showed a drastic reduction in viability after 1 month ($12 \pm 2\%$), with all spikelets dead after 3, 6 and 12 months in these conditions (Kruskal-Wallis test: $H_{4,20}$ = 18.65, $p < 0.001$) (Fig. 4a). Spikelets dry-stored at $+5$ ºC maintained $44 \pm 4\%$ of viable seeds up to 3 months, although with a significant reduction compared to the control (One-way ANOVA test: F=110.87, df=4, $p < 0.0001$) (Fig. 4b). Seeds stored wet at $+5$ ºC maintained viability for at least 6 months, decreasing after 12 months (Fig. 4c, d, e). This reduction of seed viability

over time was more pronounced in distilled water ($3 \pm 1\%$) (One-way ANOVA test: F= 56.40, df = 4, $p < 0.0001$), than in 0.3 M and 0.6 M NaCl, in which viability always remained above 40% (One-way ANOVA test: F= 24.34, df=4, $p < 0.0001$; F= 12.33, df=4, $p < 0.001$, respectively).

S. maritima viable seed germination was ca. 98% after all storage periods in all conditions (One-way ANOVA or Kruskal-Wallis test, $p > 0.05$) (Fig. 4a, b, c, d, e). *S. maritima* spikelets showed T_{50} values of 23.3 ± 1.6 days for the control treatment. Germination was significantly delayed (higher T_{50}) when spikelets were stored dry at $+20$–25 ºC for 1 month. However, spikelets stored dry at $+5$ ºC did not delay their germination (Fig. 5). In contrast, in the three wet and cold storage conditions, germination was significant and progressively accelerated (lower T_{50}) when storage time increased (Fig. 5).

Spartina densiflora seed viability was $92 \pm 2\%$ for the control treatment. Seeds dry-stored at $+20$–25 ºC maintained initial viability for 1 month, followed by a $71 \pm 1\%$ reduction in viability after 3 months, with senescence of nearly all seeds after 6 months (Kruskal-Wallis test: $H_{4,20}$ = 17.41, $p < 0.01$) (Fig. 4a). Spikelets stored dry at $+5$ ºC maintained initial viability until 6 months, with a reduction in viability to 83% after 12 months (One-way ANOVA test: F= 7.52, df=4, $p < 0.01$) (Fig. 4b). Spikelets stored wet at $+5$ ºC maintained initial viability for 6 months (Fig. 4c, d, e). Seed viability was reduced significantly in distilled water and 0.3 M NaCl solution after 12 months (One-way ANOVA test: F= 22.44, df=4, $p < 0.0001$; F= 19.35, df=4, $p < 0.0001$, respectively) (Fig. 4c, d), and viability remained constant in 0.6 M NaCl solution (One-way ANOVA test: F= 2.01, df=4, $p > 0.05$) (Fig. 4e). Viable seed germination was $52 \pm 5\%$ for the control sowing, with an initial dormancy level of 48%. Germination of spikelets storage dry at $+20$–25 ºC for 1 month increased to 81%, being maintained this high for 3 and 6 months (One-way ANOVA test: F= 10.65, df=3, $p < 0.01$) (Fig. 4a). Germination percentage remained constant during the entire storage period for dry-stored seeds at $+5$ ºC (One-way ANOVA test: F= 1.85, df=4, $p > 0.05$) (Fig. 4b). In the three wet and cold storage conditions, initial seed germinability was maintained until 3 months, increasing significantly after 6 or 12 months (Kruskal-Wallis test, 0.0 M: $H_{4,20}$ =14.32, $p < 0.01$; One-way ANOVA test, 0.3 M: F= 18.71, df=4, $p < 0.0001$; 0.6 M: F= 21.32, df=4, $p < 0.0001$) (Fig. 4c, d, e). At the time of spikelet collection (control), T_{50} was 22.9 ± 0.6 days. At dry and $+20$–25 ºC, germination was significantly delayed (higher T_{50}) after 1 month or more, whereas T_{50} was not significantly affected in dry at $+5$ ºC (Fig. 5). In contrast, germination was significantly accelerated (lower T_{50}) in the three wet and cold storage conditions in storage periods longer than 3 months (Fig. 5). No *S. maritima*

Fig. 3 Percentage of floating spikelets in each beaker over time in different salt solutions of native *S. maritima* (**a**) and invasive *S. densiflora* (**b**) (M: molar NaCl), and in the different *S. densiflora* habitats (**c**) (LM: low marsh, MM: middle marsh and HM: high marsh). Regression lines and equations are presented in each case (Spearman correlation coefficient showed in Results)

A   *S. maritima*

○ 0.0 M y = -2.4948 x + 94.926
● 0.3 M y = -2.4365 x + 95.834
▲ 0.6 M y = -2.5049 x + 95.746

B   *S. densiflora*

○ 0.0 M y = -1.2474 x + 96.156
● 0.3 M y = -1.2479 x + 97.502
▲ 0.6 M y = -1.1752 x + 98.679

C   *S. densiflora*

○ HM y = -1.2748 x + 102.580
● LM y = -1.1798 x + 85.810
▲ MM y = -1.2160 x + 103.940
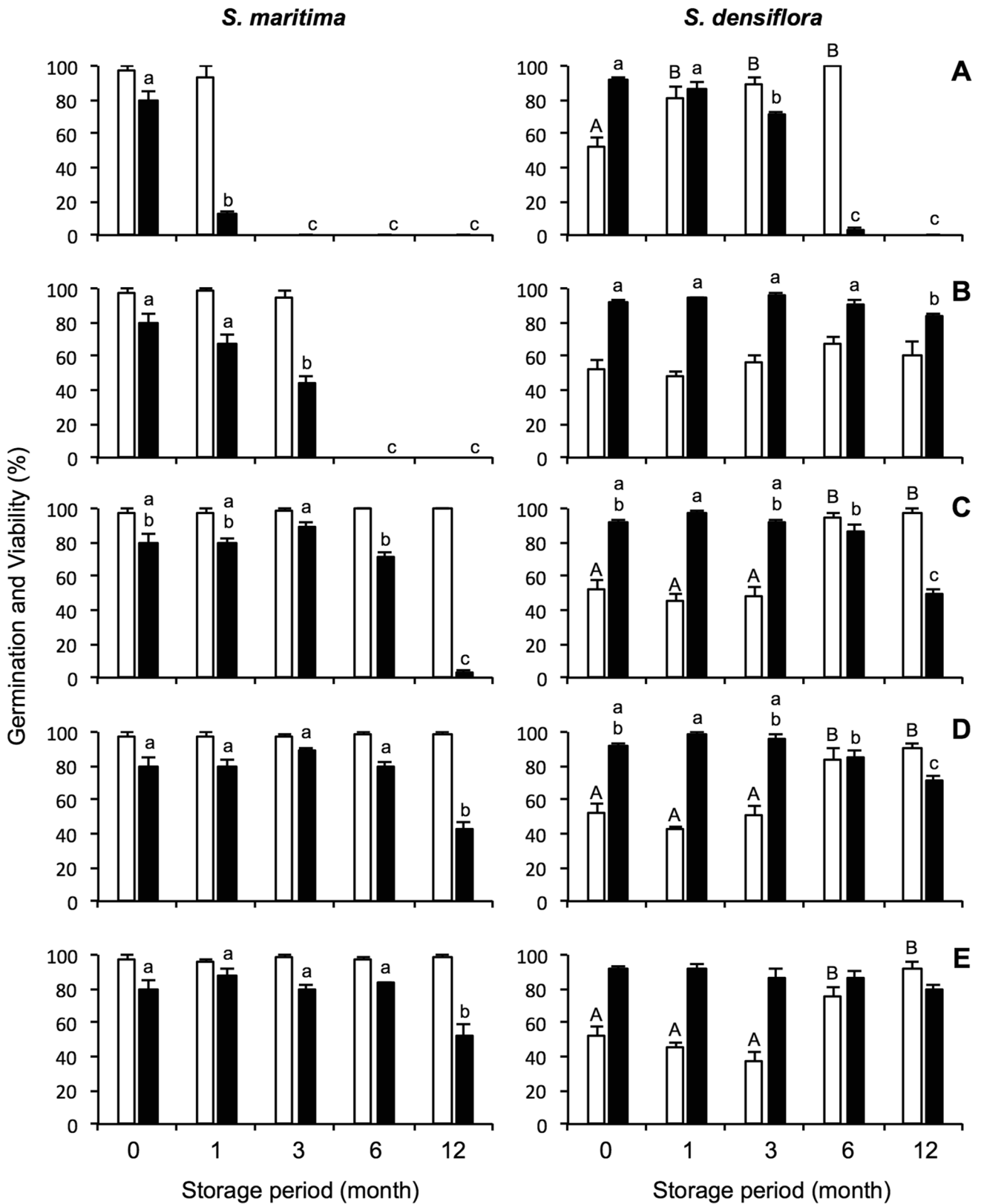
Fig. 4 Germination (white bars) and viability percentages (black bars) for seeds of *Spartina maritima* and *S. densiflora* stored under different conditions: dry at +20–25 ℃ (**a**), dry at +5 ℃ (**b**), wet (0.0 M NaCl) at +5 ℃ (**c**), wet (0.3 M NaCl) at +5 ℃ (**d**) and wet (0.6 M NaCl) at +5 ℃ (**e**); during 0 (control), 1, 3, 6 and 12 months

Fig. 5 Days necessary to reach 50% of the final germination percentage (T_{50}) for native *Spartina maritima* and invasive *S. densiflora* spikelets stored under different conditions during 0, 1, 3, 6 and 12 months. Data are means ± SE. Different letters indicate significant differences among storage periods within each storage condition (Mann-Whitney U-test or Tukey's HSD test, $p < 0.05$)
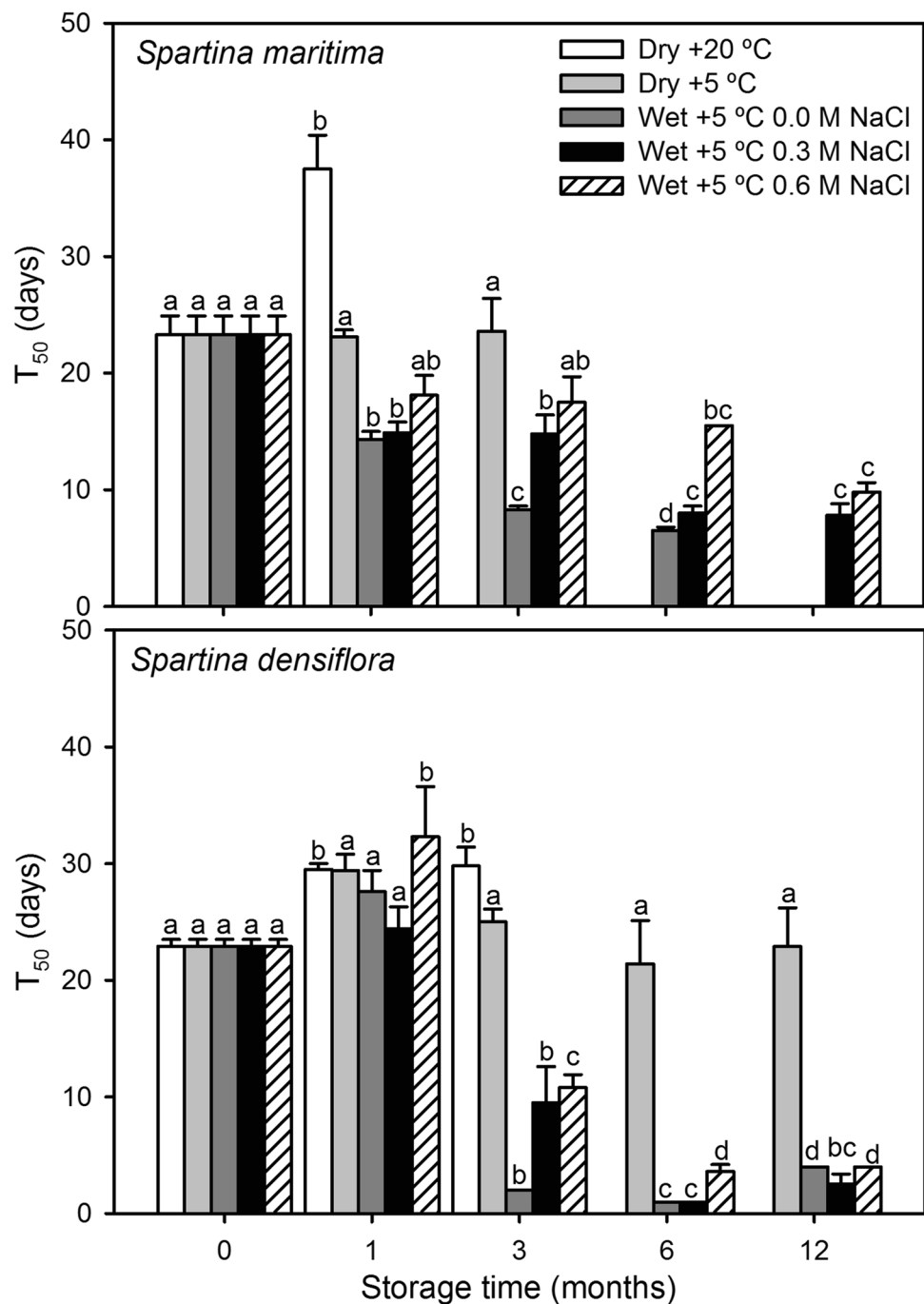
and *S. densiflora* spikelet germinated during the storage experiment.

Annual Spikelet Production (ASP)

ASP of *S. maritima* from field sampling was 7538 ± 896 total spikelets m^{-2} in Sm1, 8069 ± 1454 total spikelets m^{-2} in Sm2, 1699 ± 760 total spikelets m^{-2} in Sm3, and 6565 ± 1441 total spikelets m^{-2} in Sm4. Production of spikelets with caryopses per m^2 was 889 ± 106 in Sm1, 991 ± 178 in Sm2 562 ± 252 in Sm3, and 2177 ± 478 in Sm4; and the production of spikelets with viable caryopses per m^2 was 450 ± 201 in Sm3 and 2111 ± 463 in Sm4.

ASP of *S. densiflora* showed no significant differences among the three studied *S. densiflora* habitats in any production trait. Production of total spikelets per m^2 ranged from 1623 ± 304 to 6652 ± 1893, production of spikelets with caryopses per m^2 ranged from 533 ± 80 to 1722 ± 476 and production of spikelets with viable caryopses per m^2 oscillated between 304 ± 52 and 1390 ± 380 (Table 1).

Table 1 Density of total spikelets (total Spk m^{-2}), density of spikelet with caryopses (caryopses m^{-2}), density of spikelets with viable caryopses (Vcaryopses m^{-2}) for invasive *Spartina densiflora* at the annual spikelet production and initial and remnant seed bank in bare mudflats (BM), low marshes (LM), middle marshes (MM), and high marshes (HM) ($n = 30$)

	BM	LM	MM	HM	ANOVA (F) or KW (H) test
Annual spikelet production					
Total Spk m^{-2}	-	6652 ± 1893^a	1623 ± 304^a	1789 ± 300^a	F = 2.71, df = 2, $p > 0.05$
Caryopses m^{-2}	-	1722 ± 476^a	757 ± 124^a	533 ± 80^a	$H_{2,89} = 2.36$, $p > 0.05$
VCaryopses m^{-2}	-	1390 ± 380^a	511 ± 80^a	304 ± 52^a	$H_{2,89} = 5.64$, $p > 0.05$
Initial seed bank					
Total Spk m^{-2}	68 ± 32^a	475 ± 150^a	1783 ± 231^b	3922 ± 1101^b	$\mathbf{H_{3,120} = 60.59, p < 0.0001}$
Caryopses m^{-2}	17 ± 17^a	119 ± 47^b	187 ± 57^b	340 ± 107^b	$\mathbf{H_{3,120} = 12.50, p < 0.01}$
VCaryopses m^{-2}	0 ± 0^a	102 ± 45^b	119 ± 47^{bc}	306 ± 96^c	$\mathbf{H_{3,120} = 15.65, p < 0.01}$
GCaryopses m^{-2}	0 ± 0^a	34 ± 24^a	17 ± 17^a	170 ± 51^b	$\mathbf{H_{3,120} = 18.48, p < 0.001}$
Remnant seed bank					
Total Spk m^{-2}	17 ± 17^a	51 ± 28^a	1681 ± 400^b	1358 ± 271^b	$\mathbf{H_{3,120} = 58.41, p < 0.0001}$
Caryopses m^{-2}	0 ± 0^a	0 ± 0^a	17 ± 17^a	51 ± 28^a	$H_{3,120} = 6.16$, $p > 0.05$
VCaryopses m^{-2}	0 ± 0^a	0 ± 0^a	0 ± 0^a	17 ± 17^a	$H_{3,120} = 3.00$, $p > 0.05$
GCaryopses m^{-2}	0 ± 0	0 ± 0	0 ± 0	0 ± 0	-

Initial seed bank includes data of spikelets with germinated caryopses (Gcaryopses m^{-2}). Different letters indicate significant differences among habitats (Mann-Whitney U-test or Tukey's HSD test, $p < 0.05$). Data are means $\pm$ SE. Significant differences are marked in bold

Soil Seed Bank

Regarding *S. maritima* seed bank, we only found 153 ± 109 spikelets without caryopses m^{-2} in Sm2 and 51 ± 51 spikelets without caryopses m^{-2} in Sm3 in the initial seed bank. No spikelets with caryopses were recorded in any of the studied *S. maritima* populations neither in any studied *S. densiflora* habitat or beaches.

In the initial seed bank, density of total spikelets of *S. densiflora* was significantly higher in MM and HM than in LM and BM. Density of spikelets with caryopses was significantly higher in soils from LM, MM and HM than in BM. Density of spikelets with viable caryopses increased from BM to HM along the intertidal gradient. Finally, density of spikelets with caryopses germinating in the field at the moment of sampling was significantly higher in HM than in BM, LM and MM (Table 1).

In the remnant seed bank, density of total spikelets of *S. densiflora* was significantly higher in MM and HM than in BM and LM. We only found spikelets with caryopses at similar densities in MM and HM. Spikelets with viable caryopses were recorded only in HM. In addition, we did not find any germinated caryopsis in the remnant seed bank (Table 1).

In the studied beaches, total density of *S. densiflora* spikelets was higher at the high tide lines than at lower bare mudflats or sand beaches (Kruskal-Wallis test: $H_{2,110} = 21.72$, $p < 0.0001$). Density of spikelets with caryopses, with viable caryopses and with germinated caryopses also showed the highest values at high tide level, but without significant differences among zones (Kruskal-Wallis test, $p > 0.05$) (Table 2).

Table 2 Density of total spikelets, spikelets with caryopses, with viable caryopses and with germinated caryopses for invasive *Spartina densiflora* in soils from different zones at beaches away from source populations

	Bare mudflat	Sand beach	High tide line
Total Spikelets m^{-2}	102 ± 45^a	34 ± 24^a	550 ± 116^b
Caryopses m^{-2}	34 ± 24^a	0 ± 0^a	71 ± 38^a
Viable caryopses m^{-2}	34 ± 24^a	0 ± 0^a	51 ± 30^a
Germinated caryopses m^{-2}	0 ± 0^a	0 ± 0^a	41 ± 28^a

Different letters indicate significant differences among zones (Mann-Whitney U-test or Tukey's HSD test, $p < 0.05$). Data are mean $\pm$ SE ($n = 30$ for bare mudflats and sand beaches; $n = 50$ for mean high tide line)

Discussion

Our results about seed dispersal potential and soil seed bank dynamics are useful to explain the distribution of *S. maritima* and *S. densiflora* along the intertidal gradient in salt marshes. An important result of this work is that no seed bank was found for *S. maritima* in either its low marsh populations nor in upper areas in the intertidal gradient. However, in accordance with our hypothesis, *S. densiflora* established a viable transient seed bank in all elevation zones of the marsh. This finding reveals a seed bank life stage of the invader that is present through the salt marsh, and can contribute to its invasiveness. Results of our work show that daily submersion of inflorescences decreases the buoyancy of spikelets as predicted in our hypothesis.

In buoyancy trials, most of the native *Spartina maritima* spikelets sank within the first 24 h in water with different salinities, whereas only c. 30% of *S. densiflora* spikelets sank after 24 h and practically all *S. densiflora* spikelets were submerged after 94 h in water with different salinities. This poor buoyancy ability of *S. maritima* likely contributes to why we did not detect any *S. maritima* seeds in soil seed banks at locations away from their population sites, including sampled *S. densiflora* locations and isolated beaches. These results do provide support for the better dispersal ability of *S. densiflora* which was present in soils seed banks at isolated beaches some distance away from its populations. Moreover, the density of *S. densiflora* spikelets in the seed bank before the germination period (initial seed bank) in HM exceeded the sum of the density of remnant spikelets and the in situ annual production, meaning that the HM was a sink habitat for propagules, whereas LM and MM were propagule production areas from which propagules were exported. Our results coincide with studies on *Spartina alterniflora* Loisel. in the Yangtze Estuary (China) that have reported a higher density of spikelets in MM and HM than in LM and bare mudflats (Wang et al. 2009; Xiao et al. 2009). Upper salt marsh zones are less exposed to tides at the same time that halophytes canopies reduce the speed of tidal currents, favouring the deposition of seeds that have been dragged by the tide (Lambrinos and Bando 2008). In contrast, tidal currents favour the exportation of propagules in low marshes (Wolters and Bakker 2002).

The recorded flotation period for *S. densiflora* spikelets was similar to that found by McDonald (2014). Other cordgrasses showed longer buoyancy periods than *S. densiflora*. For example, *S. alterniflora* and *Spartina patens* (Aiton) Muhl. from the Atlantic and the Gulf Coasts of North America presented flotation times ca. 25 days (Elsey-Quirk et al. 2009) and 50% of spikelets remained afloat after 9 days and 8% after 40 days for South American *Spartina ciliata* Brongn. (Cordazzo and Davy 1994). Moreover, *S. densiflora* spikelets from LM started to sink before than those from MM and HM. This behaviour was also observed by Xiao et al. (2016) in *S. alterniflora*. Waterlogging of *Spartina* spp. propagules can cause loss of buoyancy (Morgan and Sytsma 2013). In this sense, Elsey-Quirk et al. (2009) found that *S. alterniflora* spikelets pre-treated during 30 days with wet stratification sank before than those not pre-treated. The ability of *S. densiflora* spikelets, especially from MM and HM, to remain afloat may causes that this species could be dispersed over long distances favouring the colonization of new areas (McDonald 2014). This could explain the extension of *S. densiflora* invasion from San Francisco Bay to Vancouver Island along the Pacific Coast of North America (Castillo et al. 2014) as well as its invasion from South Portugal to the Strait of Gibraltar along the Gulf of Cadiz (Nieva et al. 2001). In contrast, European native *S. maritima* spikelets capacity to

colonize new habitats located at medium and long distances would be more restricted. This limitation in spikelet dispersion may favour population isolation that could explain why *S. maritima* shows a low genetic diversity, especially in North European Marshes (Raybould et al. 1991; Yannic et al. 2004). In agreement with our results, Polo-Ávila et al. (2019) found only one *S. maritima* spikelet in all 420 soil samples analyzed along the whole intertidal gradient. Thus, due to their low buoyancy, most of *S. maritima* spikelets may be deposited on the bottom of salt marsh channels and mudflats, where they could maintain viability for longer due to humidity conditions according to our storage experiments, and where if the elevation is adequate, they could potentially germinate and establish as primary colonizers (Castellanos et al. 1994; Castillo et al. 2000).

In this context and in view of our storage results, wet *Spartina* seeds transported by currents and tides would be able to keep their viability during months in contrast with dry seeds that rapidly lost their viability, especially at moderate temperature and in the case of *S. maritima*, like many aquatic plants with recalcitrant seeds (Probert and Longley 1989; Biber and Caldwell 2008). This behaviour was also observed for *S. alterniflora* seeds that cannot withstand drying at moderate temperature, losing their viability within 40 days (Mooring et al. 1971). Cold mitigated the deleterious effects of dry storage since refrigeration prevents desiccation, being *S. maritima* able to keep some viable seeds until 3 months and *S. densiflora* until 12 months. These results are in accordance with Kittelson and Boyd (1997), who reported that *S. densiflora* caryopses did not lose viability in dry storage at 0 ℃, and with Mooring et al. (1971) who stated that *S. alterniflora* viability was preserved in cold storage, but after 8 months viability is lost.

There are numerous endogenous and exogenous types of seed dormancy. *Spartina* spp. have physiological dormancy that inhibits germination (Baskin and Baskin 2001). This dormancy can prevent germination of seeds in unfavourable conditions for seedling growth (Ungar 1978). Dormancy is an important process for establishment of persistent soil seed banks in salt marshes (Ungar 2001), and seeds of many halophytes that disperse during the fall have dormancy mechanisms (Baskin and Baskin 2001). *Spartina maritima* seeds without spikelet bracts showed dormancy levels that ranged from 9 to 19% (Infante-Izquierdo et al. 2019a), whereas, in the present study, *S. maritima* did not show dormancy sowing the entire spikelet as occurred in our study about germination at different salinities (Infante-Izquierdo et al. 2019c). This means that its seeds were ready to germinate just after being dispersed from the mother plant. In the case of *S. maritima*, its lack of dormancy and its inability to maintain viable seeds covered with bracts for long periods at moderate temperatures supports the characterization of this species as a short distance dispersal, as discussed above, and its

inability to establish even transient seed banks as recorded in this study. The lack of seedling emergence of *S. patens* and *Spartina foliosa* Trin. from seed bank assays also suggests these congeners do not maintain persistent soil seed banks (Hopkins and Parker 1984; Baldwin et al. 1996). *Spartina foliosa* can be an abundant seed producer and quickly colonizes marsh restoration sites, but a low frequency of detection in seed rain and seed banks at San Francisco Estuary is thought to be due to low seed viability, particularly at elevated salinity (Diggory and Parker 2011).

In contrast, *S. densiflora* exhibited dormancy after being dispersed from the mother plant of 47%, which would allow to disperse to long distances and establish seed banks as recorded in our study. This dormancy was broken after 1 month in dry and moderate temperature conditions, accompanied by a decrease in seed viability, and was also broken by storage over 6 months in wet and cold conditions. However, *S. densiflora* dormancy at dispersal moment was retained when spikelets were stored in dry and cold conditions. These results are similar than those recorded previously for *S. patens* (Plyler and Proseus 1996) and *S. alterniflora* (Wijte and Gallagher 1996; Biber and Caldwell 2008; Xiao et al. 2009). Thus, wet and cold conditions were the best for spikelets storage in both cordgrasses, as occurs in *S. alterniflora* (Mooring et al. 1971; Wijte and Gallagher 1996), maintaining seed viability longer, mainly in 0.6 M NaCl solutions, in which high numbers of seeds remained viable during 12 months. Moreover, germination was progressively accelerated when storage time increased in these conditions for both species. In this sense, Xiao et al. (2009) observed that a wet and chilling treatment shorten the time of onset seed germination for *S. alterniflora*. Due to its dormancy, *S. densiflora* was able to establish transient seed banks mainly at higher elevations along the intertidal gradient as recorded in this study. Abbas et al. (2021) reported that *S. densiflora* formed short-term persistent seed banks at Humboldt Bay (North California) probably related to a cooler climate than in the Odiel Marshes. Nevertheless, *S. densiflora* was unable to establish permanent seed bank in the Odiel Marshes since only 1 spikelet with viable caryopsis remained in a HM soil sample after the germination period. Transient seed bank has been reported for other *Spartina* species such as *S. alterniflora* (Wang et al. 2009; Xiao et al. 2009, 2016), *Spartina argentinensis* Parodi (Feldman et al. 2007) and allopolyploid *Spartina anglica* C.E. Hubb. (Ungar and Woodell 1993). In *S. alterniflora*, Xiao et al. (2009) found that its transient seed bank lasted less than 9 months. We recorded high germination percentages (14–56% of viable caryopses) for dispersed *S. densiflora* spikelets before the beginning of the germination period in the field (initial seed bank). Thus, all these seeds were being subtracted from the transient seed bank during and just after winter rainfalls, that reduce salinity promoting germination as occurs in most halophytes (Keiffer and Ungar 1997; Muñoz-Rodríguez et al. 2017;

Infante-Izquierdo et al. 2019c). This first germination window is followed by other germination periods later in the year (J.M. Castillo, personal observation), as *S. densiflora* seeds would lose their dormancy after 6 months in cold and wet conditions. On the other hand, the decay of non-germinated caryopses in the soil bank may be determined by the desiccation that occurs in summer, as reported by Chang et al. (2001) and as we observed. Other factors that could explain the depletion of the soil seed bank is seed transport by tides (Wang et al. 2009; Xiao et al. 2009) and seed mortality caused by microbial and fungi activity (Wagner and Mitschunas 2008; Xiao et al. 2009), or by seed predation (Espinar et al. 2004; Xiao et al. 2009).

Our results on the dynamics of seed dispersal and seed banks provide fundamental information for the conservation of native *S. maritima* and the management of invasive *S. densiflora*. The limited spikelet dispersal and the absence of soil seed banks in native *S. maritima* habitat reveals the need to maintain high levels of genetic variability in populations, which must be consider in marsh restoration works using this species. The limited dispersal ability and the absence of *S. maritima* in soil seed banks, together with the results from other studies that indicate invasive *S. densiflora* is better adapted to salinity changes expected with climate change than native *S. maritima* (Infante-Izquierdo et al. 2019c), suggest that native *S. maritima* is highly vulnerable to future changes in the littoral environment such as sea level rise. Existing natural *S. maritima* populations should be carefully preserved and used as source sites for seeds to actively create new populations. Thus, the limited dispersal and soil seed bank ecology of *S. maritima* suggests revegetation at restored salt marshes will likely require augmentative introductions since passive restoration efforts would not be enough. Ex situ collections and storage of plant species propagules to bank and preserve plant genetic resources for marsh restoration can provide an important safety net against extinction of native species in the face of climate change, invasive species, and habitat loss (Maunder et al. 2004; Millennium Ecosystem Assessment 2005). The results of storage experiments provide important information about the best way to preserve the seeds of native *S. maritima*. For invasive *S. densiflora*, attempts to manage this invasive species should focus on eliminating adult plants before annual seed release to reduce augmentation of seed banks. Also, regional weed management strategies are needed to reduce the entry of propagules from other nearby populations that will drive secondary invasions. In general, our results add useful knowledge to preserve European salt marshes using *S. maritima* as biotool and to fight exotic *S. densiflora* invasion in North American, European and African marshes.

Author Contribution Research and methodology were conceived by MDII, AFMR, JMC, supervised by AFMR. Manuscript was written and edited by MDII, AFMR, JMC and BJG. Data collection and analysis was performed by MDII, RRM, JJS and FJJN. All authors read and approved the final manuscript.

Data Availability The datasets used and analysed during the current study are available from the authors upon reasonable request.

Code Availability Not applicable.

Declarations

Ethics Approval No ethics approvals were required for this research.

Consent to Participate Not applicable.

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Competing Interests The authors declare no conflicts of interest.

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