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Persistence of *Zostera marina* L. (eelgrass) seeds in the sediment seed bank

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ABSTRACT

Two separate field experiments in the Newport River/Back Sound, North Carolina (NC) and the lower Chesapeake Bay (CB), Virginia were conducted in 2007 and 2008 to quantify the effects of time (6, 12, 15 months), seed source (mixed-annual, perennial NC; perennial CB), site (local environmental factors), and sediment type (fine, coarse) on the persistence of *Zostera marina* seeds in the sediment seed bank. It is here, at the southern limit of the species distribution along the western Atlantic, that the probability of population loss may be high and the importance of a seed bank in the resilience and recovery of these populations great. Experimental results indicate that viability of both NC and CB seeds decreased significantly after just 6 months in the sediment following the seasonal period of maximum germination and continued to decline over time with no seeds viable remaining in CB cores and <5% of seeds remaining viable after 15 months in NC treatments. In these experiments time was the overriding factor affecting the persistence *Z. marina* seed banks for all treatments in both NC and CB and viability was not significantly affected by seed source, site, or sediment type. Based on the results of the in situ experiments, mixed-annual and perennial *Zostera marina* populations in North Carolina and perennial populations in Virginia produce transient seed banks (seeds viable <12 months). The lack of a persistent seed bank may reduce the resilience of *Z. marina* at the limits of the species distribution to repeated stress events. As a result these populations may be particularly susceptible to disturbance with only a limited capacity for recovery if sexual reproduction is impaired.

1. Introduction

Zostera marina (eelgrass) is a dominant and ecologically important seagrass species distributed circumglobally in temperate coastal environments in the Northern Hemisphere (den Hartog, 1970; Green and Short, 2003; Short and Moore, 2006). Eelgrass meadows provide essential habitat for both economically and ecologically important faunal species (Heck et al., 2008; Thayer et al., 1984), improve local water quality conditions (Dennison et al., 1993), and can be an important carbon sink in coastal environments (Fourqurean et al., 2012). Many seagrass populations, including those containing *Z. marina*, are rapidly declining in response to anthropogenic disturbances (Costello and Kenworthy, 2011; Orth et al., 2006; Short and Wyllie-Echeverria, 1996; Waycott et al., 2009). During the past several decades it has become increasingly important to understand what factors are influencing seagrass population fluctuations and their resilience to stressors, as well as their recovery from chronic and short term disturbances.

Across their broad geographic distribution, *Z. marina* populations express a range of different life history strategies from perennial to annual growth forms (den Hartog, 1970; Jarvis et al., 2012; Setchell, 1929; van Lent and Verschuure, 1994). While asexual reproduction is important for sustaining persistent beds on local scales (den Hartog, 1970; Setchell, 1929; Thayer et al., 1984; Tomlinson, 1974), sexual reproduction via seed production is critical for maintaining genetic diversity (Reynolds et al., 2013), dispersing populations over short (m) and long (km) distances (Harwell and Orth, 2002; Kendrick et al., 2012; Orth et al., 1994), and as a source of propagules for the sediment seed bank that can replenish populations following acute disturbances or long term declines (Jarvis and Moore, 2010; Lee et al., 2007; Plus et al., 2003; Riddin and Adamns, 2009). Of these three features of sexual reproduction, the least understood for eelgrass is the sediment seed bank (Orth et al., 2000).

Sediment seed banks are formed when seeds are deposited on or in the sediment (Fenner and Thompson, 2005). Negatively buoyant *Z. marina* seeds that are not removed from the parent bed via dispersal (Harwell and Orth, 2002; Kendrick et al., 2012) are deposited on the sediment surface and generally do not move more than a few meters

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(Orth et al., 1994). Seeds are incorporated into the sediment through burial by physical processes and bioturbation (Blackburn and Orth, 2013; Delefosse and Kristensen, 2012; Inglis, 2000; Valdemarsen et al., 2011), removed by mortality and predation (Fishman and Orth, 1996; Sumoski and Orth, 2012) or physically relocated in sediment bed load transported by strong currents and waves (Bell et al., 2008; Inglis, 2000). Seed banks in perennial *Z. marina* meadows have been reported to contain between 0 and 1,200 seeds m^{-2} while in mixed/annual meadows seed-bank densities range from 1,300 to 30,000 seeds m^{-2} (Harrison, 1993; Harwell and Orth, 2002; Lee et al., 2007; Morita et al., 2007).

Regardless of size, for a seed bank to function some proportion of the seeds must be maintained in a suitable physiological state conducive for germination to occur at an ecologically advantageous time (Baskin and Baskin, 1998; Murdoch and Ellis, 2000; Thompson, 2000). Therefore, seed banks are classified based on how long seeds persist, or remain capable of germinating under favorable conditions, while in the sediment (Fenner and Thompson, 2005). Thompson et al. (1997) divided sediment seed banks into three main categories 1) transient containing seeds that persist in soil for less than 1 year, 2) short-term persistent with seeds that persist in the sediment for at least 1 but no more than 5 years, and 3) long-term persistent with seeds that persist in the sediment for > 5 years. Seed persistence can be determined by measuring seed viability. A viable seed is defined as an embryo which maintains the physiological capability to germinate given the appropriate cues (Murdoch and Ellis, 2000). Persistence, measured as viability, can be affected by many biotic (i.e. seed source, predation) and abiotic (i.e. burial depth, sediment type, temperature, salinity) factors in the surrounding environment (Baskin and Baskin, 1998; Moore et al., 1993; Murdoch and Ellis, 2000).

Research indicates that the development of a persistent or transient seed bank in terrestrial habitats is species and not habitat specific (Fenner and Thompson, 2005; Thompson and Grime, 1979; Thompson et al., 1997); however, there is some evidence that persistence can be modified by the environment (Ibrahim and Roberts, 1983; Pakeman et al., 2012). In particular, the local microenvironment associated with the soil has been shown to significantly impact seed longevity in terrestrial systems (Pakeman et al., 2012). The development of anoxic conditions (Ibrahim and Roberts, 1983) and the increased association of fungal pathogens associated with the soil (Blaney and Kotanen, 2001) are two characteristics that have been documented to reduce terrestrial seed viability over time. For *Z. marina,* seed germination has been shown to increase significantly in anoxic compared to oxygenated sediments (Moore et al., 1993; Probert and Brenchley, 1999). Additionally, sediment conditions such as percent organic content (Boer, 2007; Koch, 2001) and grain size distribution (Wicks et al., 2009) have been shown to significantly impact plant growth and survival. Direct measurements of the effects of variation in the surrounding sediment environment on seed-bank persistence, and therefore function, are essential to identify the type of seed bank produced within *Z. marina* beds.

One function of sediment seed banks is to offset the consequences of environmental heterogeneity and avoid the catastrophic loss of a plant population (Venable and Brown, 1988). Therefore, it is predicted that the greater the probability that a terrestrial plant population will experience catastrophic loss in one year, the more energy it devotes to seed production (Choen, 1966) as well as the longer the persistence of seeds in the soil seed bank (Fenner and Thompson, 2005; Thompson et al., 1997). In marine seagrasses this can be seen in the greater number of seeds produced in annual and mixed-annual populations of *Z. marina* compared to perennial populations (Jarvis et al., 2012; Morita et al., 2007). While the safeguard against population loss created by the sediment seed bank in terrestrial habitats is also reflected in the greater persistence of annual seed banks compared to perennial populations (Thompson et al., 1998), it is not known if this characteristic also applies to seagrasses.

Most sediment seed bank studies provide only snapshots of seed bank composition, density, and diversity (Fenner and Thompson, 2005). Snapshot *in situ* measurements of *Z. marina* seed bank characteristics have shown significant site specific variations in density (Cabaço and Santos, 2010; Harwell and Orth, 2002) and viability, with greatest numbers of viable seeds in the seed bank documented immediately following seed release (Jarvis et al., 2012; Lee et al., 2007; Morita et al., 2007). While these studies are important to describe the sediment seed bank, this approach does not provide information on how seed bank characteristics may change over time (Fenner and Thompson, 2005). To understand and fully characterize the persistence and function of the sediment seed bank, a cohort of seeds must be followed temporally (Hyatt and Evans, 1998). As it is difficult to determine when seeds were deposited within the seagrass bed from sampling the sediment seed bank alone, experimental, not observational field studies are required to provide evidence for the production of transient or persistent seed banks within *Z. marina* meadows.

Zostera marina populations located at the southern limit of the species distribution along the western Atlantic in the lower Chesapeake Bay, Virginia and in the Newport River and Back Sound, North Carolina were selected to investigate the effects of seed source, sediment type, and local environmental conditions on seed bank persistence over time with an *in situ* viability experiment. *Zostera marina* growth at these sites is thermally stressed in the late summer and the populations typically experience high mortality rates when water temperatures exceed 30 °C (Jarvis et al., 2012; Moore and Jarvis, 2008; Setchell, 1929; Thayer et al., 1984). Life history strategies documented for this area include perennial and mixed-annual populations (Jarvis et al., 2012). Mixed-annual populations experience a complete loss of biomass and are reestablished by seedlings on an annual basis. A portion of the newly established seedlings reproduce asexually through clonal growth, similar to populations with a perennial life history; however, some seedlings produce only reproductive shoots during their first growing season, a characteristic of annual plants. Experimental sites were selected in North Carolina and Virginia to represent all life history strategies and the full range of sediment conditions observed in this region (Jarvis et al., 2012; Orth et al., 2006; Thayer et al., 1984).

This study paired two separate manipulative field experiments that used reciprocal seed transplants to quantify the *in situ* effects of seed source (perennial, mixed-annual), sediment type (coarse, fine) and site (local environmental conditions) on *Z. marina* seed bank persistence over time (6, 12, 15 months). At the thermally stressed southern limit of the species range the probability of population loss may be high and the importance of a seed bank in the resilience and recovery of these populations great. We hypothesized that (1) seeds in mixed-annual populations would persist for longer periods of time than seeds from perennial populations due to their reliance on regeneration from seeds on an annual basis, (2) that seed viability would be significantly greater in coarse sediment sites compared to fine sediments due to the greater germination rates in fine sediments, and (3) that, based on *in situ* observations, seed viability would significantly decrease over time with a complete loss of viability after 12 months in all treatments.

2. Methods

2.1. Site selection

The effects of sediment type (coarse, fine), seed source, (mixed-annual and perennial), site (local environmental conditions) and time (0, 6, 12, 15 months) on the viability of *Z. marina* seed-banks were quantified in two separate *in situ* experiments in the Newport River/Back Sound, North Carolina and in the lower Chesapeake Bay, Virginia in 2007 and 2008 (Fig. 1). In North Carolina, Phillips Island (NC1) and Morgans Island (NC2) were selected based on the presence of a mixed annual population in fine sediment (>20% silt) at NC1 and a perennial population in coarse sediment (<10% silt) at NC2 (Fig. 1; Jarvis et al., 2012). In Virginia two historically persistent perennial beds in fine sediment at Allens Island (CB1, 37° 15′ N; 76° 25′ W) and

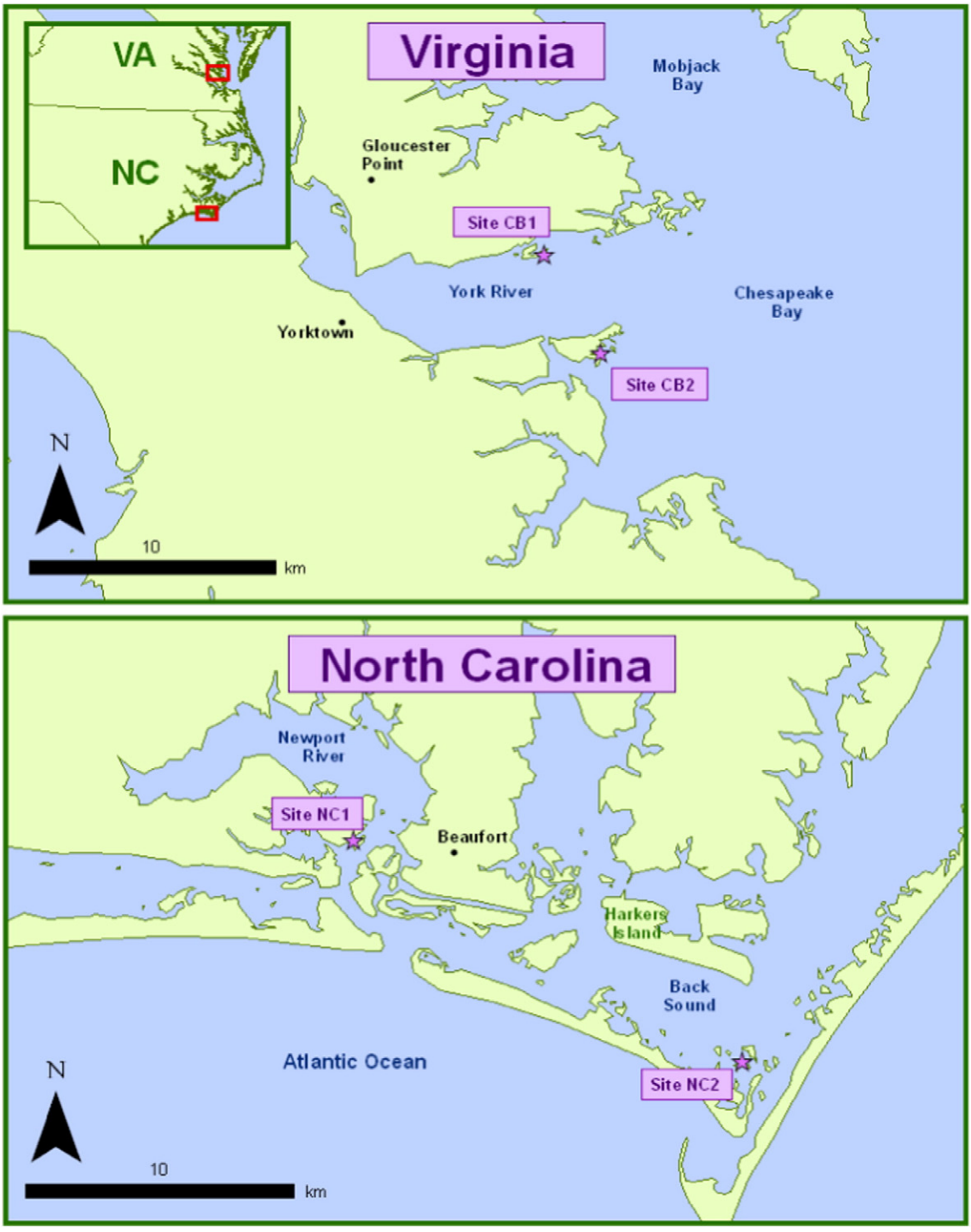


Fig. 1. Site locations for both the Virginia (CB1 and CB2) and North Carolina (NC1 and NC2) experiments. Site locations are denoted with a star.

in coarse sediment at Goodwin Island (CB2, 37° 13′ N; 76° 23′ W) in the lower York River, a tributary to the Chesapeake Bay, were selected (Fig. 1; Moore, 2009).

2.2. Seed collection and seed viability

Reproductive shoots were collected from all four *Z. marina* sites during the period of maximum seed release; early to late May in North Carolina (Jarvis et al., 2012; Thayer et al., 1984) and late May and early June in Chesapeake Bay (Orth and Moore, 1986; Silberhorn et al., 1983). Reproductive shoots from each site were kept in separate aerated flow-through seawater tanks (2.4 m × 1.2 m × 1.0 m) until seeds dehisced from the reproductive shoots approximately one month later (Marion and Orth, 2010). The seeds were then collected following methods in Marion and Orth (2010) and kept in separate containers in aerated recirculating tanks (2.4 m × 1.2 m × 1.0 m) in filtered seawater at 20 °C for one month until placement in experimental cores.

Prior to both experiments, a sub-sample of 100 seeds from each seed source was tested for viability using tetrazolium chloride (Conacher et al., 1994; Lakon, 1949; Sawma and Mohler, 2002). Tetrazolium chloride was used due to increased accuracy and greater time efficiency compared to traditional germination tests (AOSA, 1981; Conacher

et al., 1994; Lakon, 1949). Seed embryos were removed from their seed coats and soaked in a 1 % tetrazolium chloride solution for 24 hours before examination on a dissecting scope at 10x magnification (Conacher et al., 1994). Seeds with a pink to brown stained cotyledon and axial hypocotyl were considered viable (Conacher et al., 1994; Harrison, 1993; Taylor, 1957).

2.3. Seed viability experiments

Sediment for all experimental treatment cores was collected from each site and stored at 7 to 9 °C for no longer than two weeks. Prior to initiating the experiment, all sediment was dry sieved (710 μm mesh) to remove any *Z. marina* seeds and then homogenized manually.

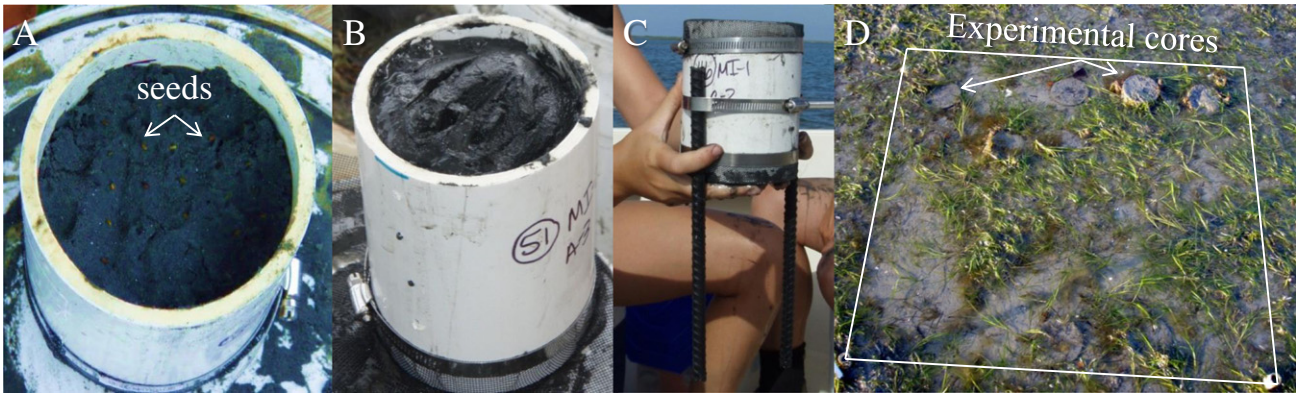
2.3.1. Seed viability and sediment cores – North Carolina

Experimental treatments at each site (NC1, NC2) consisted of four experimental cores (coarse sediment: NC1/mixed annual seeds; coarse sediment NC2/perennial seeds; fine sediment NC1/mixed annual seeds; fine sediment: NC2/perennial seeds) and two sediment only cores (fine sediment only; coarse sediment only) for each sampling time (6 months December 2007, 12 months June 2008, and 15 months September 2008). Experimental cores (PVC; 10.2 cm diameter × 15.2 cm) were partially filled with sieved sediment, stocked with 50 seeds from the treatment seed source placed at depths between 3 and 5 cm in the sediment, and then filled to the top of the core with the remainder of the sieved sediment (Figs. 2 A and B). Seed depth was selected based on observed vertical distributions of viable *Z. marina* seeds in established seed-banks (Harrison, 1991; Harwell and Orth, 2002). Once the seeds were planted, the cores were capped with sediment and covered with plastic mesh screening (0.5 cm) on both ends (Fig. 2 C) to reduce potential effects of seed predation (Fishman and Orth, 1996; Sumoski and Orth, 2012) and bioturbation (Blackburn and Orth, 2013; Delefosse and Kristensen, 2012). Sediment only cores were assembled identically to the experimental cores, minus the seeds to quantify changes in sediment conditions within the PVC cores throughout the duration of the experiment. Separate cores were used to quantify sediment effects due to the destructive nature of both seed and sediment sample processing. All cores (experimental and sediment only) were replicated three times for each sampling period.

All cores were placed in a randomized block design within a three 1 m^2 replicate plots established in June 2007 within vegetated areas at both NC1 and NC2. Within each plot, a sediment plug was extracted and replaced with one replicate seed viability/sediment only core for each treatment and buried flush with the sediment surface in randomly selected 20 cm^2 quadrats within the plots (Fig. 2 D). During sampling, one replicate treatment core and one control core for each sediment type were removed at each of three sampling times after installing the cores in the sediment (N = 12 treatment cores and 6 sediment only cores $site^{-1}$ $sampling\ period^{-1}$, Fig. 2 E).

2.3.2. Seed viability and sediment cores; Virginia

Virginia treatments and sediment cores were identical to the North Carolina experiment except that sediment and seeds were collected from CB1 and CB2 in the lower York River and seed source treatments did not differ in reproductive strategy as both sources were derived from known perennial populations (Orth and Moore, 1986) in coarse (CB1; Buzzelli, 1998) and fine (CB2; Hobbs, 1994) sediments.



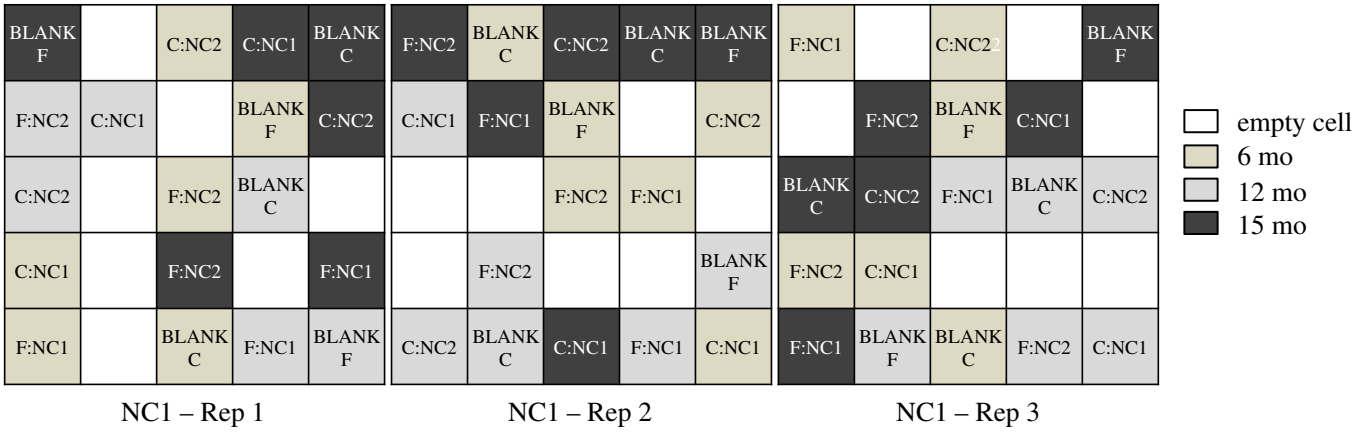


Fig. 2. Experimental core construction and experimental design for seed viability experiment. (A) Seeds are placed in a core at 3–5 cm depth, (B) covered with sediment, (C) and capped with a mesh screen on both ends and rebar anchors to reduce core loss, (D) then placed flush with the sediment surface. (E) Example experimental design for site NC1. All cores were placed in 1 m^2 plots (3 replicate plots per site) and labeled as sediment type:seed source. F = fine sediments, C = coarse sediments, blank = sediment only cores. Experimental designs were similar at NC2 and both Virginia sites.

Experimental treatments at each site (CB1, CB2) consisted of four experimental cores (coarse sediment: CB1 seeds; coarse sediment CB2 seeds; fine sediment: CB1 seeds; fine sediment: CB2 seeds) and two sediment only cores (fine sediment only; coarse sediment only) for each sampling time (6 months January 2008, 12 months August 2008, and 15 months October 2008; Fig. 2). Three 1 m^2 plots were established at each Virginia site in June 2008 following the methods described for the North Carolina experiment and one replicate set of treatment and sediment only cores were collected during each time step (N = 12 treatment cores and 6 sediment cores $site^{-1}$ sampling $period^{-1}$; Fig. 2). The collection of seeds and initiation of the Virginia experiment was delayed one month compared to the North Carolina experiment due to the effect of latitude on the development of flowering shoots and production of seeds (Phillips et al., 1983b; Silberhorn et al., 1983). Timing of both experiments was selected to mirror the timing of maximum seed production and influx of seeds to the *in situ* seed bank (Jarvis and Moore, 2010; Jarvis et al., 2012; Silberhorn et al., 1983; Thayer et al., 1984).

2.3.3. Experimental core collection

For both North Carolina and Virginia, at each sampling time step the corresponding experimental seed viability cores were located and extracted from the sediments. The cores were placed in a cooler filled with site water and processed within 2 hours. Daytime vertical redox (Eh) profiles were quantified for the collected experimental and control cores with a 21 cm platinum electrode. The probe was inserted into the top of the core and redox was measured at 0.0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, and 10.0 cm. Final readings were corrected for temperature relative to the reference electrode (Hinchey and Schaffner, 2005).

Following redox measurements, all experimental cores were wet sieved (710 μm mesh) to remove all seeds. Seeds (intact seed coat with embryo), germinated seeds (cotyledon extended through the seed coat, still attached to the seed), and seed coats (seed coat split open with missing embryo) were sorted and counted. Intact seeds were tested for viability using tetrazolium staining and divided into viable and non-viable categories. Seeds that were not accounted for at the completion of the experiment were considered to be lost from autonomous death and decay (Harrison, 1993) and, along with germinated seeds, were placed in the non-viable category for all statistical analysis. Total seed density, viability, and changes in viability were quantified over time and presented as means ± SE.

2.3.4. Experimental sediment characterization

For each time step the three of the experimental sediment cores were divided into sections at 3 cm and 6 cm depths; all sections were then halved following redox measurements. Percent organic matter was determined by drying a sediment sub-section at 60 ºC until a constant dry weight was reached. Samples were then weighed, combusted at 500 ºC for five hours, and weighed again. Percent organic matter was calculated as the difference in weights (Erftemeijer and Koch, 2001). Sediment exchangeable nutrients were extracted with a volume of KCl (2 M) equal to twice the sediment volume, shaken on a rotary shaker for 1 h at room temperature, centrifuged 6 minutes at 4000 RPM, filtered (Gelman Supor, 0.45 μm), and frozen in sterile Whirlpak® bags until analyzed. NH_4^+ was determined by the technique of Solorzano (1969). DIN (NO_x) and DIP (PO_4^{-3}) were determined on a Lachat Instruments auto analyzer (Liao, 2001; revised 2002, Knepel and Bogren, 2001; revised 2002, Smith and Bogren, 2001; revised 2002). Percent sand, silt, and clay fractions were determined using a wet sieve (Plumb, 1981). Although samples were collected during each sampling date, percent sand/silt/clay analysis was only completed on the 6 month samples (December 2007 NC, January 2008 CB).

2.4. Data analysis

Prior to the beginning of the experiments a set of analytical models were developed to describe the relationship between seed viability, seed source, time, sediment type, and site (Table 1). The models were used for both North Carolina and Virginia treatments, although all experimental and sediment seed-bank data were analyzed separately for each of the sites. For all analyses it was assumed that viability of one seed did not significantly affect the viability of surrounding seeds (Orth et al., 2000). Therefore, each seed was considered an independent Bernoulli trial and analyzed separately.

Experimental core seed viability was analyzed using logistic regression using a binomial distribution with time, seed source, sediment type, and site as factors (GLM; R Core Team, 2013). Logistic regression was selected due to the binary response variable, the large number of observations (600 per treatment), and a high number of zeros recorded throughout the course of the experiment (Zuur et al., 2009). Site seed-bank viability was analyzed using logistic regression with a Poisson distribution using time and site as factors.

To determine the best fitting model the second-order Akaike Information Criterion (AIC_c) was calculated using log likelihood ratios derived from all regression analyses (Burnham and Anderson, 2002). AIC_c differences (Δ_i) between all models were then calculated and the models were ranked. The model with the smallest Δ_i was selected as the best fitting model. For all significant model terms ($p < 0.05$) odds ratios were calculated using Wald chi square statistics (SAS; SAS Institute Inc.). Likelihood ratio tests for all parameter estimates were also calculated and compared to the Wald chi Square Statistics (Allison, 1999).

To quantify the effects of time, sediment type, site and sediment pore water nutrient concentrations (NH_4^+, PO_4^{-3}) all data was log transformed, normality was confirmed, homogeneity of variance verified with Cochran's test (Zar, 1999), then analyzed with Analysis of Variance (ANOVA) (aov; R Core Team, 2013). *Post hoc* comparisons between treatments were made with Tukey's HSD test (TukeyHSD, R Core Team, 2013). Redox data was also analyzed using ANOVA to test the effects of time, sediment type, site, and depth on redox values (aov; R Core Team, 2013). *Post hoc* analyses of redox data were performed with Tukey's test (TukeyHSD; R Core Team, 2013.). Sediment percent organic matter data and percent sand/silt/clay for experimental cores was analyzed with logistic regression using a binomial distribution with time (percent organic only), sediment, depth, sediment type, and site as factors (GLM; R Core Team, 2013).

3. Results

3.1. Seed viability experiments

3.1.1. North Carolina experimental seed viability

Viability of North Carolina seeds in the experimental cores decreased significantly from initial seed viabilities of 80% with increasing time in the sediment (likelihood χ^2 estimate $p < 0.001$; Table 2; Figs. 3 and 4). The decline occurred within the first 6 months as there were 2.97 times more viable seeds at the beginning of the experiment compared to 6 months later ($p < 0.001$; Table 3). Seed viability of the samples collected after 12 and 15 months remained low (Figs. 3 and 4).

There was no significant effect of site, sediment or seed source on overall seed viability ($p = 1.000$ for all; Table 3). There were significant interactions between time and both sediment type ($p < 0.001$) and seed source ($p = 0.002$; Table 3) after 6 months in the sediment. The significant sediment interaction was a result of more viable seeds in the fine than coarse sediment treatment (Table 3, Figs. 3 and 4) while the source interaction was attributed to 14 times more viable mixed-annual (NC1) than perennial (NC2) seeds remaining in the experimental cores after 6 months in the sediment (Figs. 3 and 4). This significant trend did not continue at the 12 and 15 month sampling dates. Site and time had a significant interaction with greater viability of seeds at NC1 (3.50 ± 3.15%) compared to NC2 (2.73 ± 1.69%) after 15 months. During the course of the experiment, the proportion of unaccounted for or lost seeds had an inverse relationship with seed viability (Fig. 3). As time elapsed the percentage of lost seeds increased from 0 % to 61 ±

Table 1
A priori equation selection for both North Carolina and Virginia seed core viability. k is the number of estimable parameters in the model.

Experimental Core Model	k
$V_1 = \alpha + \text{time}$	2
$V_2 = \alpha + \text{time} + \text{sediment}$	3
$V_3 = \alpha + \text{time} + \text{site}$	3
$V_4 = \alpha + \text{time} + \text{source}$	3
$V_5 = \alpha + \text{time} + \text{source} + \text{time*source}$	4
$V_6 = \alpha + \text{time} + \text{sediment} + \text{site}$	4
$V_7 = \alpha + \text{time} + \text{sediment} + \text{source}$	4
$V_8 = \alpha + \text{time} + \text{site} + \text{source}$	4
$V_9 = \alpha + \text{time} + \text{site} + \text{sediment} + \text{source}$	5
$V_{10} = \alpha + \text{time} + \text{site} + \text{source} + \text{time*site}$	5
$V_{11} = \alpha + \text{time} + \text{site} + \text{source} + \text{time*source}$	5
$V_{12} = \alpha + \text{time} + \text{site} + \text{source} + \text{time*site} + \text{time*source}$	6
$V_{13} = \alpha + \text{time} + \text{sediment} + \text{source} + \text{time*sediment}$	5
$V_{14} = \alpha + \text{time} + \text{sediment} + \text{source} + \text{time*source}$	5
$V_{15} = \alpha + \text{time} + \text{sediment} + \text{source} + \text{time*sediment} + \text{time*source}$	6
$V_{16} = \alpha + \text{time} + \text{site} + \text{sediment} + \text{source} + \text{time*site} + \text{time*sediment} + \text{time*source}$	8
$V_{17} = \alpha + \text{time} + \text{site} + \text{sediment} + \text{source} + \text{time*site} + \text{time*sediment} + \text{time*source} + \text{site*sediment} + \text{site*source} + \text{sediment*source} + \text{time*site*sediment} + \text{time*site*source} + \text{site*sediment*source} + \text{time*site*sediment*source}$	15

10 % for fine sediment treatments and from 0 % to 68 ± 5 % for coarse sediment treatments.

3.1.2. Virginia experimental seed viability

The model used in analysis of the Virginia experimental core data did not include sediment type; therefore, these variables were not included in any further analysis (Table 2). As with the North Carolina seeds, viability of Virginia seeds decreased with time from initial viabilities of 75% for CB2 (likelihood ratio estimate $p < 0.001$; Figs. 3 and 4). Viability decreased significantly within 6 months ($p = 0.050$; Table 3), decreasing from 75% at the start of the experiment to 4 % at 6 months (Fig. 3). The trend continued over time with a significant decline in seed viability after 12 and 15 months in the sediment.

There was no significant effect of seed source on seed viability ($p = 1.000$; Table 3), but there was a significant interaction between time and seed source ($p < 0.001$). Seeds collected from CB2 were 13.8 times more viable than seeds collected from the CB1 after 6 months in the sediment (Fig. 3). This difference did not hold throughout the experiment due to a decline in viability across all treatments after 12 months in the sediment (Figs. 3 and 4). The proportion of unaccounted seeds increased with time from 0 % of total seeds to 38 ± 15 % by 15 months for fine sediment treatments and from 0 % to 30 ± 10 % for coarse sediment treatments.

3.2. Sediment characteristics

3.2.1. North Carolina experimental sediment cores

Sediment percent organic matter differed significantly between sediment types ($p = <0.001$) but did not differ significantly with depth ($p = 0.614$), between sites ($p = 0.635$), or sampling dates ($p = 0.415$). There was a significant interaction between site NC1 and sampling date ($p = 0.007$) and between fine sediment and sampling date ($p = 0.046$) due to an increase in organic matter in NC1 cores between 6 and 12 months that was greater in the cores with fine sediment compared to coarse sediment. Differences in organic content between sites are reflective of ambient site sediment conditions (Jarvis et al., 2012) and may be related to sediment deposition within the cores throughout the course of the experiment (Table 4). Due to the lack of a significant difference between depth and sampling date sediment organic data are averaged across depths and dates and presented as mean ± SE (Table 4).

Sediment redox profiles were also significantly affected by site ($p < 0.001$) and sediment type ($p < 0.001$) as sediments were more reduced in fine compared to coarse sediments and at NC1 compared to NC2. In addition, redox values were significantly different over time ($p = 0.038$). For all treatments except for the sand treatment at NC1, redox values were less reduced at the 12 month sampling (December 2007) when water temperatures were at their lowest (Jarvis et al., 2012). Depth did not significantly affect redox values ($p = 0.212$). The overall sand/silt/clay ratios were similar between coarse and fine treatments at each site ($p = 0.482$).

There were no significant differences in sediment exchangeable pore water nutrients between sites (NH_4^+ $p = 0.241$; PO_4^{-3} $p = 0.511$) or sediment treatments (NH_4^+ $p = 0.550$; PO_4^{-3} $p = 0.147$). However, there was a significant effect of time for both NH_4^+ and PO_4^{-3} ($p < 0.001$ for both). Tukey's post hoc test shows that all dates are significantly different from each other for both NH_4^+ and PO_4^{-3} with the greatest values recorded in December 2007 for all treatments (Table 4). Sediment PO_4^{-3} values showed a significant date and site interaction ($p = 0.003$) due to the large increase in PO_4^{-3} in September compared to December 2007 and June 2008 that did not increase equally across all sites (Table 4).

Table 2
Top ranked models describing the viability of experimental seed cores for both North Carolina and Virginia experiments. Rankings were based on differences in QAICc values. k is the number of estimable parameters in the model. (†) denotes model used in data analysis.

Model	k	Log likelihood	QAIC	QAICc	Δi
North Carolina					
$V_{15} = \alpha + \text{time} + \text{site} + \text{sediment} + \text{source} + \text{time*site} + \text{time*sediment} + \text{time*source}^{\dagger}$	8	−941.3	1898.6	1898.7	0.0
$V_{14} = \alpha + \text{time} + \text{sediment} + \text{source} + \text{time*sediment} + \text{time*source}$	6	−948.7	1909.4	1909.4	10.7
$V_{12} = \alpha + \text{time} + \text{sediment} + \text{source} + \text{time*sediment}$	5	−953.1	1916.2	1916.2	17.5
$V_{11} = \alpha + \text{time} + \text{site} + \text{source} + \text{time*site} + \text{time*source}$	6	−953.7	1919.4	1919.4	20.7
Virginia					
$V_5 = \alpha + \text{time} + \text{source} + \text{time*source}^{\dagger}$	4	−965.5	1939.1	1939.1	0.0
$V_{14} = \alpha + \text{time} + \text{sediment} + \text{source} + \text{time*source}$	5	−965.4	1940.7	1940.8	1.7
$V_{11} = \alpha + \text{time} + \text{site} + \text{source} + \text{time*source}$	5	−965.5	1941.0	1941.0	1.9
$V_{10} = \alpha + \text{time} + \text{site} + \text{source} + \text{time*site}$	5	−977.9	1965.9	1965.9	6.8

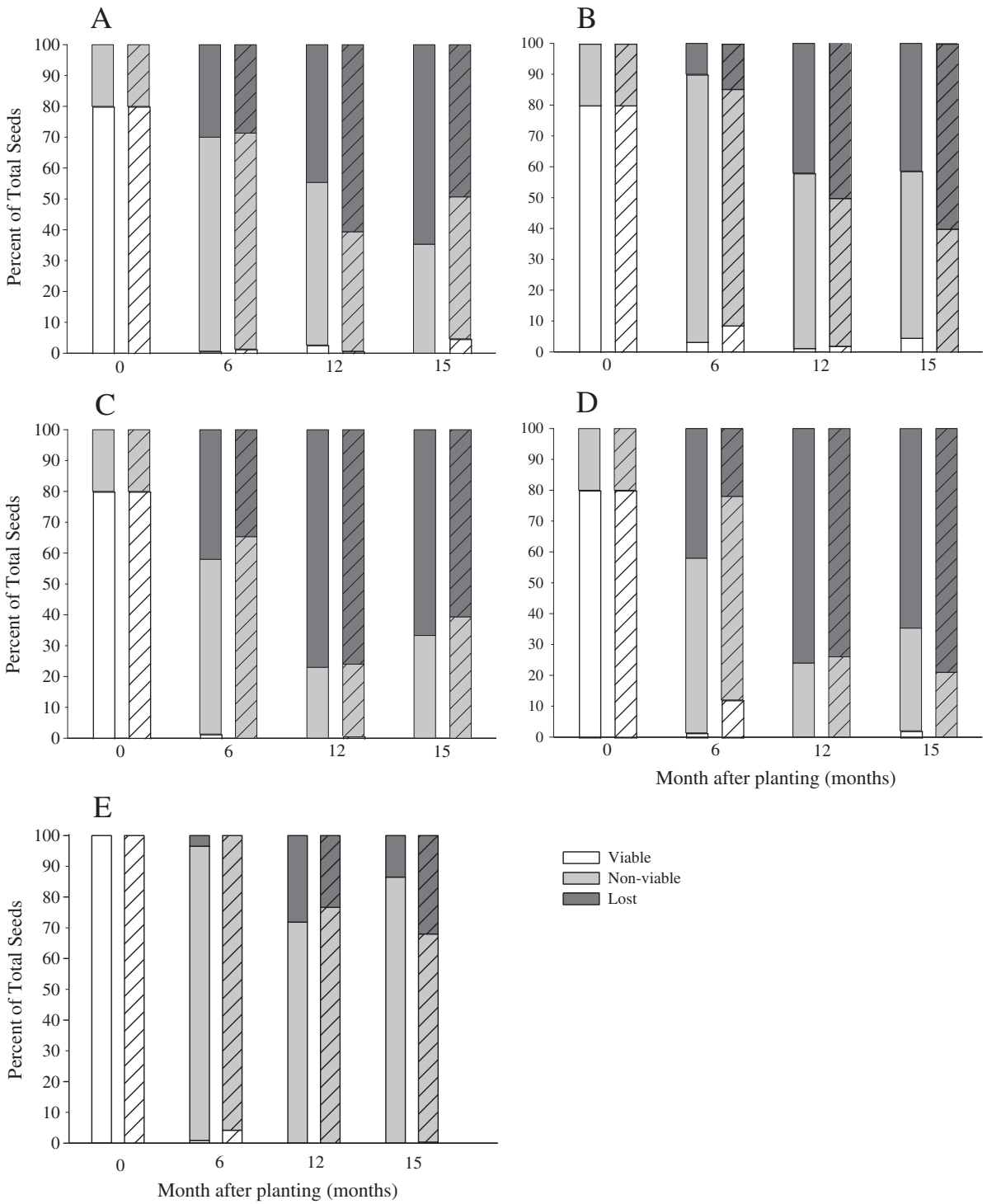


Fig. 3. Mean percent of total seeds removed from each experimental sediment core in North Carolina and Virginia sites that were viable, non-viable, or lost to germination or mortality. Experimental treatments are (A) seeds at NC1 in coarse sediment, (B) seeds at NC1 in fine sediment, (C) seeds at NC2 in coarse sediment, (D) seeds at NC2 in fine sediment, and (E) seeds in CB experiments combined across sediment types and sites.

3.2.2. Virginia experimental sediment cores

Sediment percent organic matter in the experimental cores differed significantly between sediment types ($p < 0.001$) but did not differ significantly between sites ($p = 0.079$), with depth ($p = 0.810$) or between sampling dates ($p = 0.654$, Table 4). Due to the lack of a significant difference between depth and sampling date, CB experimental data was sediment organic data are averaged across depths and dates and presented as mean ± SE (Table 4). Sediment redox profiles were also significantly affected by site ($p < 0.001$) and sediment type ($p < 0.001$) as sediments were more reduced in fine compared to coarse sediment treatments and in both sediment treatments at CB1 compared to CB2. In addition, redox values were significantly different over time ($p < 0.001$) and depth ($p < 0.001$) and there was a significant time and site interaction ($p < 0.001$) and a time and depth interaction ($p < 0.001$). Redox values were significantly different between all sampling dates with the most reduced values in August 2007. Redox

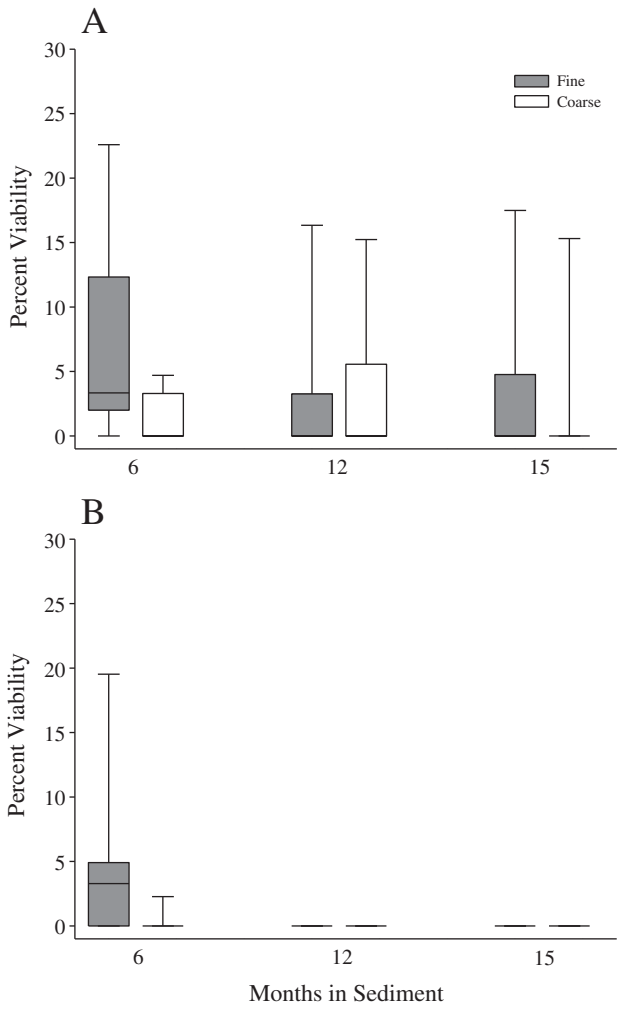


Fig. 4. Boxplots of combined viability of remaining seeds in both (A) North Carolina and (B) Virginia experimental seed cores. Dark gray bars represent seeds in silt/clay treatments and white bars represent seeds in sand treatments. Lines within the bars represent the median and whiskers represent minimum and maximum values of the data.

values shallower than 3 cm were significantly less reduced than depths > 4 cm.

The percentage of clay in the sediments was similar in sediment treatments between sites ($p = 0.461$); however percent sand was significantly greater at CB2 ($p = 0.032$) and percent silt was significantly greater at CB1 ($p = 0.032$). Differences in percent silt and sand between sites are reflective of ambient site sediment conditions and may be related to sediment deposition within the cores during the first 6 months of the experiment (Jarvis and Moore, 2010). Sediment exchangeable pore water PO_4^{-3} was significantly greater ($p = 0.035$) in CB1 sediments than CB2; however for both sites PO_4^{-3} values were less than 1 μM (Table 4). There was no significant difference between sediment pore water NH_4^+ ($p = 0.808$) between sites. There was also no significant difference in sediment exchangeable pore water nutrients between sediment treatments (NH_4^+ $p = 0.411$; PO_4^{-3} $p = 0.947$) or in sediment PO_4^{-3} concentrations between dates ($p = 0.164$). However, there was a significant difference in NH_4^+ over time ($p < 0.001$) with the greatest NH_4^+ concentrations occurring after 6 months in fine sediments and after 12 months in the coarse sediments, resulting in a significant time and sediment interaction ($p < 0.001$; Table 4). There was also a significant time and site interaction ($p < 0.001$) in sediment pore water NH_4^+ due to the large increase concentrations in month 12 at CB2 compared to CB1 (Table 4).

Table 3
Logistic regression model for (A) North Carolina and (B) Virginia experimental seed viability cores. All significant results are denoted with an (*).

Parameter	DF	Est	SE	X^2	p
North Carolina					
Intercept	1	1.39	0.14	9.61	<0.001*
Time 6 mo	1	−5.70	0.50	−11.30	<0.001*
Time 12 mo	1	−7.83	1.08	−7.25	<0.001*
Time 15 mo	1	−6.98	0.73	−9.95	<0.001*
Site	1	0.00	0.14	0.00	1.000
Sediment	1	0.00	0.14	0.00	1.000
Source	1	0.00	0.14	0.00	1.000
Site*Time 6 mo	1	−0.05	0.34	−0.14	0.885
Site*Time 12 mo	1	1.98	1.08	1.85	0.065
Site*Time 15 mo	1	1.47	0.66	2.25	0.025*
Sed*Time 6 mo	1	2.10	0.51	4.19	<0.001*
Sed*Time 12 mo	1	−0.62	0.73	−0.85	0.396
Sed*Time 15 mo	1	0.39	0.52	0.76	0.450
Source*Time 6 mo	1	−1.26	0.40	−3.18	0.002*
Source*Time 12 mo	1	0.62	0.73	0.85	0.396
Source*Time 15 mo	1	0.33	0.52	0.63	0.531
Virginia					
Intercept	1	−0.36	0.19	3.85	0.254
Time 6 mo	1	0.85	0.72	1.30	0.050*
Time 12 mo	1	−4.46	1.14	15.32	<0.001*
Time 15 mo	1	16.1	0.71	509.08	<0.001*
Source	1	0.00	0.12	0.00	1.000
Source*Time 6 mo	1	−2.55	0.63	16.47	<0.001*
Source*Time 12 mo	1	0.09	0.72	0.01	0.903
Source*Time 15 mo	1	−21.05	–	–	–

4. Discussion

The results of two separate *in situ* field experiments have shown that *Zostera marina* populations at the species southern limit in the western Atlantic can produce transient (seeds viable for <12 months) seed banks within the range of seed sources, sediment types and local environmental conditions that we studied here. While several observational field studies indicate that *Z. marina* populations produce transient seed banks in this region, (Harwell and Orth, 2002; Jarvis et al., 2012; Orth et al., 2000), this is the first study to follow a cohort of seeds over time and directly measure changes in viability under *in situ* conditions. Understanding how long seeds remain viable in the sediment is imperative to increase our understanding of the role of sexual reproduction in the maintenance and recovery of *Z. marina* populations (Jarvis and Moore, 2010). These findings suggest that quantifying sexual reproduction within *Z. marina* meadows requires moving beyond enumerating densities of flowering shoots and seeds to include additional temporal measurements of seed bank viability for more efficient and effective management of these important coastal habitats.

The lack of a long-term persistent seed bank could have a significant impact on the resilience of *Z. marina* populations to disturbance (Greve et al., 2005; Keddy and Patriquin, 1978; Plus et al., 2003), as germination from the sediment seed bank is essential for yearly reestablishment of annual and mixed annual populations (Jarvis et al., 2012; Keddy and Patriquin, 1978; Phillips et al., 1983a; Santamaría-Gallegos et al., 2000; van Lent and Verschuure, 1994) and provides a primary mechanism for population recovery following seasonal or large scale disturbance related declines (Greve et al., 2005; Jarvis and Moore, 2010; Lee et al., 2007; Plus et al., 2003). Due to an increase in frequency of large scale declines of *Z. marina* related to short term excessive heat events in this region (Moore et al., 2013), the lack of a persistent seed bank may limit the capacity for resilience and recovery within these populations if sexual reproduction is impaired.

4.1. Time

Time was the only factor that significantly affected seed viability in both *in situ* field experiments. However, the effect of time was not

equal on the persistence of sediment seed banks due to the presence of viable seeds after 12 months in the NC experimental cores, but not in the CB experiment. Despite differences in seed persistence, loss of viability after 6 months in the sediment was similar across all sites as mean seed viability in all experimental treatments decreased by 62 % between time 0 (July/August 2007) and 6 months in the sediment (December 2007/January 2008; Fig. 4). Similar reductions in seed viability (80% over 7 months) were reported in annual *Z. marina* sediment seed banks in the Zandreek embayment in the Netherlands (Harrison, 1993). The initial loss in the number of viable seeds in the NC and CB experimental cores coincided with the period of maximum seed germination for *Z. marina* beds in these regions (Jarvis et al., 2012; Moore et al., 1993; Silberhorn et al., 1983; Thayer et al., 1984). Once seeds have germinated, they are removed from the seed-bank; therefore, the number of viable seeds in the seed-bank decreases (Leck et al., 1989). Due to the presence of germinated seeds in all cores collected after 6 months in the sediment, the large decrease in viability observed in both the NC and CB experiments between time 0 and 6 months may be attributed primarily to germination. Those seeds that did not germinate lost their viability by 12 months in the CB experiment and were reduced to < 5 % viability after 15 months in the NC treatments. While germination in *Z. marina* beds is still possible after 6 months (Moore et al., 1993; Reusch, 2006), the additional loss of viability reported here may also be due to damage to the seed coat, disease, or decay (Harrison, 1993; Keddy and Patriquin, 1978).

Interestingly, the persistence of NC seeds over 15 months in the experimental results was not supported by site seed bank viability data which showed a complete loss of persistence in the ambient seed bank after the period of maximum germination (Jarvis et al., 2012). The lack of viable seeds in the ambient seed bank after 12 months may be due to factors such as seed predation (Fishman and Orth, 1996; Sumoski and Orth, 2012) or removal by physical processes (Hammerstrom et al., 2006). Experiments have shown that while *Z. marina* seeds can maintain viability following the consumption of a guild of seed predators, predation can remove viable seeds from the seed bank through consumption and by germination enhancement (Sumoski and Orth, 2012). The experimental results presented here indicate that although NC *Z. marina* seeds have the capacity to remain viable for >12 months in the sediment seed bank, factors such as low viable seed densities (Jarvis et al., 2012) and predation pressures may reduce seed persistence and result in the development of a transient rather than persistent seed bank.

4.2. Seed source

We hypothesized that seeds derived from mixed-annual populations in the North Carolina experiment would remain viable for longer time periods in the sediment than seeds from perennial populations; however, across all sampling times seed source did not have a significant effect on seed viability. There was a significant interaction between seed source and time as a result of a greater viability of mixed-annual seeds (NC1, 6.33 ± 2.04 %) than perennial seeds (NC2, 0.83 ± 0.39%) after 6 months in the sediment. The greater viability of mixed-annual seeds coincides with the period of maximum germination and may provide an advantage for populations that rely on seeds for bed re-establishment on a yearly basis (Jarvis et al., 2012; Phillips et al., 1983a; Santamaría-Gallegos et al., 2000; van Lent and Verschuure, 1994). In a separate study Jarvis et al. (2012) documented 27.2 times more viable seeds in the sediment seed bank at the mixed-annual (NC1) compared to perennial (NC2) sites in 2007 and 2008. Similar differences in ambient seed-bank densities were reported for perennial (0–1,200 seeds m^{-2}) and annual (1,300–30,000 seeds m^{-2}) *Z. marina* populations in other locations across within the species geographic range (Harrison, 1993; Harwell and Orth, 2002; Lee et al., 2007; Morita et al., 2007). Although mixed-annual seeds do not support seed banks that maintain viability over longer time scales than regional perennial populations, greater seed abundances and higher viability during periods of peak germination within the mixed-annual *Z. marina* bed would increase the ability of the bed to re-establish from seed on a yearly basis (Inglis, 2000). Therefore by maintaining greater seed viabilities during times of maximum germination than perennial populations, and producing significantly greater numbers of seeds, semi-annual *Z. marina* populations may have a greater regeneration potential from the seed-bank than perennial beds.

4.3. Sediment and environmental conditions

Unexpectedly, neither site nor sediment type had a significant effect on *Z. marina* seed viability in either experiment, despite the fact that both redox potential and organic content were significantly different between sediment treatments. Anoxia is a cue for *Z. marina* germination (Churchill, 1992; Moore et al., 1993; Probert and Brenchley, 1999) and sediments with greater organic content and a greater proportion of fine (silt and clay) sediments are more reduced than lower organic content sandy sediments (Boer, 2007; Koch, 2001). In addition, coarse sediments typically allow more oxygen transport deeper into the sediment,

Table 4
Mean experimental sediment conditions for both (A) North Carolina and (B) Virginia sites. Except for sand/silt/clay analyses, all sediment characteristic values are averaged across depths and over time, and are presented as means ± standard errors. Sand/silt/clay is reported for June 2007 only for each treatment. Mean ± SE values are used due to no significant difference in sediment characteristics between dates or over depths. – denotes samples that were lost during processing.

Location	North Carolina				Virginia			
Site	NC1		NC2		CB1		CB2	
Sediment Type	Coarse	Fine	Coarse	Fine	Coarse	Fine	Coarse	Fine
% Organic	2.7 ± 0.2	2.3 ± 0.3	2.7 ± 0.2	2.1 ± 0.2	0.8 ± 0.1	2.1 ± 0.2	0.8 ± 0.1	1.8 ± 0.2
Sand:Silt:Clay								
% Sand	81.0 ± 1.9	90.1 ± 2.5	81.0 ± 1.9	65.4 ± 1.7	88.7 ± 4.7	65.4 ± 1.7	88.7 ± 4.7	81.4 ± 2.8
% Silt	13.5 ± 1.3	6.8 ± 1.9	13.5 ± 1.3	26.4 ± 2.0	8.4 ± 4.1	26.4 ± 2.0	8.4 ± 4.1	12.8 ± 2.4
% Clay	5.6 ± 1.8	3.1 ± 0.7	5.6 ± 1.8	8.3 ± 0.8	3.0 ± 0.6	8.3 ± 0.8	3.0 ± 0.6	5.7 ± 0.8
Nutrients								
$NH4^+$ μM								
6 mo	–	53.5 ± 11.2	48.5 ± 9.4	42.3 ± 4.3	27.4 ± 4.4	97.3 ± 16.7	33.6 ± 1.1	53.7 ± 15.2
12 mo	50.7 ± 10.8	37.1 ± 8.7	23.6 ± 2.9	39.3 ± 8.9	61.0 ± 7.1	35.8 ± 3.9	152.2 ± 32.1	49.5 ± 7.0
15 mo	24.2 ± 3.2	24.0 ± 4.2	23.8 ± 1.4	30.1 ± 5.9	22.8 ± 2.9	43.2 ± 16.5	19.5 ± 1.4	24.8 ± 2.9
PO_4^{-3} μM								
6 mo	0.27 ± 0.04	0.34. ± 0.08	0.61 ± 0.23	–	0.40 ± 0.24	0.05 ± 0.01	0.60 ± 0.26	0.31 ± 0.08
12 mo	1.23 ± 0.36	1.62 ± 0.30	0.52 ± 0.09	1.37 ± 0.75	0.36 ± 0.15	0.19 ± 0.01	0.28 ± 0.12	0.95 ± 0.43
15 mo	0.33 ± 0.26	0.03 ± 0.02	0.00 ± 0.00	0.00 ± 0.00	0.14 ± 0.03	0.40 ± 0.29	0.23 ± 0.03	0.18 ± 0.01

increasing the depth at which anoxic conditions develop (Folmer et al., 2012; Koch, 2001). Despite the differences in organic content and sand: silt:clay ratios between experimental sediment treatments, a reduced or anoxic environment did not develop until depths ≥ 3 cm across all treatments. The similar depths of the redox layer may have been an artifact of the processing required to remove seeds from sediment collected in existing beds. Under *in situ* conditions seagrass seeds in the sediment seed bank are not static and can be moved to shallower or deeper depths through both physical (Hammerstrom et al., 2006) and biological processes (Blackburn and Orth, 2013) potentially exposing seeds to a range of redox conditions. Therefore, despite the lack of a significant impact of redox on seed viability reported here, the impact of variable redox conditions on long term seed viability needs to be considered across a broad range of sediment burial depths and sediment types.

5. Conclusions

Zostera marina populations at the southern limit of the species distribution along the western Atlantic produce transient seed banks regardless of seed source, sediment type, or local environmental conditions. Viability of all seed sources in both the Virginia and North Carolina field experiments were reduced significantly after only 6 months in the sediment and were further reduced to 0 and <5% of initial viability by 15 months, respectively. The lack of a persistent seed bank may limit the ability of *Z. marina* meadows to naturally recover from multiple and/or consecutive stressors. Timing may be critical, with a potentially greater negative impact of a transient seed bank occurring on meadow recovery if biomass is lost before mature viable seeds are released (May/June) to replenish the seed bank. This may have significant implications for the management of *Z. marina* within this region. Future research should focus on developing effective management strategies that include monitoring sexual reproductive output and seed bank viability to determine meadow resilience. Finally, while seed addition has been successfully used to restore *Z. marina* beds in the coastal bays of Virginia (Orth et al., 2012), the effects of "seeding" existing meadows in this region to increase the resilience or recovery potential of these populations is unknown and requires further investigation.

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