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APPLIED WETLAND SCIENCE

High Reinvasion Potential of *Phragmites australis* in a Delaware River (USA) Tidal Freshwater Marsh Following Chemical Treatment: the Role of the Seedbank

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Abstract
Management of the invasive *Phragmites australis* haplotype has focused on controlling its abundance in wetlands where it reduces biodiversity. However, little information is available on establishment of native communities and reinvasion by seed following removal using herbicides. The potential for reinvasion and development of native vegetation were evaluated using a seedbank assay and a vegetation survey along gradients from the channel edge to the marsh interior (0, 5 and 20 m distance) in three tidal freshwater marsh sites - Natural, treatment 6 months prior (Treated), and untreated (Phragmites). Recolonization potential from the seedbank was high with >18,500 seedlings m^{-2} in Treated samples. Richness and density of native species were low in the interior of Treated and Phragmites sites as compared to the Natural marsh. Few species were present in Treated site vegetation 11 months following treatment where *P. australis* litter comprised a large proportion of the cover. Results indicate that planting native vegetation to outcompete *P. australis* seedlings and total removal of *P. australis* to cut off the seed supply may be necessary for successful longer-term restoration and establishment of native species.

Keywords Seedbank · Delaware Estuary · Native plant community · Restoration · Invasive species

Introduction

Controlling invasive plant species is a major land management priority and successful programs tend to integrate knowledge of the characteristics of the invading plant species, the structure and dynamics of the invaded ecosystem, and the role of human activities in facilitating the invasion (Hobbs and Humphries 1995). In the United States, there has been a large effort to reduce populations and control the spread of an invasive haplotype of the common reed, *Phragmites australis* (Cav.) Trin. ex Steudel (Marks et al. 1994; Martin and Blossey 2013; Hazelton et al. 2014). Despite costing ~US$4.6 million per year (2005–2009), *P. australis* control efforts, similar to those for many other invasive species, have generally not resulted in strong long-term ecological outcomes (Martin and Blossey 2013; Reid et al. 2009). One of the key uncertainties and potential limitations to success is whether current management approaches are effective in promoting the reestablishment of native plant communities (Hazelton et al. 2014). With few studies on vegetation community development post-removal (Kettenring and Adams 2011), it is difficult to ascertain potential limitations for native species re-establishment. Native species colonization in areas where *P. australis* has been removed may be limited by the lack of a seedbank, altered or unfavorable abiotic conditions, and/or competition with *P. australis* propagules. The same conditions that can limit native vegetation colonization can also foster reinvasion of *P. australis*, which is a common outcome of many restoration attempts (Myers et al. 2000).

Wetland ecosystems tend to be prone to plant invasions, particularly when coupled to human landscape disturbances (Zedler and Kercher 2004). A Eurasian lineage of *Phragmites australis* (haplotype M) is a common invader of well-drained areas of tidal marshes and has dramatically expanded its range in North America in both coastal and inland habitats since the

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mid-1900s (Saltonstall 2002). Although some studies identify positive attributes of the invasive *P. australis*, such as its ability to rapidly accumulate organic material and sediment (Rooth et al. 2003), contributing to both carbon sequestration and marsh capacity to keep up with sea-level rise, negative impacts include outcompeting native species and reducing biodiversity overall (Benoit and Askins 1999; Keller 2000; Meyerson et al. 2000). As such it is considered one of the most problematic invasive species in North American wetlands (Galatowitsch et al. 1999). Successful invasion has been associated with its ability to spread rapidly via rhizome, and through this underground conduit, *P. australis* transports oxygen to clones spreading into lower more anaerobic elevations (Amsberry et al. 2000). While once thought to produce largely non-viable seeds (*in* Zedler and Kercher 2004) and spread mainly through asexual fragments and underground rhizomes (Saltonstall et al. 2010), there is increasing evidence that colonization by seed is an important means of *P. australis* invasion (Belzile et al. 2010; McCormick et al. 2010; Kettenring and Mock 2012).

Seedbanks, therefore, may play a critical role in *P. australis* recolonization following chemical treatment. In brackish marshes of Chesapeake Bay, an average of between 10 and 698 *P. australis* seedlings m^{-2} emerged from the seedbank of *P. australis*-dominated areas indicating that colonization from the seedbank is possible (Baldwin et al. 2010). This may be challenging from a management perspective because successful restoration also includes recolonization by native species, which, in areas once dominated by *P. australis*, may be largely by seed dispersal along tidal channels and germination from the soil seedbank. Identifying the environmental conditions that reduce the success of *P. australis* reinvasion by seed and facilitate the development of a diverse native seedbank is therefore important for developing long-term management plans.

Here, we investigate the potential for *P. australis* to reinvade a tidal freshwater marsh immediately following herbicide treatment using a seedbank assay and vegetation survey. We hypothesized that species richness and density of native species that emerge from the seedbank are greater in a natural tidal freshwater marsh community than an area where *P. australis* was removed using herbicide treatment and an intact *P. australis* stand. For all sites, we predicted that the diversity and density of seedlings that emerged from the seedbank would be influenced by proximity to a tidal channel. Specifically, we hypothesized that the seedbank of the natural marsh would be most abundant and diverse in the marsh interior in contrast to the previously and currently *P. australis*-dominated sites, where dense stands in the marsh interior may limit seed and propagule dispersal. *Phragmites* seed germination and seedling survivorship have been shown to be extremely sensitive to flooding (Carlson et al. 2009; Baldwin et al. 2010) and therefore, the flooding dynamics following treatment were predicted to be an important determinant of the community that develops (Leck 1996; Leck 2003).

Methods

Study Location and Sites

The Abbott Marshlands include the northernmost tidal freshwater wetland on the Delaware River (New Jersey, USA; 40.172477, −74.713873). The tidal marsh has been the location for numerous studies (e.g., Whigham and Simpson 1975; Leck and Graveline 1979; Parker and Leck 1985; Leck and Simpson 1995; Elsey-Quirk and Leck 2015). The tidal range is 2 to 3.5 m. Since the 1950s, tidal range has increased >1 m, as a result of sea level rise, channelization and deepening of the Delaware River for commercial navigation, and downriver wetland loss (Leck et al. 1988).

The study area is located in Roebling Park at the northern edge of the Abbott Marshlands, and is about 3.2 km south of Trenton, NJ. The tidal portion of Roebling Park lies at the headwater of Watson's Creek, a first order tributary of the Delaware River. The study site is ~16.2 ha, a tenth of Roebling Park, and is owned by Mercer County, NJ. An early study of plant communities of the marshland had no vegetation type dominated by *Phragmites* (Whigham and Simpson 1975). However, the study area had a high susceptibility to *P. australis* invasion because of anthropogenic disturbances, such as along the edges of Spring Lake, which had been damned to provide swimming and boating for an amusement park (Colello 2013), the establishment of an adjacent landfill, and small isolated disturbances along the wetland edges (Supplementary Fig. 1; Leck, pers. obs.). In addition, an electrical transmission line traverses the wetland and *P. australis* has colonized the old road that connected the transmission towers as well as the area around the base of each tower.

Herbicide treatment was initiated by Mercer County to control *P. australis*, enhance the wetland for wildlife, and provide additional recreational opportunities such as bird watching. In 2018, two *P. australis*-dominated sites and one with no *P. australis*, a reference site, were selected from aerial photographs taken in 2012. For control, the herbicide Imazapyr was selected, which necessitated educational public meetings and trail closings during treatment.

In all 16 ha were treated. Due to permitting delays and logistical problems involving tides and winds, only half of the area was sprayed in autumn 2018 (Fig. 1). This provided both treated and non-treated *P. australis* sites along with a nearby natural site for the study (Fig. 1). The intact

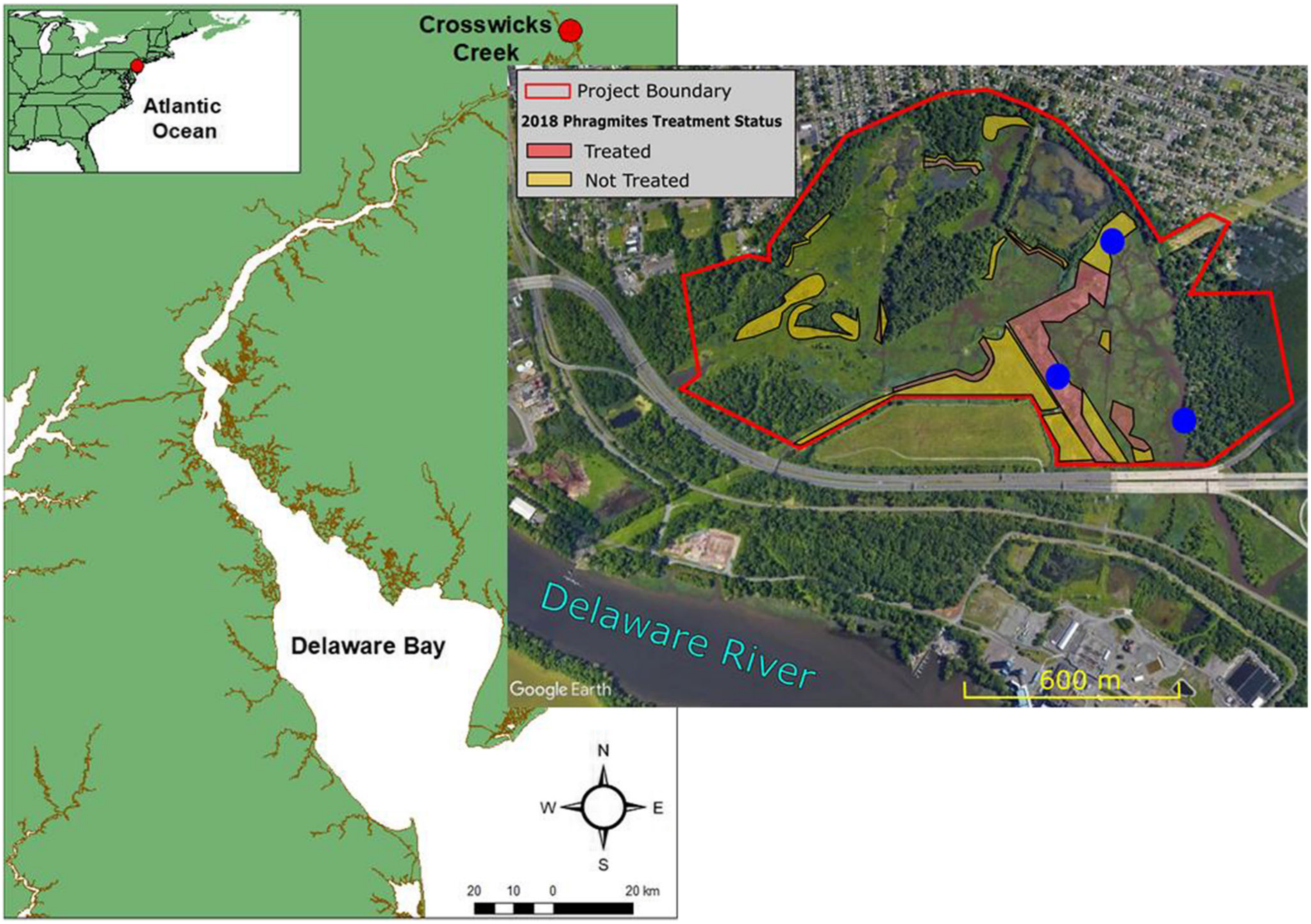


Fig. 1 Map of the Delaware Bay region study area. The red polygon represents the study location along a tributary to Crosswicks Creek. Aerial imagery of the project area and treatment status map from 2018 is shown, courtesy of Princeton Hydro (Princeton, NJ). Blue circles represent the midpoint of Natural, Treated, and Phragmites sites where transects and plots were established for soil collection and vegetation surveys. The Natural marsh is southeast of the Treated and Phragmites sites adjacent to a forested area. Note the transmission line that crosses the marsh, from NE to SW

Phragmites site and remaining areas of *P. australis* were subsequently treated in Fall 2019.

Sampling

Seedbank Assay On March 20, 2019, soil samples (10 × 10 × 3 cm deep) were collected in a natural tidal freshwater marsh community (Natural), an area treated with herbicide in October 2018 (Treated), and an untreated area dominated by *P. australis* (Phragmites; Fig. 1). New Jersey Department of Transportation allowed site access. Five 20 m long transects, 5 m apart, running perpendicular to the tidal channel were established at each site. One soil sample was collected at 0, 5 and 20 m distances from the channel along each transect for a total of 15 samples at each site ($n = 5$). An additional three were collected at 0 and 20 m at each site to determine the effects of flooding on seedling recruitment ($n = 3$).

Samples were placed into plastic bags, stored overnight outdoors, and set up in the greenhouse on March 21. Each sample was spread on ~2 cm of Perlite in a 20 cm × 20 cm × 4 cm deep aluminum pan with drainage holes. These samples were kept at about field capacity: they were surface watered 2 or 3 times weekly and water level on the benches, lined with plastic, was maintained at ~1 cm. The flooded samples were placed into pans without drainage holes or Perlite; water level was maintained at approximately 1 cm above the soil surface. Locations of samples were randomized on two greenhouse benches.

Seedlings were identified to species if possible. Once seedlings were identified, they were removed to prevent overcrowding. Cross-contamination was avoided by removing seedlings before they dispersed seeds. Some seedlings were transplanted to pots with potting soil until identification was possible. Samples were maintained until mid-August after germination had ceased.

Vegetation Survey Seedlings in field plots were documented along three transects at 0 and 20 m on May 18 (Natural), May 22 (Treated), and June 2 (Phragmites) sites. Because few seedlings were encountered in plots and some plots could not be relocated, we only noted species presence. To determine the impact of treatment and evaluate biodiversity, species cover was evaluated on August 4, 2019. Plots at each location were relocated using GPS coordinates. At each site 15 1-m^2 plots were surveyed at the locations where soil samples had been obtained. In addition, elevation was determined for each plot using a Leica GNSS RTK (Projection: NJ NAD83; Local Ellipsoid: GRS1980; Geoid model 12B). The cover of each species was measured using range midpoints from a modified Daubenmire protocol (Brower et al. 1998). All species present were recorded.

Nomenclature follows USDA ITIS (2020)- https://www.itis.gov/ <https://www.itis.gov/> (July 2020) except *Nuphar advena* (Aiton) Aiton f. (Padgett 2007).

Statistical Analyses

The effects of site, distance from the channel, and their interaction were tested on seedbank species richness, seedling density, and percent cover of plant species using a two-way analysis of variance (ANOVA). Species richness and seedling density data were $\log(x + 1)$ transformed to meet the normality assumptions of analysis of variance. The Honestly Significantly Difference Tukey test was conducted for all post-hoc comparisons. Separate analyses were conducted for *P. australis* and for all other species combined. The effect of flooding was also tested on seedling density of individual species and all species other than *P. australis*. JMP SAS (v.15) was used for all univariate analyses. To test whether the species composition of seedlings that emerged from the seedbank differed among sites and distances from the channel, we used Permutational multivariate analysis of variance in a two-factor crossed design (PERMANOVA, Primer 6; Clarke and Gorley 2006). Non-metric multidimensional scaling (MDS) plots were developed to illustrate separation in community structure across transect distances. Pre-treatment of community data included a global logarithmic transformation and a Bray-Curtis similarity resemblance matrix using Primer 6 (Primer-E Ltd., Clarke and Warwick 2001).

Results

Seedbank

Species Richness A total of 44 species emerged from the seedbank of the Natural marsh. The Treated and Phragmites sites had fewer total species emerge from the seedbank with 37 and 26 species, respectively. Several species that emerged from the Natural marsh were absent in the seedbank samples of the Treated and Phragmites sites, such as *Artemesia vulgaris*, *Alnus incana*, *Hypericum mutilum*, *Impatiens capensis*, *Juncus tenuis*, *Peltandra virginica*, *Saururus cernuus*, *Scutellaria lateriflora*, and *Zizania aquatica* (Supplementary Table 1). However, there were also species that emerged from the Treated and/or Phragmites sites that were not present in the Natural marsh such as *Alisma subcordatum*, *Duchesnea indica*, *Erechtites hieracifolius*, *Eupatorium serotinum*, *Plantago rugelii*, *Pontederia cordata*, *Solidago canadensis,* and *Toxicodendron radicans*.

An average of 10 species emerged from the seedbank of the Natural marsh, which did not vary significantly by distance (Fig. 2). In the Treated site, species richness was similar to that in the Natural marsh at the channel edge (0 m) and 5 m from the channel, but was over 50% lower than the Natural marsh at 20 m. In the Phragmites site, species richness was similar to the Natural marsh at the channel edge but was significantly lower at both 5 and 20 m distances from the channel (Fig. 2). The Treated and Phragmites sites had similar species richness overall and at each distance from the channel.

Seedling Density *Phragmites australis* is not present in the vegetation in the Natural marsh yet was found in the seedbank with densities ranging from an average of 460 seedlings m^{-2} at the channel edge to 1320 seedlings m^{-2} in the marsh interior, 20 m from the edge (Fig. 3a). However, seedling density of *P. australis* in the Natural marsh was significantly lower than that in the Treated and Phragmites sites (Fig. 3a). Interestingly, the Treated site had over three times greater density of *P. australis* seedlings (18,546 ± 5662 seedlings m^{-2}) than the

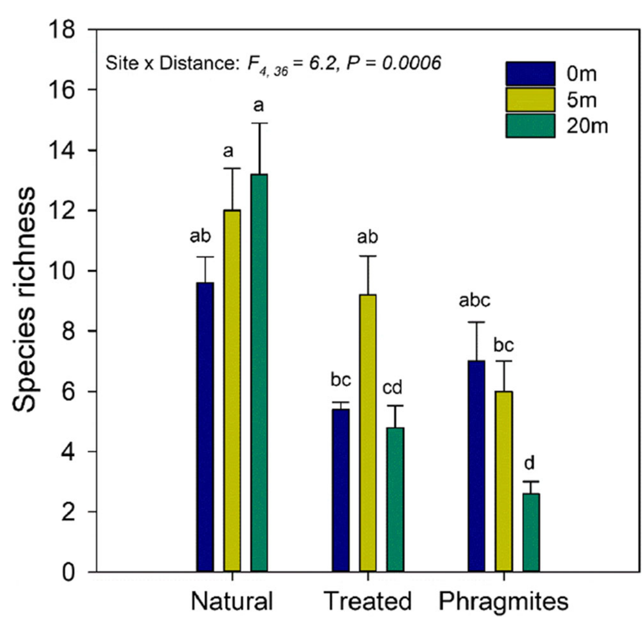


Fig. 2 Species richness of seedbank samples collected at three distances (0, 5, and 20 m) from a channel in a natural tidal freshwater wetland (Natural), a former *Phragmites*-dominated area that had been treated with herbicide (Treated), and a *Phragmites*-dominated site (Phragmites) ($n = 5$, mean ± standard error)

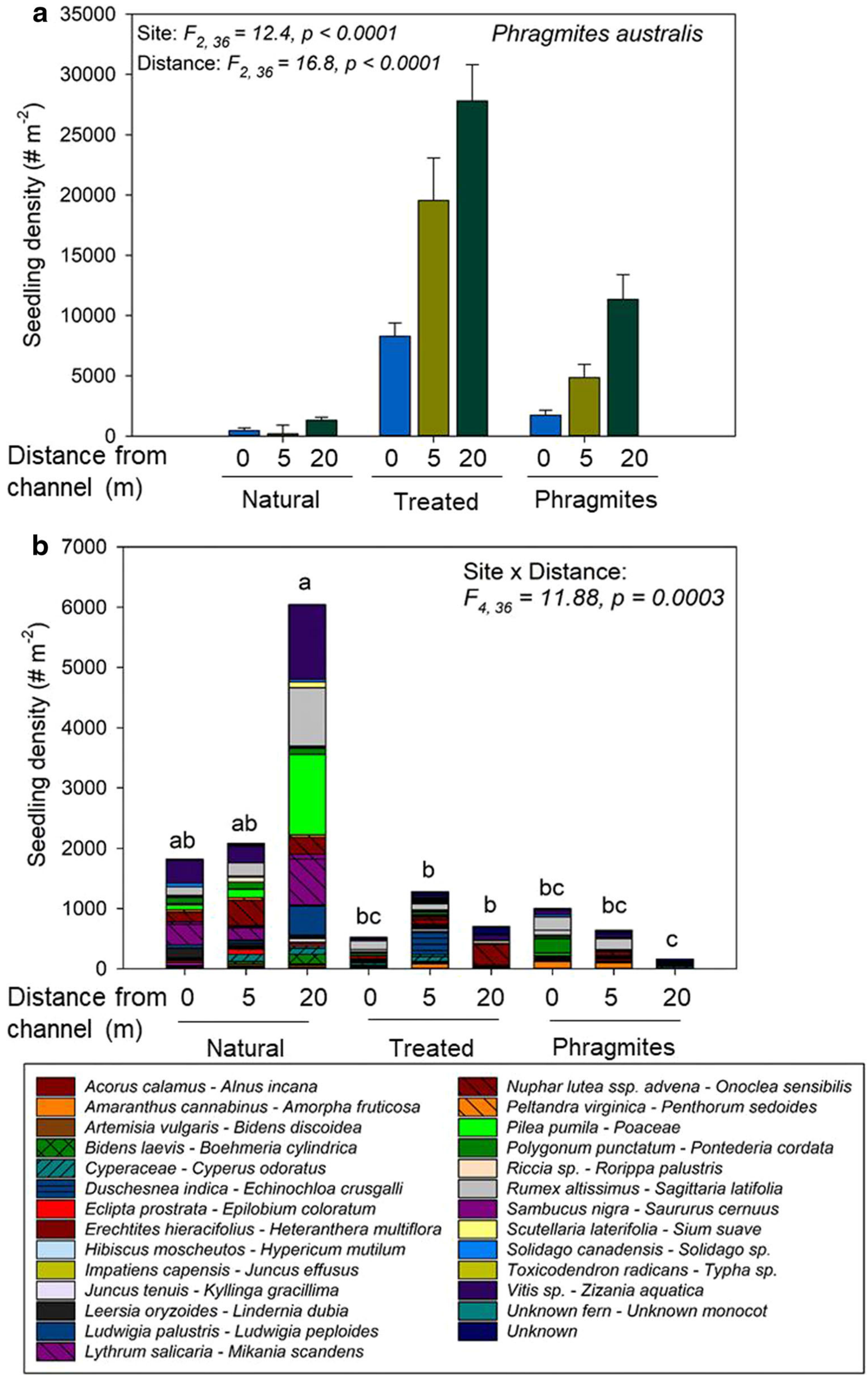


Fig. 3 Density of seedlings of *Phragmites australis* (**a**) and other species (**b**) emerging under freely-drained conditions from soil samples collected from a natural tidal freshwater site (Natural), a *Phragmites*-dominated area treated with herbicide (Treated), and a *Phragmites*-dominated site (Phragmites). Samples were collected at three distances (0, 5, and 20 m) from a tidal channel. Note the different y-axis scales for *P. australis* (**a**) and other species (**b**) seedling densities. Values for *P. australis* are means ± standard errors ($n = 5$). Values are means for each species and two consecutive species share a color but are separated by lines (**b**). Means and standard errors for the other species are reported in Supplementary Table 1. (Note: for completeness, also included are non-seed plants, *Riccia* (a liverwort) and ferns, including *Onoclea*)

intact Phragmites site (5973 ± 2829 seedlings m^{-2}). *Phragmites australis* seedling density increased from the marsh edge to the interior at all sites (Fig. 3a).

Seedling densities of species other than *P. australis*, including seedlings of native and invasive species such as *Lythrum salicaria*, ranged from an average of 1840 near the channel edge to 6140 at 20 m in the Natural marsh (Fig. 3b). For simplicity, we are referring to the seedbank of species other than *P. australis* as the "native" seedbank. The native seedbank was comparatively low in the Treated and Phragmites sites, particularly in the marsh interior where seedbank densities averaged 760 and 200 seedlings m^{-2} in the Treated and Phragmites sites, respectively (Fig. 3b).

Species Composition Species composition of seedlings differed among sites depending on distance from the channel (Fig. 4). The Natural marsh had a seedbank composition that clearly differed from the Treated and Phragmites sites, where the seedbank was dominated by *P. australis*. The channel edge of the Phragmites site had relatively fewer seedlings of *P. australis* resulting in a seedbank composition that was slightly more diverse than those in the interior and in the Treated site at all distances. In contrast, in the Natural marsh, the greatest diversity based on both richness and abundance was in the marsh interior at a 20 m distance from the channel.

The Effect of Permanent Flooding The effect of flooding on germination from the seedbank was tested on samples collected at the channel edge (0 m) and the marsh interior (20 m). A total of 44 species emerged from the seedbank at those distances under freely-drained conditions, while only 21 species emerged under flooded conditions (Supplementary Table 1).

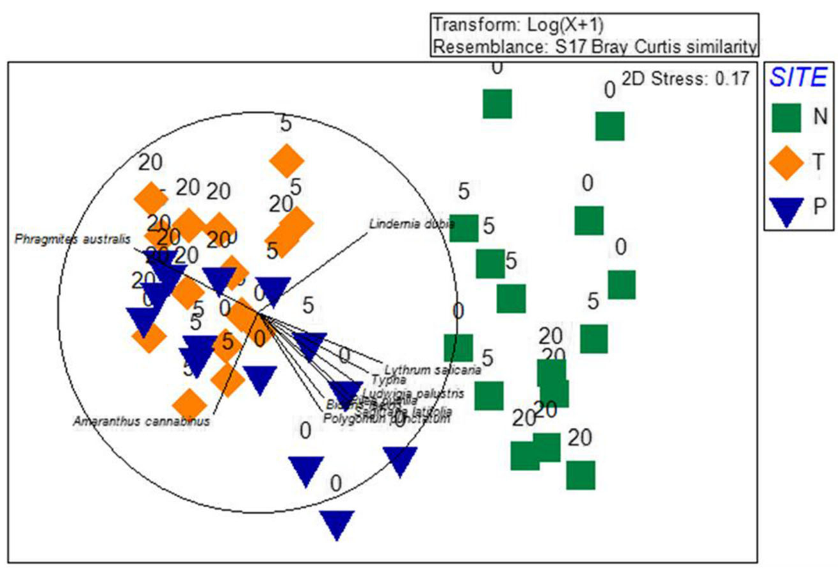
Flooding had a negative effect on the emergence of *P. australis* seedlings across all sites and distances, reducing abundance from an average of 8493 ± 1860 to 44 ± 18 seedlings m^{-2} ($p < 0.0001$; Table 1). Flooding also reduced seedling emergence of other species from an average of 1777 ± 468 to 872 ± 239 seedlings m^{-2} ($p = 0.0419$). However, high densities of seedlings of other species emerged from samples collected at the channel edge of the Treated and Phragmites sites under flooded conditions (Table 1).

Field Seedlings

In May 2019, seedlings at 0 m in the Natural site included *Zizania aquatica* (1), *Amaranthus cannabinus* (1), *Polygonum punctatum* (6), and an unknown monocot (1): and at 20 m *Bidens laevis* (1), and *Pilea pumila* (1). Other seedlings were *Ambrosia trifida* and several *Impatiens capensis* elevated on litter. At the Treated site, no seedlings were observed in plots, but elsewhere along a survey path or at the edge of the channel were seedlings of *Amaranthus cannabinus*, Apiaceae, *Bidens laevis, Bidens* sp., possibly *Heteranthera multiflora, Impatiens capensis*, *Pilea pumila*, and *Zizania aquatica*. Of these only *Z. aquatica* was present in appreciable numbers along the channel edge. At the Phragmites site, seedlings, not in plots, were Apiaceae, *Bidens laevis, Bidens* sp., *Peltandra virginica*, *Pilea pumila, Polygonum punctatum*, and *Typha* sp. No *P. australis* seedlings were observed.

Marsh Elevation and Vegetation

The elevations of the three sites were similar, all having lower elevations near the channel (0 and 5 m) and higher elevations in the marsh interior, 20 m from the channel, by an average of approximately 30 cm ($F_{2,\ 19} = 18.0$, $p < 0.0001$; Fig. 5).



Permanova results
Site x Distance:
Pseudo-$F_{4,\ 43}$ = 2.64, P(perm) = 0.001

Fig. 4 Results of non-metric multi-dimensional scaling and Permanova analyses examining the species composition of seedlings that emerged from seed bank samples collected from Natural (N), Treated (T) and Phragmites (P) sites. The numbers adject to symbols indicate distances from the channel in meters. Vectors correspond to species contributing to the scaling based on correlations >0.5

Table 1 The effect of flooding on seedling density of *P. australis* and other species from seedbanks collected from a Natural, Treated, and intact Phragmites tidal freshwater marsh sites. Samples were collected from the channel edge and marsh interior. Values are means ± standard errors (n = 5, drained; n = 3, flooded). (Note: also included are non-seed plants, *Riccia* (a liverwort) and ferns, including *Onoclea*)

		Distance from a channel			
		0 m		20 m	
Site	Vegetation type	Drained	Flooded	Drained	Flooded
Natural	*P. australis*	460±216	33±33	1320±240	0
	Other species	1840±160	833±33	6140±1789	2500±794
Treated	*P. australis*	8280±1104	0	27,820±2992	133±88
	Other species	620±171	800±231	760±238	33±33
Phragmites	*P. australis*	1740±406	33±33	11,340±2055	67±33
	Other species	1100±369	1000±458	200±54	67±33

In August 2019, the Natural site generally had over 60% open canopy with the majority of cover dominated by *Nuphar lutea* var. *advena*, *Bolboschoenus fluviatilis*, and *Sagittaria latifolia* in the high marsh (Fig. 6). The Treated site had no live *P. australis*, and a similar percentage of open canopy as the Natural marsh. *Peltandra virginica*, *Polygonum punctatum*, and *Zizania aquatica* were all present in the vegetation although litter of *P. australis* comprised the majority of cover. Most of the standing dead *P. australis* that was present during seedbank sampling in March was represented by litter on the marsh surface by August (Fig. 6). The total cover of vegetation was highest in the Phragmites site compared to the Natural and Treated sites, where live *P. australis* made up the majority of the cover (Fig. 6).

Discussion

Phragmites australis invasion coincides with localized disturbances to the vegetation and hydrologic alteration and seems to be largely initiated by colonization from seed followed by expansion by vegetative reproduction (Amsberry et al. 2000; Bart and Hartman 2002, 2003; Minchinton and Bertness 2003). The importance of sexual reproduction of *P. australis* to the initial invasion and subsequent removal and longer-term restoration is beginning to be appreciated. Here, we show that *P. australis* produces a large viable seedbank. Our estimates of seedling densities emerging from the seedbank are greater than ever reported previously (> 27,000 per m^{-2}). Therefore, at this location, the potential for reinvasion following herbicide treatment is high, particularly in areas with less flooding, as flooding dramatically reduced seedling emergence. Additionally, the interior of *P. australis* stands had a very limited native seedbank compared to the natural marsh, which also reduces the potential for a diverse community to establish. The vegetation community that developed in the summer following treatment was absent of live *P. australis*, but differed from that of the Natural marsh, with seedling emergence potentially limited by a dense *P. australis* litter layer or other factors.

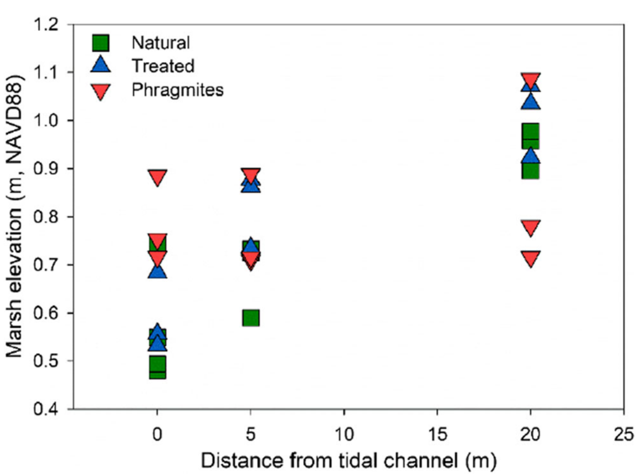


Fig. 5 Marsh elevations at the channel edge (0) and at 5 and 20 m from the channel in a natural tidal freshwater (Natural), a Phragmites-dominated site treated with herbicide (Treated), and an untreated *Phragmites*-dominated site (Phragmites)

Phragmites Reinvasion Potential

This study establishes that *P. australis* produces viable seeds in the tidal freshwater marshes of the Delaware Estuary as found in Chesapeake Bay marshes (Kettenring and Whigham 2009; Baldwin et al. 2010) and in riparian wetlands of the Platte River, Nebraska (Galatowitsch et al. 2016), among other places. Seedling density in tidal freshwater marshes in the Delaware Estuary is over an order of magnitude greater than the density measured from *P. australis*-dominated brackish marshes in Chesapeake Bay (Baldwin et al. 2010), potentially due to the lower salinity at our sites. Elevated nutrient conditions have also been implicated in promoting a greater number of inflorescences per plant, thus increasing the number of seeds that can form a seedbank and disperse (Kettenring et al. 2011). Seed viability is further enhanced by genetic diversity, which has been shown to be higher in more developed watersheds than in forested watersheds (Kettenring et al. 2011). Thus, the watershed conditions present in this relatively developed region

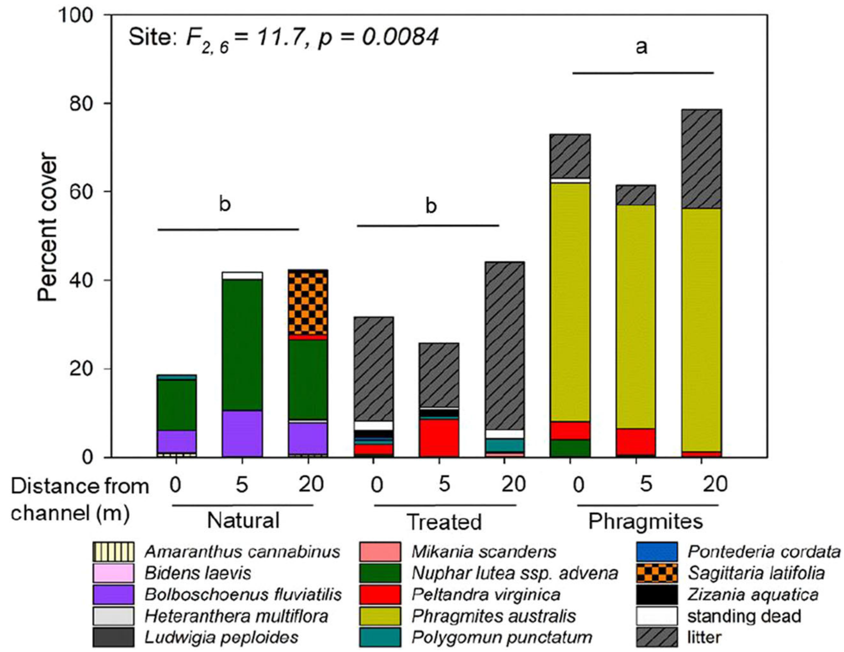


Fig. 6 The percent cover of plant species in Natural, Treated, and Phragmites-dominated marsh sites. Values represented by different letters represent significant differences ($p < 0.05$)

of the Delaware Estuary may promote viable seed set and reproduction by seed. The high density of germinable seeds in the seedbank and the ability of seeds to germinate under a wide range of hydrologic conditions (Galatowitsch et al. 2016), makes these areas susceptible to reinvasion by seed.

Phragmites australis seedlings emerged from the Natural marsh samples at densities greater than that of any other species (860 ± 139 m^{-2}), yet it is not present in the vegetation community. Propagule pressure from other species is therefore not likely to be limiting *P. australis* establishment in the Natural marsh. The predominant plant species during the vegetation survey were *N. lutea* ssp. *advena*, *Bolboschoenus fluvilatilis*, and *S. latifolia* in the high marsh. All three of these species are perennial and not reliant on a seedbank for regeneration. They tend to grow rapidly from rhizomes and may outcompete seedlings for resources such as light, nutrients and space. *Phragmites australis* is particularly sensitive to light limitation with seedlings strongly inhibited by shade (Ailstock et al. 2001; Kettenring and Whigham 2018). Similarly, despite extremely high seedling densities in soils of the Treated and Phragmites sites, no seedlings were observed in the field. In the Treated site, a dense *P. australis* litter layer may be preventing seedling establishment, while the establishment of seedlings in the intact Phragmites site is likely very limited by shade from both the surface litter and live *P. australis*. Disturbance to the vegetation in the Natural marsh and removal of the litter in Treated marsh may create opportunities for reinvasion of *P. australis* by seed (Kettenring et al. 2015). Additionally, marshes are typically spatially heterogeneous and gaps or openings can create windows of opportunity for germination (Kettenring et al. 2015). At this site, the presence of human modified higher elevation areas, including unimproved roads, a landfill, and power line right of way, may facilitate re-establishment by seed.

Natural colonization of native species from the seedbank in the Treated site is likely constrained by propagule pressure from *P. australis* as well as the dense litter layer of Phragmites. The seedling density of *P. australis* was two orders of magnitude greater than that of native seedlings in the marsh interior indicating that under appropriate field conditions for recruitment, native seedlings would likely be outcompeted. Our results also show more *P. australis* seeds in the seedbank when standing vegetation is removed than when it is present. It is not clear whether spraying in the autumn allowed greater seed rain in-situ and accumulation in the seedbank or whether dispersal was facilitated into the site following *P. australis* removal. Nonetheless, the abundance of *P. australis* in the interior of the Treated site illustrates the need to continue to manage for possible reinvasion from the seedbank.

Native Seedbank

Following invasive species removal, recruitment from a diverse and abundant native seedbank can facilitate the rapid establishment of plants that reflect the local surrounding matrix vegetation and genetic stock (van der Valk and Pederson 1989). In brackish wetlands of Chesapeake Bay, a functionally diverse seedbank was found under *P. australis* monocultures and in areas where it was removed; however, the community composition and the relative abundance of native seedlings that emerged varied across locations of the sub-estuary (Hazelton et al. 2018). Similarly, a relatively high species richness was found under intact stands of *P. australis* from Chesapeake Bay (Baldwin et al. 2010). Successful passive revegetation of native species has also been documented over a five-year period in a *P. australis* removal area along the tidal brackish portion of the Raritan River in New Jersey (Hallinger and Shisler 2009). Our initial findings in the year following removal and in intact stands of *P. australis* is that species richness and abundance of other species is significantly lower in the interior of these habitats than in a nearby natural marsh. While studies have illustrated that native species colonization can be limited by the surrounding matrix of native vegetation (Rohal et al. 2019), here, we show that recolonization is limited by seed and propagule dispersal into intact *P. australis* stands.

Proximity to a tidal channel played a strong role in the composition and abundance of species in the seedbank. Tidal channels facilitate the dispersal of propagules from other sites, and these channel connections may be important in facilitating the colonization by native species following *P. australis* removal. Propagules dispersing via hydrochory may be preferentially deposited close to the channel bank or along the wrack zone just inland from the channel (Elsey-Quirk and Leck 2015). Farther into the marsh interior, dense stands of *P. australis* along with a large quantity of surface litter may limit dispersal and establishment of all species (Leck and Simpson 1995; Leck 2013). With high reproductive output via seeds and rhizomes and the overall competitive dominance of *P. australis*, the likelihood for other species to establish and contribute to the seedbank is low (Leck 2013). When *P. australis* is removed, native species may initially colonize near the channel and expand gradually. The seedbank was less species-rich in the marsh interior in Phragmites and Treated sites than in the Natural site indicating that additional management may be required to promote a species-rich community following herbicide treatment. It is not clear whether a diverse seedbank can develop in the marsh interior over time once *P. australis* is removed. A high density of *P. australis* seedlings emerging from the seedbank would likely outcompete native species especially at higher elevations with less flooding.

Flooding Impact

Deep and prolonged flooding in large stands of *P. australis* has been associated with landscape-scale hydrologic disturbance, which promotes reinvasion and limits native species colonization (Rohal et al. 2019). Flooding dramatically reduced *P. australis* seedling emergence (600–37,100 to 0–300 seedlings m^{-2}) and changed the composition of the seedbank favoring more flood-tolerant species in all sites. *Phragmites australis* typically grows at or above mean high water and seed germination and young seedlings are particularly sensitive to flooding. While seeds require a moist substrate for germination, continuous flooding can prevent seedling emergence (Carlson et al. 2009; Baldwin et al. 2010). For example, recruitment of *P. australis* from the seedbank of brackish marshes was prevented with 3.5 cm of continuous flooding (Baldwin et al. 2010) and in the present study with approximately 1 cm of water above the surface. This indicates that *P. australis* regeneration from seed will likely occur in areas where flooding is infrequent or of short duration in the spring. In tidal wetlands, where river and tidal flooding can create high water conditions in the spring, reinvasion by seed will likely be minimal at low elevations. At periods of low water and at the many higher elevation microsites, *P. australis* may be able to emerge from the seedbank. The timing of submergence relative to *P. australis* life stage is also important (Galatowitsch et al. 2016). Young seedlings submerged to depths of 4 to 12 cm also experience a large reduction in growth; however, they can recover rapidly when the water table is lowered (Armstrong et al. 1992). Emergence from rhizome fragments is also limited in poorly drained soils (Bart and Hartman 2002). Tolerance to submergence increases with age and at 40 days old, *P. australis* can survive a month of continuous submergence (Mauchamp et al. 2001). Thus, the window of opportunity for flooding to limit *P. australis* reinvasion by seed is relatively short.

Despite a diverse seedbank, the Natural marsh vegetation is dominated by flood-tolerant species. Flooding the seedbank of the Natural marsh resulted in a composition of seedlings that was similar to the adult vegetation such as *Nuphar lutea ssp. advena* (although without *Bolboschoenus fluviatilis*, which is not found in the seedbank; Leck and Simpson 1994; Elsey-Quirk and Leck 2015). Species richness declined overall, but the density of seedlings of species other than *P. australis* was not statistically reduced by flooding. Many studies have illustrated the negative effects of flooding on species diversity in tidal marshes (Leck and Simpson 1987; Leck 2003; Neff et al. 2009; Baldwin et al. 2010). Here, we suggest that although flooding reduces the diversity of species emerging from the seedbank, *P. australis* seedlings are all but eliminated, and flood tolerant species in similar densities to non-flooded conditions have the potential to colonize. The future trajectory of the community post-treatment may depend on both flooding dynamics across the site and vectors for propagule transport.

Field Seedlings and Vegetation

Few seedlings of native species and none of *P. australis* were observed in late spring at any site during 2019. This may be related to dispersal limitations or lack of suitable germination and/or establishment conditions. However, *P. australis* seeds may be long lived (e.g., Thompson et al. 1997) and appear to accumulate with stand age, further complicating restoration efforts. In the Treated site, surface litter may have limited seedling emergence (Minchinton et al. 2006; White 2014). Removing *P. australis* surface litter by burning following herbicide treatment in a non-tidal marsh enhanced the pace of colonization of a diverse marsh community as compared to a non-burned site (Ailstock et al. 2001). The site treated with herbicide alone experienced a very slow recovery.

Management Implications

The findings of this study highlight several important management considerations for eliminating *P. australis* reinvasion and promoting a more diverse community post-herbicide treatment. First, control strategies that limit the potential reinvasion of *P. australis* by seed will be necessary following treatment. Regeneration from seed may contribute to the spread and reinvasion of *P. australis* in the tidal freshwater marshes of the Delaware Estuary, particularly at higher, less flooded elevations. In general, persistent seedbanks of invasive species complicate control efforts by increasing the likelihood of invasion over longer-time periods (Hallinger and Shisler 2009). Here, we suggest that the strategic management of higher elevations and interior areas where *P. australis* was dominant is necessary. At higher elevations, it is more likely for *P. australis* to germinate from seed and outcompete native species. In the interior of *P. australis* stands, the density of *P. australis* seeds is high and the density of native species is initially low. In addition to edge elevations that could be optimal for germination, higher microsites, caused, for example, by litter accumulation or footsteps, could provide chance colonization opportunities. Additionally, complete removal of *P. australis* from the surrounding areas may be necessary to sever seed supply. Chemical treatment in these locations may be phased out over time as the *P. australis* seedbank is reduced.

Second, seedling densities of native species were very low compared to *P. australis* suggesting that post-treatment management must consider ways to tip the competitive balance from *P. australis* to that of other species. Thus, planting native species, particularly in the marsh interior, after chemical treatment to outcompete seedlings of *P. australis* may be required at this site. We found an important influence of tidal channel connections on the diversity of the seedbank. Often, *P. australis* invades disturbed soils where the hydrology has been modified. Ensuring hydrologic connectivity with the larger wetland landscape may be key to facilitating dispersal and establishment of native species. Further, once *P. australis* has colonized, it can raise wetland surface levels by developing a rhizome mat and contributing high quantities of organic matter to the soil (Windham and Lathrop 1999; Rooth et al. 2003). These altered soil conditions have a positive feedback on *P. australis* growth, while reducing flooding frequency and limiting the potential for hydrochory. In this case, it may be necessary to regrade the marsh interior to enhance connectivity of the marsh to the channel.

The cover of vegetation in the Treated site was dominated by surface litter of *P. australis*, which may limit the recruitment of both *P. australis* and native species (Minchinton et al. 2006). While we observed few seedlings and adult plants in the Treated site, others have shown the negative effects of litter on species colonization following herbicide treatment (Ailstock et al. 2001). Removal of surface litter may occur naturally through tidal action or may need to be removed manually to allow sufficient light availability for the growth of seedlings.

This study and those in which viability may exceed 5 years (5 of 14 studies cited by Thompson et al. 1997), indicate that long-lived seed banks can contribute to recolonization and must be factored into management plans. Moreover, evaluation of restoration success must be long-term and continuing as noted by Hazelton et al. (2014); *P. australis* in a constructed wetland (Leck 2013) near these study areas was not a vegetation component for the first 7 years of monitoring in one of three locations; frequency across all three sites was 28% during the first 5 years and 98% in the 17th year (Leck pers. obs.)

In conclusion, we found in Phragmites sites (Treated and Phragmites) that there was a sizable readily germinable *P. australis* seedbank that could reinvade following herbicide treatment. In addition, there was a diverse seedbank of native species mainly at the channel edge (0 m) of the Treated and Phragmites sites indicating limited dispersal to the interior of *Phragmites*-dominated sites. A flooding treatment of 1 cm, markedly reduced germinability of *P. australis* seeds suggesting that flooding could be used to reduce recolonization from seed; native species were not similarly affected. As predicted, species richness was greatest in Natural site samples. Additional studies that examine changes in the seedbank of *P. australis* over a longer period following treatment would be helpful in planning for control.

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Authors' Contributions T. Elsey-Quirk and M.A. Leck conceived of, designed and implemented the study and wrote the manuscript. M.A. Leck maintained the greenhouse and identified the seedlings. Field surveys were conducted by T. Elsey-Quirk and M.A Leck. T. Elsey-Quirk analyzed the data.

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Data Availability Seedling data is in the Supplemental Table and vegetation data is available upon request.

Code Availability Not applicable.

Code Availability Not applicable.

Compliance with Ethical Standards

Conflicts of Interest/Competing Interests None.

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