

Project Title: OLIGOHALINE TIDAL WETLAND PLANT COMMUNITY RESTORATION AND RESPONSE TO CHANGES IN TIDAL FLOODING AND SALINITY

Primary Investigators: Sarah Kidd and Alan Yeakley, Portland State University

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Note: Portions of this report are excerpted and condensed from Sarah Kidd's dissertation and manuscripts in prep, expected 2017.

Project Abstract: In a collaborative project between Portland State University (PSU) and the National Park Service (NPS) the composition of seed banks and seed bank emergence were assessed among common native and non-native dominant plant communities from tidally reconnected oligohaline (0.5-5 ppt) wetlands within Lewis & Clark National Historical Park (LEWI). The ability of native plants (*Carex lyngbyei*, *Schoenoplectus lacustris*) and non-native plants (*Phalaris arundinacea*, *Juncus effusus* subsp. *effusus*) to dominate different areas within a wetland was hypothesized to be related to their abundance in the seed bank and germination responses to soil conditions created by the restored frequency and duration of tidal flooding and salinity. To identify how seed bank viability changes in response to these abiotic factors, seed bank emergence was examined under a gradient of tidal flooding and salinity treatments in a greenhouse setting. Seed bank composition and seed densities were also determined through manual seed ID. Overall, non-native species were found to be the most abundant seed type identified across both native and non-native seed banks. Non-native species, *P. arundinacea* and *J. effusus*, were also found to germinate more readily out of the seed bank under high marsh flooding (1 hour daily) and freshwater treatments as compared to mid and low marsh flooding (3 and 6 hours twice daily) oligohaline (3 ppt) treatments and all brackish salinity (10 ppt) treatments. Native species, *C. lyngbyei* and *S. lacustris*, germinated at similar densities across all flooding and salinity treatments. These results indicate that the newly created salinity and flooding gradient of restored oligohaline marshes act to suppress these non-native species' germination in the low-mid marsh regions but not in the high marsh, where they are likely able to outcompete the native wetland species due to their overwhelming dominance in the seed bank. These results indicate that sea level rise induced increases in tidal flooding and salinity within the high marsh zones of restored wetlands may drive *P. arundinacea* and *J. effusus* further up the wetland elevation gradient. Further research is needed, however, to identify how the established plant communities of these species respond to changes in tidal flooding and salinity and to monitor the spread of other, currently less abundant, non-native species such as *Typha angustifolia* and *Phragmites australis*, which are known to be more tolerant of oligohaline-brackish low marsh conditions.

INTRODUCTION

Restoring and maintaining native tidal wetland plant communities is one of the main goals of tidal wetland restoration and conservation efforts. The general ubiquity of invasive wetland plant species has, however, made this an especially difficult goal to achieve. Plant communities dominated by invasive species *Phalaris arundinacea* (*P. arundinacea*), reed canarygrass, and *Juncus effusus subsp effusus* (*J. effusus*), common rush, have been observed within and among restored wetlands throughout the Columbia River Estuary (CRE) and other wetland habitats throughout North America (Christy 2004, Borde et al. 2012). These invasive plant species can dominate wetland plant communities, reduce wetland biodiversity, degrade wetland habitat value, and stall native plant community recovery (Keddy 2004, Suding et al. 2004, Borde et al. 2012, Roman and Burdick 2012, Kidd and Yeakley 2015 and in prep). Understanding the mechanisms promoting these non-native plant community invasions within restored tidal wetlands is necessary for evolving the ecological theories that guide wetland restoration efforts. This is key for furthering our understanding of wetland plant community development in restored tidal wetlands and broadening our understanding of how climate change and sea level rise will impact these ecosystems in the future.

Generally, researchers have established strong correlations between *in situ* wetland elevation, tidal flooding, soil moisture, soil oxidation - reduction (redox) potential, and salinity gradients, and wetland plant community distributions (e.g., Eicher 1987, Zedler et al. 1999, Keddy and Fraser 2000, Baldwin et al. 2001, Davy 2011, Spencer and Harvey 2012, Weilhoefer et al. 2012, Janousek et al. 2013a, Janousek et al. 2013b, Moeslund et al. 2014). Specifically, the hydrologic and salinity gradients found in salt marshes have often been used to explain patterns of plant community assemblages; however, less work has been done to understand the role of these gradients in more dynamic restored fresh to brackish transitional oligohaline (0.5-5ppt) tidal wetlands (Engles 2010, Janousek and Folger 2013b). Experimental work in tidal marshes has provided evidence that observed *in situ* plant community patterns are representative of species-specific seed germination responses to abiotic gradients (Baldwin et al. 1996, 2001, Keammerer 2011, Janousek and Folger 2013a). These researchers have found the role of plant competition and exclusion secondary to plant species' germination and growth requirements for determining plant species distributions (Baldwin et al. 1996, 2001, Keammerer 2011, Janousek and Folger 2013a). These studies provide evidence supporting van der Valk's (1981) adapted Gleasonian theory for ecological succession in wetlands which proposes that hydrologic

conditions, i.e. timing and duration of wetland flooding, and seed bank response are the determining factors in plant community persistence and change over time. This theory further posits that the first species in the seed bank to respond favorably and in high densities to soil conditions (through germination) becomes locally dominant and suppresses further seed bank response and/or seedling establishment (Leck 2003, Wilcox 2004, Keammerer 2011). In turn, these newly dominant plant species fill the local seed bank with their own seeds/propagules thus enhancing the likelihood of their continued existence (Leck 2003). This theory implies that restored soil conditions, seed bank composition, and seed bank viability play a significant role in determining the development and long-term persistence of plant communities within and among restored tidal wetlands. Although this theory was developed in non-tidal wetlands, its transferability appears to be more universal (van der Valk's 1981). It is the aim of this study to apply and test this theory in restored tidal oligohaline wetlands, in an attempt to identify the mechanisms driving non-native plant community development in those systems.

Tidal restoration subjects the soil and seed bank to a dramatic change in environmental conditions through the creation of a hydrologic stress gradient (from tidal flooding) resulting in chemical and hydrological shifts in a restoration site's soil environment (Davy 2011). Prior to tidal reconnection, agricultural fields typically host well-drained soils with high oxygen penetration (Portnoy 1999). Once tidal flooding is reintroduced soils become saturated with water and oxygen levels drop because oxygen diffusion is dramatically reduced between the soil/water interfaces (Armstrong 1979, Portnoy 1999). Additionally, respiration from plants and organisms (in the soil) use up the oxygen faster than it can be replenished under these saturated conditions. Low oxygen levels in the saturated soil can lead to a shift in soil biota and respiration pathways from aerobic (where oxygen is the primary electron acceptor) to anaerobic (in which other ions such as nitrate, iron, magnesium, sulfate, and carbon are used as electron acceptors in the place of oxygen and each other) (Schlesinger and Bernhart 2013). The salinity of the water reintroduced can also effect these biogeochemical pathways, high salinities promote respiration through sulfate reduction which can reduce the soil pH, reduce organic matter accumulation, and nutrient availability (Schlesinger and Bernhart 2013). The goal of reintroducing tidal flooding to these sites is to restore these tidal wetland hydrologic gradients and associated environmental conditions. Very few plants are adapted to survive in sustained anoxic and/or saline conditions, making the transition from agricultural to tidal wetland plant communities seemingly swift (Davy et al. 2011).

The passive restoration approach of breaching dikes and reintroducing tidal flow comes with the assumption that after tidal reconnection, non-desirable pre-restoration agricultural plant species will die off and the seed bank will respond to these new environmental conditions through germination and growth of desired wetland plant species specifically adapted to the tidal flooding environment (high soil moisture, low oxygen conditions, and increase in salinity). Little attention has been paid however, to the precise conditions that need to be restored to promote the development of desired native plant communities over invasive plant communities out of restored tidal wetland seed banks.

As compared to naturally occurring tidal wetlands, a restored tidal wetland's seed bank harbors plant species both from a legacy of agricultural land use and historic wetland status. These restored seed banks also hold newly dispersed seeds from nearby wetlands and riparian areas. The newly created environmental conditions associated with tidal reconnection are responsible for stimulating the germination, growth, and establishment of this myriad of plant species stored in the seed banks. The exact environmental conditions and/or signature of conditions that control germination from the seed bank are genetically defined for each species. This is an adaptation of each species to its environment with germination only taking place when the environmental conditions present are likely to provide a suitable opportunity for the species to grow and become established (Finch-Savage and Leubner-Metzger 2006). Environmental conditions such as water, salinity, oxygen, temperature, and light levels are all known to influence seed germination.

The exact requirements and biological mechanisms driving seed germination are complex and vary from one plant species to another. Most plant species require a particular combination of water, oxygen, and temperature changes for germination to take place (Deberry and Perry 2000). Some wetland plant species have also shown germination responses to a change in light and soil-water chemistry (Leck 1989, Kettenring et al. 2006, McCormick and Gibble 2014). All of these environmental shifts can result in external changes in the physical thickness, character, and/or moisture level of the seed coat and internal changes to germination suppressing hormones and abscisic acid production which can result in a break of seed dormancy and subsequent germination (Finch-Savage and Leubner-Metzger 2006). Before the seed coat is broken and exposed to the outer environmental conditions, seedling respiration is assumed to be primarily anaerobic, with a switch to aerobic respiration after emergence (Deberry and Perry 2000). In all wetland environments, germinating seedlings must be adapted to the continued (or fluctuating) anaerobic conditions (low oxygen, low ORP) which persist in saturated soils (Deberry and Perry

2000). In estuarine tidal wetland environments germinating seedlings must also be adapted to fluctuating salinity conditions. Increases in salinity are related to a shift in osmotic potential which reduces the ability of a seed and seedling to uptake water. Generally, this shift in osmotic potential acts to suppress germination and growth, with only a few species adapted to germinate under high salinity conditions (Ungar 1978, Janosek and Floger 2013a). Understanding different wetland plant species' abilities to germinate and grow under varying restored wetland conditions, from saturated soils to high salinities, is essential to explaining the plant community outcomes seen in restored tidal wetlands.

In Young's Bay, Oregon, and throughout the CRE, non-native plant communities dominated by *P. arundinacea* and *J. effusus* have been found abundant in restored oligohaline and freshwater tidal wetland high marsh zones (Borde et al. 2012, Kidd and Yeakley, in prep). These areas are higher in elevation, less frequently flooded, and have lower soil salinity than desired native plant assemblages dominated by *C. lyngbyei* and *S. lacustris*, which are found most frequently occupying the restored low marsh tidal wetland zones (Borde et al. 2012, Kidd and Yeakley, in prep). These same zonation patterns are less apparent in natural reference marshes where *C. lyngbyei* and *S. lacustris* are commonly found co-occupying the high - low marsh zones and *P. arundinacea* and *J. effusus* are much less abundant overall (Borde et al. 2012, Kidd and Yeakley, in prep). It is possible that the non-native wetland species *P. arundinacea* and *J. effusus* are more abundant in the seed bank and/or more successful at germinating in these newly created high marsh conditions than the common native species, giving them a competitive advantage. This would allow them to dilute the native component of the seed bank with their own seed production, further reducing the likelihood of successful native plant community recovery (Suding et al. 2004). This survival strategy could create resilience to ongoing management efforts, suppress native plant community establishment, and in turn facilitate their continued spread throughout the system (Suding et al. 2004). This study uses a combination of *in situ* and greenhouse observations of existing native and non-native plant community distributions (and seed bank compositions, viability) with corresponding wetland soil, tidal flooding, and salinity conditions to determine whether variations in these restored wetland conditions are responsible for promoting or suppressing native and non-native plant community establishment.

The goal of this study is to develop a better understanding of the environmental and ecological mechanisms driving these commonly observed native and non-native plant community zonation patterns, specifically focusing on the importance of restored *in situ*

environmental conditions and seed bank composition. These relationships and mechanisms were evaluated by 1) conducting *in situ* field surveys of dominant plant community species composition and environmental conditions, 2) determining plant community seed bank compositions through direct seed identification, and 3) testing seed bank composition and germination response to a gradient of tidal flooding and salinity regimes. These data provided a foundation for understanding the importance of restored environmental conditions and seed bank composition on dominant native and non-native plant community development and resilience in these wetlands.

ECOLOGY OF FOCAL PLANT COMMUNITIES

C. lyngbyei, and *S. lacustris* are both native perennial species commonly found dominating reference tidal wetland plant communities throughout the PNW and are valued as cultural resources by Native Americans and for providing habitat and forage for local fauna (Morgan and Sytsma 2009). *C. lyngbyei* is a grass-like sedge that grows in dense stands which spread through both rhizome and seed. Regionally, it is found most commonly in high to low coastal salt marsh and brackish marsh conditions (Morgan and Sytsma 2009). *S. lacustris* (L.) Palla is a species classification that encompasses both the hard stem, *S. acutus* var. *acutus* and soft stem bulrush sub species, *S. tabernaemontani* and their hybrids (Morgan and Sytsma 2009)¹. *S. lacustris* is a rush with a growth form that consists of dense narrow erect stems up to 3 meters tall (Morgan and Sytsma 2009). *S. lacustris* spreads through rhizome and seed and is commonly found in low and high tidal fresh and brackish marsh conditions (Morgan and Sytsma 2009). Native tidal wetland species commonly occurring as sub-dominants to *C. lyngbyei* and *S. lacustris* include *Scirpus microcarpus* (*S. microcarpus*), small-fruited bulrush, *Oenanthe sarmetosa* (*O. sarmetosa*), water parsley, *Potentilla anserine* (*P. anserine*), silverweed cinquefoil, (*G. triflorum*), sweet smelling bedstraw, *Typha latifolia* (*T. latifolia*), broadleaf cattail, *Eleocharis palustris* (*E. palustris*), creeping spike rush, (*A. plantago-aquatica*), American water plantain, and *Lilaeopsis occidentalis* (*L. occidentalis*), Western grasswort (Kidd and Yeakley, in prep, Appendix A). Most of these native species have been documented requiring moister (saturated/flooded) soil growing and germinating conditions than the common non-native tidal wetland species with the (Appendix A). The requirement for saturated/flooded

¹ It should be noted that the genus for bulrush species has recently changed from *Scirpus* to *Schoenoplectus*.

conditions aligns with these plant species' common occurrence in the low marsh zone of the restored tidal wetlands.

P. arundinacea and *J. effusus* are both perennial non-native wetland species introduced from Europe, and commonly found in wet pasture habitats. Both species have historically hybridized and/or replaced similar but less aggressive native species' populations (USDA 2002, Zika 2003, Lavergne and Molosky 2006). *P. arundinacea* is a highly invasive grass, known to become dominant in wetland environments, reducing local species richness and habitat quality (Lavergne and Molosky 2006, Jenkins et al. 2008, Hanson et al. 2016). *P. arundinacea* control efforts are very time and energy intensive with few successful eradications documented (Lavergne and Molosky 2006, Jenkins et al. 2008). *J. effusus* is a grass-like rush that grows in dense tussocks, commonly found dominating wetlands, ditches, and slow-moving waterways in agricultural areas with high soil nutrient levels (McCorry and Renou 2003). *J. effusus* is well known as an agricultural weed throughout Europe and parts of North America however very little information is available regarding its biology or invasive control (McCorry and Renou 2003). Both *P. arundinacea* and *J. effusus* spread successfully through rhizome and seed (Zika 2003, Lavergne and Molosky 2006).

In a study of PNW palustrine wetlands (seasonally flooded) researchers found both *P. arundinacea* and *J. effusus* most commonly associated with higher wetland elevations subjected to fluctuating dry and moist soil conditions during the growing season (Kellogg et al. 2003). *P. arundinacea* and *J. effusus* commonly co-dominant upland tidal wetland areas, characterized by low frequency and short durations of tidal flooding (Kidd and Yeakley, in prep). Non-native species which commonly occur as sub-dominants to both *P. arundinacea* and *J. effusus* include *Agrostis stolonifera* (*A. stolonifera*), creeping bent grass, *Holcus spp*, velvet grass species, *Lotus corniculatus* (*L. corniculatus*), birdsfoot trefoil, and *Ranunculus repens* (*R. repens*), creeping buttercup (Kidd and Yeakley, in prep). Most of these non-native species including *P. arundinacea* and *J. effusus* have been documented requiring dryer and less saline soil growing and germinating conditions than the common native tidal wetland species (Appendix A).

STUDY PURPOSE AND HYPOTHESES

The purpose of this study is to 1) investigate the importance of tidal flooding regimes and salinity on native vs. non-native seed bank germination and plant community development in oligohaline tidally restored wetlands, and 2) anticipate the impacts of salinity intrusion and

changes in tidal flooding from sea level rise on existing native and non-native oligohaline wetland seed bank emergence.

Following van der Valk's (1981) adapted Gleasonian theory of wetland plant community succession and the known ecology of the target plant species it was hypothesized that the existing native, *C. lyngbyei* and *S. lacustris*, and non-native, *P. arundinacea* and *J. effusus*, tidal wetland plant community distributions in the field are reflective of both their abundance in the seed bank and their environmental tolerance to the restored tidal wetland flooding and salinity conditions. Specifically, it was expected **H1**) that native and non-native seed densities would be significantly higher in their own respective seed banks. This difference in seed densities among the seed banks indicating self-seeding by these species and the promotion of long-term plant community resilience. Additionally, it was expected that these species would show proportionally greater germination success out of the seed bank when subjected to the tidal flooding and salinity conditions characteristic of these standing plant communities in the field. Specifically, it was expected **H2**) that non-native plant species, *P. arundinacea* and *J. effusus*, would germinate more successfully out of the seed bank (higher density of germinating seeds) when treated with high marsh tidal flooding conditions as compared to the native plant species, *C. lyngbyei* and *S. lacustris*, which would comparatively germinate more successfully out of the seed bank when treated with low marsh tidal flooding conditions. Overall, it was expected that increases in flooding and salinity, such as those expected from sea level rise, would reduce seed bank germination of non-native species, *P. arundinacea* and *J. effusus*, and comparatively increase germination of native species, *C. lyngbyei* and *S. lacustris*.

These hypotheses provide a framework (Figure 1) for evaluating the drivers of plant community development and the applicability of van der Valk's (1981) theory of wetland plant community succession in these systems. van der Valk's (1981) theory predicts that both the seed densities in the seed bank and the salinity and flooding gradient are the primary drivers of plant community development (Figure 1). If, however, seed bank germination was not found to be affected by tidal flooding and salinity conditions and/or if seed bank densities were found to be similar among the plant communities then other mechanisms, such as competition, may be the primary driver of the observed patterns of plant community development (Figure 1).

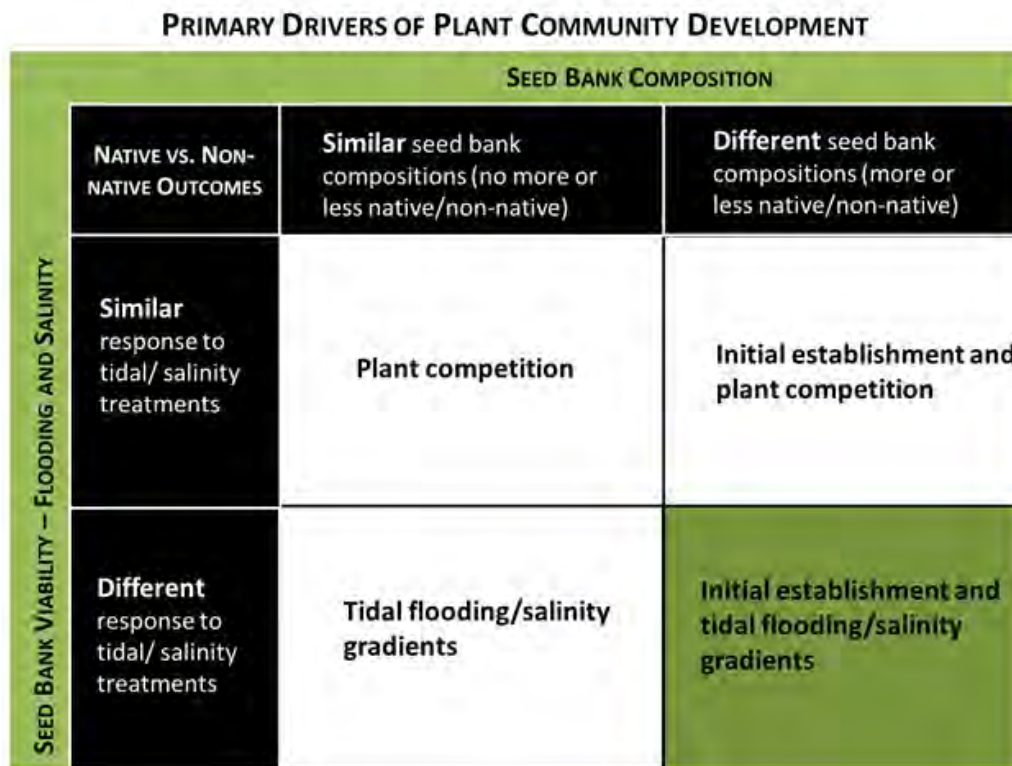


Figure 1: Framework for determining plant community drivers based on seed bank composition and germination outcomes.

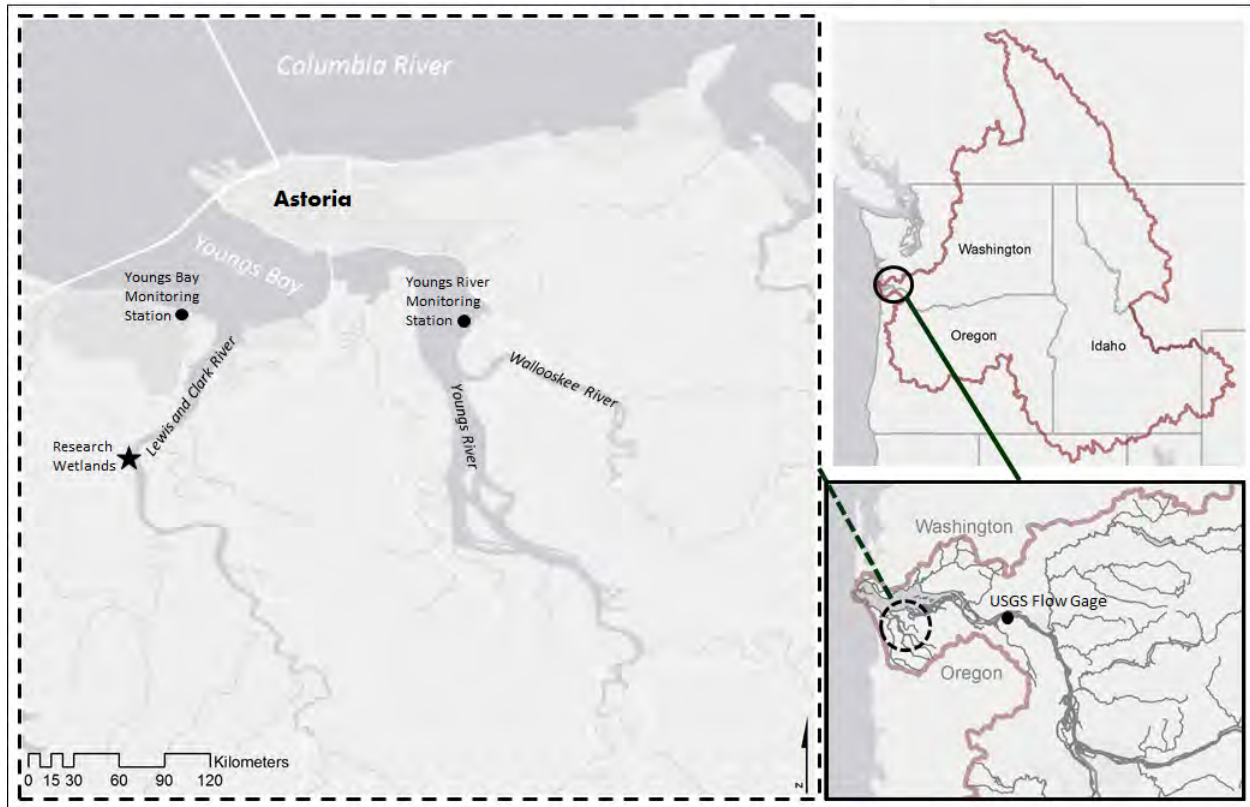
METHODS

OVERVIEW

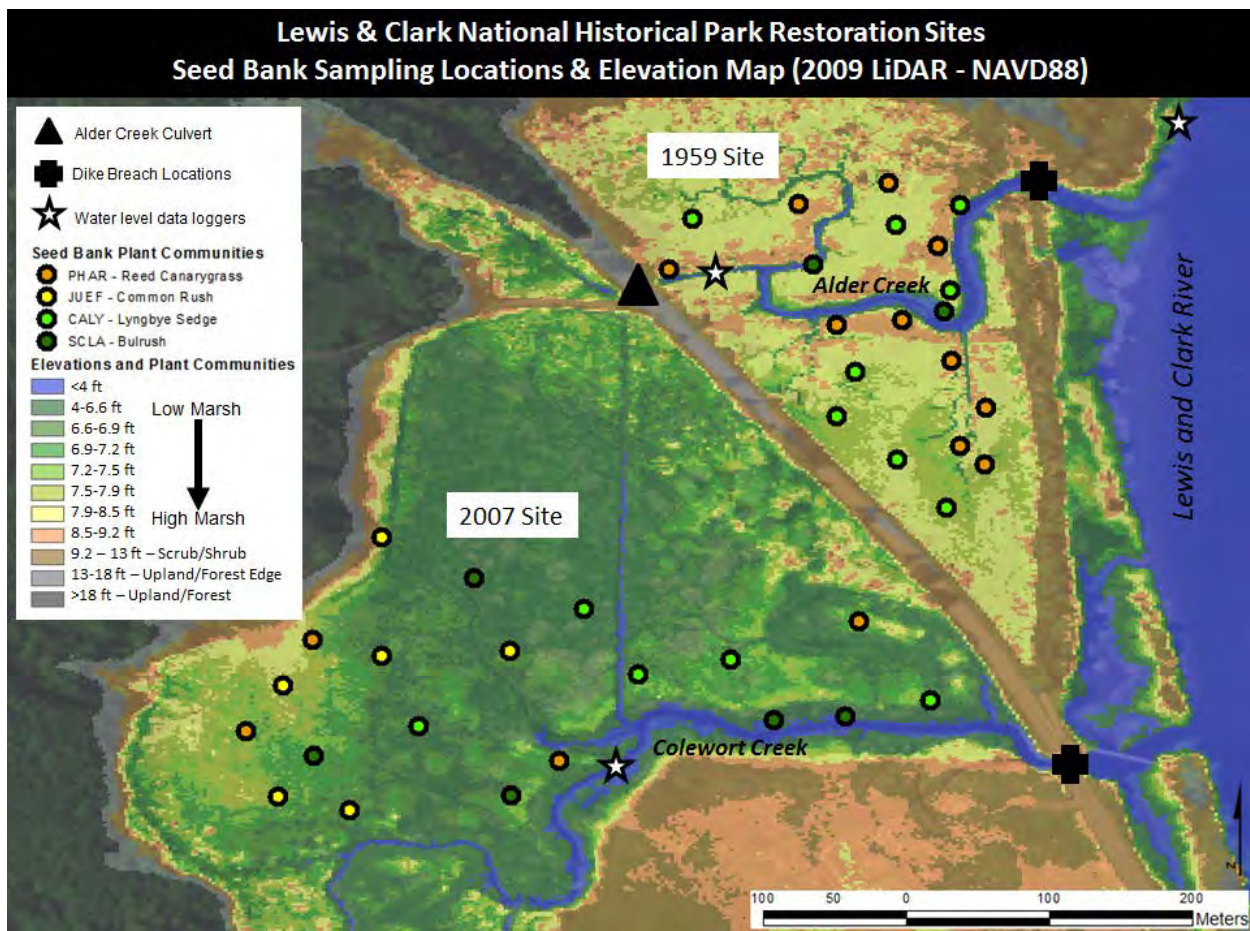
Study Sites -The restoration sites used in this study are located in the Lewis and Clark National Historical Park in the Youngs Bay watershed, near Astoria, Oregon in the lower Columbia River Estuary (Map 1). These restoration sites were diked and drained for agricultural use in the early 1900's. Each site has its own tidally influenced main channel which connects it to the Lewis and Clark River (Map 2). Historically, Alder Creek was blocked with a dike and tide gate, but the tide gate failed (no longer held back tidal exchange) in 1959, and the failed tide gate was replaced with a bridge in 1962. Prior to 2007, Colewort Creek was also blocked from tidal exchange with a dike and functioning tide gate, however in 2007 the tide gate was removed and replaced with a bridge to restore tidal flooding to the site. Currently, the 2007 site's main channel, Colewort creek, maintains freshwater flow during low tide events while the 1959 site's main channel, Alder Creek, drains completely. During high tide events the sites are hydrologically connected through Alder Creek via culverts installed in 1962 which run under the highway and maintenance road that separate the sites (Map 2). In 2011 the north end of the 2007 site was lowered to increase flooding in this portion of the site and further promote tidal

connectivity with the 1959 site. To avoid ambiguity in the results this newly altered portion of the 2007 site was avoided during sampling.

YOUNGS BAY RESEARCH LOCATIONS MAP



Map 1: Youngs Bay watershed map including the location of the seed bank study wetlands “research wetlands” relative to the other monitoring stations located in the watershed and the USGS flow gage (river mile 53).



Map 2: Map of U.S. National Parks Service Lewis and Clark National Historical Park restoration sites surveyed in this study including historical dike breach locations, culvert placement, and the location of the seed bank/vegetation sampling throughout each site and water level data loggers. Map elevation data derived from 2009 LiDAR data. For a watershed perspective see Map 1.

Approach - Previous vegetation and elevation surveys of the restoration sites enabled identification of the dominant plant communities and their general locations throughout the sites (Kidd and Yeakley, in prep). These observations informed the overall study design including the target seed bank plant communities and the character of tidal flooding throughout the sites. Water level and water salinity monitoring began on the site before seed bank sampling in the summer of 2014. During seed bank sampling, in April 2015, plant community species richness and cover (%), elevation, and soil characteristics (salinity, ORP, pH, temperature, bulk density, organic matter, and nutrient content) were collected at each seed bank location to help characterize environmental conditions among the plant communities. Site water level monitoring data and plant community elevation data were then used to develop the tidal flooding and salinity treatments for the seed germination experiment. Flooding treatments represent low, mid, and high marsh elevations observed on the site and salinity treatments represent the fresh to oligohaline (0 ppt-3 ppt) conditions currently characteristic of the site during the winter and

spring seasons and brackish (10 ppt) conditions which represent the upper range of salinity the sites are exposed to in late summer (Appendix B).

FIELD DATA COLLECTION

Seed Bank Collection - A total of 40 composite seed bank samples were collected across the two restoration sites (n=20 each) in April 2015. Sampling locations were randomly stratified throughout the sites' dominant (100% cover) plant communities maintaining a distance of >10 m between sample locations (Map 2). This was done by visually identifying and mapping dominant plant community locations, assigning numbers to these, and then using a random number generator to determine survey locations. This resulted in 10 native: 8, *C. lyngbyei*, and 2, *S. lacustris*, and 10 non-native: 10, *P. arundinacea* plant community sample locations being used in the 1959 site and 10 native: 5, *C. lyngbyei*, and 5, *S. lacustris*, and 10 non-native: 4, *P. arundinacea*, and 6, *J. effusus*, plant community sample locations being used in the 2007 site (Map 2). Overall, this summarized to 20 native: 13, *C. lyngbyei*, and 7, *S. lacustris* and 20 non-native: 14, *P. arundinacea*, and 6, *J. effusus*, plant community sampling locations across both sites (Map 2). Within each dominant plant community sampling location composite samples were collected by taking five seed bank samples (5 diameter x10 cm depth) haphazardly distributed within a 1 m² quadrat and combining them to make one composite sample (total approx. volume of soil sampled per composite was 1,029.6 cm³, ~1,250 grams). This was done to account for the high variability in seed bank composition. Post processing (see **Processing, seed density, and composition**) each composite seed bank sample was approximately 1000 ml in volume, representing 98.15 cm² of soil surface sampled per each 1 m² sample area. The 1000 ml sample was then divided into 10, 100 ml subsamples, each representing 9.815 cm² of surface soil sampled. Seed density per 1 m² was calculated for each 100 ml subsample by multiplying by 1,018.84, this conversion representing the number of seeds anticipated to be present in the entire top layer of a 1 m² (x 10 cm deep) sample of soil. A subsample of each composite was reserved for seed bank composition analysis (seed counts to determine species presence and density), and the remaining soil was used for determining seed bank viability under experimental tidal flooding and salinity conditions. All seed bank collection and preparation was conducted following the methods outlined by Mcfarland and Shafer 2011 and Steigerwalt and Adams 2011.

Plant community - At each seed bank sample location standing plant community percent cover and species richness was recorded within the 1m² quadrat. Due to dense canopy overlap the overall total percent cover within each quadrat could be greater than 100%. For analysis these

data were summarized to relative cover which is the percent cover of each species in a quadrat divided by the total cover of all species in that quadrat. Taxonomic guides to regional flora were consulted (e.g., Hitchcock and Cronquist 1973, Guard 1995, Cooke 1997) to help with species identification. Native, non-native and wetland indicator status determination for each plant species was identified using the online NRCS PLANTS database (<http://plants.usda.gov>). Generally, if plant status was ambiguous or locally contested, its status was listed as unknown for analysis purposes.

Soil survey - Within each quadrat *in situ* surface soil salinity, conductivity, soil redox potential (ORP), pH, and temperature data were also collected using Extech soil probes placed 5 cm below the soil surface (Bledsoe and Shear 2000, Neckles et al. 2002, Davy et al. 2011, Mossman et al. 2012, Gerla et al. 2013). All soil surveys were conducted in saturated soil conditions, timed near peak low tide, and surveyed in order from highest to lowest elevation. Although these soil parameters are dynamic over time depending on the precise environmental conditions present and the duration of tidal flooding the logic in taking these *in situ* samples was to capture the general gradient that exists among the different plant communities. If all samples were collected under similar conditions and at similar intervals of time, they become comparable amongst each other. In addition, one soil core sample (5 cm diameter by 10 cm deep) was collected at each seed bank sample location and analyzed for bulk density, organic matter content (loss on ignition), texture, and salinity following the standard methods described by Kalra and Maynard (1991). Total soil N and P were analyzed using methods described by Bremner (1995) and Taylor (2000).

Topography- Real-time kinematic (RTK) and handheld GPS surveying equipment were used to establish field benchmarks and collect high-resolution positional data of each sample's location (USGS 2012a, 2012b). This position data was also used to compare and correct site wide elevations identified through LiDAR (Light Detection and Ranging) data. These data were used to characterize the plant community elevation ranges and overall topography for each site (Map 2). The LiDAR was collected in 2009 by Watershed Sciences and is publically available through OpenTopography.org. Elevation extraction from the LiDAR data used the ground point cloud processed into a high-resolution TIN (1 ft) using the online data services of

OpenTopography.org². All elevations within this document are reported using the North American Vertical Datum of 1988 (NAVD88).

Tidal flooding and salinity- Tidal flooding characteristics (timing, frequency, amplitude and duration of high/low water levels) were monitored to account for any potential site to site variability within these parameters. Monitoring was done using depth recording data loggers (monitoring at 30 min intervals) installed into the main wetland tidal channels and in the adjacent tributary below the elevation of wetland vegetation establishment (Figure 2, Map 2). Along with these water level data loggers, a conductivity data logger was used to identify major trends in water salinity in the Lewis and Clark River (adjacent to the sites). Salinity data was collected using HOBO (U24) Salt Water Conductivity/Salinity Data Logger measuring salinity (± 0.1 ppt) every 30 mins in the main Lewis and Clark River at the same location as the water level data logger (Map 2). Additionally, point measurements using a YSI probe (± 0.1 ppt) of water salinity, temperature, and depth (using a meter stick) were collected for comparison during data logger deployment and retrieval. Due to a malfunction with the conductivity data logger, salinity data was not recorded post-April 2015 on the sites. To supplement this data gap salinity and water level data collected at two other locations in the watershed were also used (Kidd and Yeakley, in prep) to characterize seasonal conditions. Tidal flooding conditions were monitored from July 2014 through Sept 2015 following USGS protocols (USGS 2012c).

Water depth data were combined with site elevation data and atmospheric pressure data to determine tidal flooding characteristics for each vegetation quadrat surveyed (Farrelly 2012, USGS 2012c). The level of flooding for each vegetation quadrat was related to the elevation of water within the site at the location of monitoring (tidal channel). The assumption being that if the water level (elevation) is above that of the vegetation quadrat elevation, then the quadrat is flooded by the difference in these elevations (elevation of tidal channel water - elevation of vegetation quadrat = depth of water above vegetation quadrat) (Roegner et al. 2009, Farrelly 2012). These data have been calibrated by collecting flooding depth data throughout the sites throughout different tidal periods and comparing these data to those calculated using the above method for that same time period. Tidal flooding data were summarized by averaging the duration and frequency of tidal flooding over a 12-hour cycle during the month of March (2015). Data were summarized for the month of March to represent tidal flooding during the early growing season when most seeds would begin naturally germinating in the tidal wetland. These

² This material is based on [data, processing] services provided by the OpenTopography Facility with support from the National Science Foundation under NSF Award Numbers 1226353 & 1225810.

parameters were calculated for the dominant plant communities surveyed and were used to define the experimental greenhouse tidal flooding treatments.

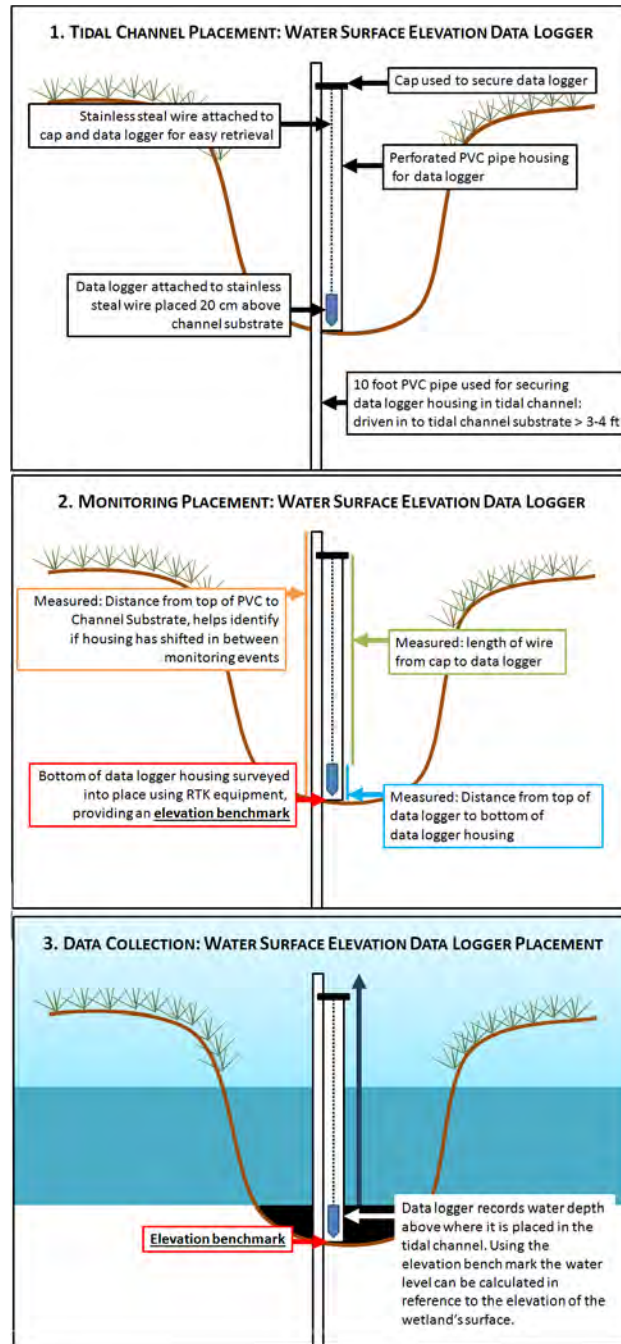


Figure 2: Schematics for the placement and monitoring of water surface elevation data loggers in the wetland tidal channel.

SEED BANK EXPERIMENT

Processing, seed density, and composition - To prepare samples for greenhouse viability analysis and seed identification, composite samples were broken down, rinsed, and sieved to remove organic matter and debris (Photo 1: A). During soil processing, all water used for rinsing

and cleaning organic matter removed was reserved and passed through a 0.063 mm mesh sieve (small enough to retain *Juncus* species). Processing produced approximately 1 liter of soil/seed slurry for each seed bank sample. Post processing this seed bank sample slurry was mixed by hand for one minute, and ten 100 ml subsamples were separated for further analysis. Nine of these subsamples were reserved for the greenhouse, and the tenth sample was reserved for seed identification and density counts.

Seed identification through direct counts – Direct seed counts and species identification was conducted by the Oregon State University Certified Seed Laboratory (Corvallis, Oregon). Before seed counting and identification, the 100 ml subsample was dried and separated into 5 size classes. Seeds were then removed and identified from these dried subsamples for a total of 3.5 hours for each sample. The same intensity and duration of seed identification were maintained across all size classes and among all 40 seed bank samples. Seed counts only included undamaged seeds to avoid overestimating viable seeds within the soil samples. When possible all seeds were identified to species.

Seed bank viability – experiment rationale - Other researchers have examined wetland seed bank responses to differences in flooding and salinity (e.g., Baldwin et al. 1996, 2001, 2012), however, this study is unique in both its field-based approach to identifying patterns of plant community development and tidal flooding and in its simulation of those flooding conditions in the greenhouse. Commonly the influence of tidal flooding and salinity are simulated in an experimental setting through the use of long-term ponded mesocosms of different water depths, however, the biogeochemical effects of using the same standing water levels over time versus daily fluctuating water levels over time are significant. Keeping stagnant ponded water over time promotes much lower average soil ORP conditions than fluctuating water levels, as draining the soil promotes soil oxygenation and shifts the soil biogeochemistry. These differences in soil conditions are important when trying to develop a better understanding of seed germination and species distributions within a dynamic tidally flooded system.

Seed bank viability –experimental setup- In a controlled greenhouse environment, seed bank samples were subjected to a gradient of 3 tidal flooding regimes (low marsh: twice a day – high marsh: once a day) and 3 salinity (fresh, oligohaline, brackish) - for a total of 360 treated subsamples (Figure 3). Tidal flooding in Youngs Bay is semidiurnal (different high and low tide levels twice each day) which is challenging to replicate in a greenhouse setting. Given this, average frequencies and durations of flooding were calculated for each sampling elevation (see ***Tidal flooding and salinity methods above and calculations in Appendix B***) and these average

times and durations characterize the 24 hr tidal cycles used in the greenhouse to replicate field conditions. These flooding treatments included low elevation tidal wetland areas, which in the greenhouse were flooded twice a day for 6 hours each tide, the first tide occurring at 1-7 pm and the second at 1-7 am, mid-elevation tidal wetland areas, which were flooded twice a day for 3 hours each tide, the first tide occurring at 1-4 pm and 1-4 am, and high elevation tidal wetland areas which were flooded once a day for 1 hour from 1-2pm. Filling and draining occurred rapidly, taking less than 2 mins. These treatments were developed based on the dominant plant community assemblages and their assessed tidal flooding frequency (Figure 3, Appendix B). In addition to the three tidal flooding treatments, seed bank samples were also subjected to three different salinity treatments including freshwater conditions (<1 ppt), oligohaline (3 ppt), and brackish (10 ppt). These salinity treatments were also determined based on locally observed salinity conditions, with fresh and oligohaline treatments representing commonly existing salinity conditions and brackish representing more extreme observed salinity conditions (Appendix B). Given brackish conditions occur but are currently uncommon on the sites, this treatment was used as a sea level rise scenario, as its occurrence may become more frequent in the future (Glick et al. 2007, Jay et al. 2011, Tebaldi et al. 2012, Appendix B). Treatment water salinity conditions were adjusted using Instant Ocean (Aquarium Systems, Inc., Mentor, OH, U.S.A.) sea salt and monitored bi-weekly using a Milwaukee M 887 Seawater Refractometer (± 1 ppt).

A total of 40 independent seed bank samples were tested per flooding x salinity treatment (Figure 3, n=360). All seed bank samples were randomly stratified through 3 treatment tub/reservoir systems which were placed at random locations along the greenhouse bench to reduce the influence of variable greenhouse conditions (Photo 1: B, F). Seed bank treatment pots were all filled with 3 cm of sand to promote draining between flooding, with approx. 2 cm (100 ml) of composite seed bank sample layered on top (Steigerwalt and Adams 2011). To keep each potted seed bank sample independent from one another, each pot was lined with landscaping fabric which allowed tidal exchange but prevented cross-contamination of the seed bank samples during flooding (Photo 1: C). During flooding, treatments maintained 10 cm of water above the seed bank samples and during low tide treatments water completely drained from the treatment tubs. Drains were used to prevent overtopping and cross contamination during high tide treatments. Control pots filled with only sand were randomly placed in each treatment tub to identify whether any greenhouse weeds or cross-contamination occurred during the study. No seedlings were found growing out of these control tubs at any point during the study. Pumps

were used to regulate treatments and treatment tubs were connected to individual water reservoirs (Photo 1: D). The pumps were set on a timer calibrated to provide a consistent level, duration, and timing of flooding for each subsample and treatment. Pumps were under 24 hr surveillance using motion sensor and video camera technology to prevent any disruptions in flooding regimes. During the 5 month study period (June-November 2016), primarily natural light was used. Natural light was supplemented with greenhouse lights starting in Oct-Nov to maintain light duration (12 hr/day) and to promote seedling maturation for successful species identification. Greenhouse temperature was regulated, preventing extreme high or low-temperature conditions, with the intention to mirror the temperature range found in the study wetlands during the growing season (60-85°F).

| GREENHOUSE STUDY | | | | |
|-------------------------|--|---------------------------------|---------------------------------|---------------------------------|
| Salinity Gradient | | | | |
| | Treatments | Fresh <1 ppt | Oligohaline 3 ppt | Brackish 10 ppt |
| Tidal Flooding Gradient | High Marsh (Flooded 1 hr x 1 day) | Native n=20, Non-native n=20 | Native n=20, Non-native n=20 | Native n=20, Non-native n=20 |
| | Mid-Marsh (Flooded 3 hr x 2 day) | Native n=20, Non-native n=20 | Native n=20, Non-native n=20 | Native n=20, Non-native n=20 |
| | Low Marsh (Flooded 6 hr x 2 day) | Native n=20, Non-native n=20 | Native n=20, Non-native n=20 | Native n=20, Non-native n=20 |

Figure 3: Figure outlining study design including the gradient of inundation and salinity treatments tested on seed bank samples collected from National Park Service sites 2007, and 1959 year of tidal reconnection (Map 1). Each treatment had 20 seed bank subsamples from native and non-native dominant plant communities. Inundation treatments were based on field observations of in situ plant community tidal flooding and salinity data (Appendix C).

METHODS: SEED BANK PROCESSING & GREENHOUSE SETUP

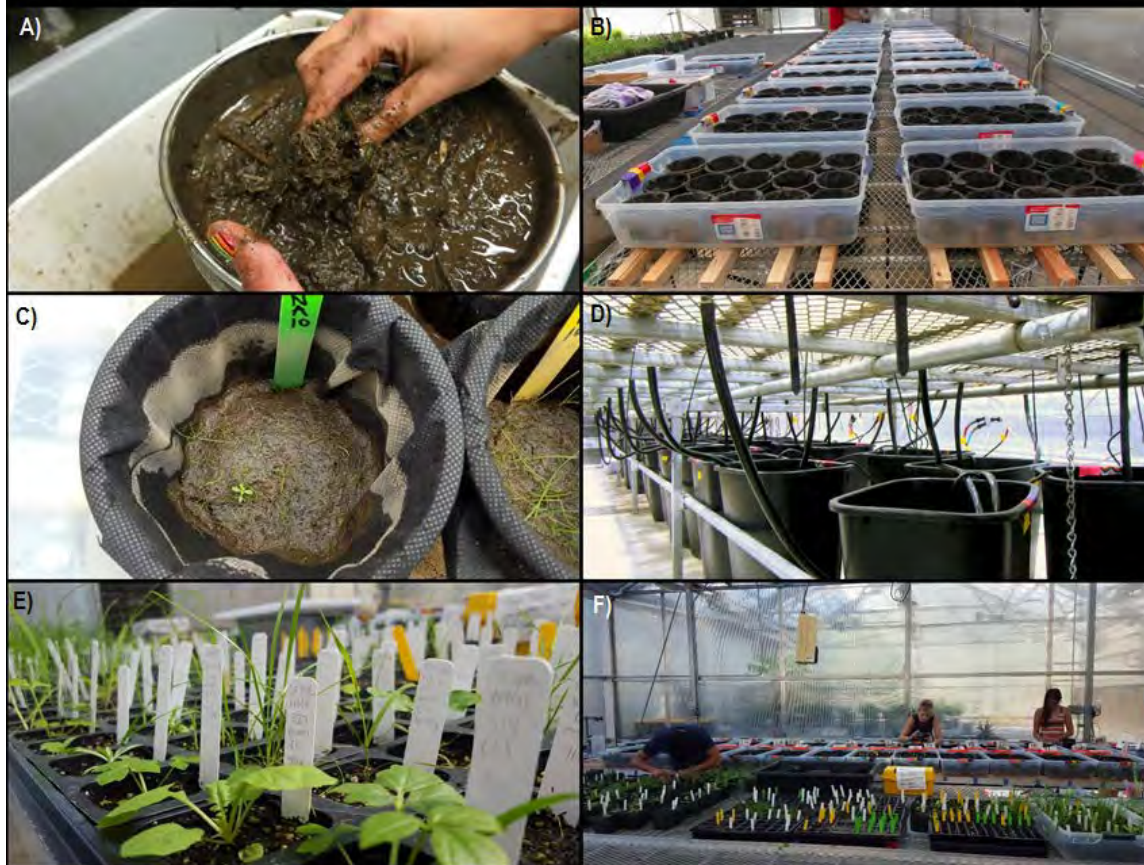


Photo 1: A) processing seed bank samples by removing large pieces of organic matter, B) greenhouse set up of treatment tanks with samples inside, C) treatment subsample with germinating seedlings, D) reservoirs and pumps used in the greenhouse to fill the treatment tubs, E) transplanted seedlings being tracked for positive species identification, F) greenhouse monitoring.

Greenhouse data collection - Data collection consisted of recording the number and species of new seedlings on a weekly and then bi-monthly basis over a five month period for each sample in each treatment. Weekly monitoring was replaced by bi-monthly monitoring after ten weeks reflecting a decline in overall germination during that time. Counted and identified seedlings were removed from the sample to prevent competition from impeding further seedling emergence. Representative seedlings were removed from samples and grown separately until a positive species ID could be confirmed (Steigerwalt and Adams 2011, Photo 1: E, F). If time and conditions allowed, representative species were grown to flowering and then preserved as an herbarium specimen for later reference.

DATA ANALYSIS

Field and Seed Count Data - Field data including plant community (relative abundance and species richness), elevations, flooding, soil conditions, and direct seed count data were summarized and compared among and within the restoration sites and across the dominant plant community sampling locations (native vs. non-native, *P. arundinacea*, *J. effusus*, *C. lyngbyei*, *S. lacustris*). Seed count data were summarized to relative frequency (%) by dividing the number of seeds identified for each species in a sample by the total number of seeds identified in the sample overall. Transformation to normality was not possible for these data and Kruskal-Wallis one-way non-parametric analysis of variance by ranks and pair-wise Man-Whitney U matched pairs signed ranks test were used to evaluate for significant differences (Wilcoxon 1945, Mann and Whitney 1947, Kruskal and Wallis 1952). Significant levels were adjusted for multiple comparisons using a Bonferroni correction (Mossman et al. 2012).

Germination - To evaluate differences in germination across treatments and among species seedling germination count data were summarized to relative frequency (%) of occurrence for each species across all treatments (n=9) for each seed bank sample. This was done by dividing the number of germinated seedlings for each species in each treatment pot (subsample) by the total number of germinated seedlings for that species identified across all treatment pots (9 subsamples) for that seed bank sample (total of 100% when adding together all 9 subsamples). Transformation to normality was not possible for these data and non-parametric analysis was conducted to evaluate significant differences. The seed bank germination species richness and relative germination frequency data were compared among the flooding treatments and salinity treatments using the Kruskal-Wallis one-way non-parametric analysis of variance by ranks (Kruskal and Wallis 1952). When significant differences were detected, the non-parametric Dunn's multiple comparison post hoc tests with a false discovery rate correction was used to determine significant differences between treatments combinations and dominant species (Dunn 1961, Pike 2010).

Multivariate analysis - To explore trends in standing plant community, seed bank, and germination species composition relative species cover and relative frequency data were used to calculate the Bray-Curtis Index (BCI). This was then used to evaluate the composition similarity among the sites, dominant plant community seed bank types, and treatments. Analysis of similarities (ANOSIM) was used to evaluate significant differences among sites and seed banks and Permutational Multivariate Analysis of Variance (PERMANOVA) were used to determine

significant differences and interaction effects among the treatment combinations (Anderson 2001, Mossman et al. 2012). The BCI data matrix of standing, seed bank, and germination species data was used to create non-metric multidimensional scaling (NMDS) ordination plots of the sites, seed banks, and treatments with distances (on the NMDS plot) representing similarity/dissimilarity in species composition. Linear vectors of field and treatment conditions were fitted to the NMDS plots to identify whether these factors were significantly ($p < 0.05$) associated with similarity/dissimilarity among the samples (NMDS space). Proportional bubble plots were used with the NMDS vector plots to explore trends in the plant community, seed count, and germination characteristics such as native and non-native species abundance/frequency. All data analysis was conducted using R 2.15.3 statistical software and associated packages (R 2015).

RESULTS

FIELD OBSERVATIONS: STANDING PLANT COMMUNITY AND SEED BANK

Standing Plant Community and Seed Bank Data by Native and Non-native Seed Bank Sample Locations - Native and non-native plant community sampling occurred in areas that had greater than 75% standing cover of native (*C. lyngbyei* and *S. lacustris*, $n=20$) or non-native (*P. arundinacea* and *J. effusus*, $n=20$) plant species. Within the overall standing native plant community a total of 15 different species were observed including 11 native and 3 non-native species, and within the standing non-native plant community, there were also a total of 15 species observed, 10 native and 4 non-native (Table 1). In comparison, the native plant community seed bank samples (taken from these same field survey locations) were composed of a total of 26 species, 13 native and 10 non-native, and the non-native plant community seed bank samples were composed of a total of 29 species, 10 native and 14 non-native species (Table 2). Overall there was an overall lap of 7 species (1 non-native and 6 natives species) found in both the native seed bank and in the standing plant community and 8 species (4 non-native, 4 native) found both in the non-native seed bank and standing plant community (Appendix C).

By study design, significantly more dominant native species cover (*C. lyngbyei* and *S. lacustris*) was observed in the standing native plant communities and significantly more dominant non-native plant cover (*P. arundinacea* and *J. effusus*) was observed among the standing non-native plant communities sampled (Table 1 and 2). The dominant non-native species *P. arundinacea* was also observed growing at low levels ($0.6 \pm 1.5\%$ relative cover on

average) within the overall native plant community and the dominant native species *C. lyngbyei* was observed growing within the non-native plant community ($2.5 \pm 7.9\%$ relative cover on average) (Table 1). Other species that were found growing within the dominant native and non-native plant communities at mean levels greater than 1% relative cover included 5 native species (*E. palustris*, *O. sarmetosa*, and *P. anserine*), 1 non-native species (*L. corniculatus*), and 1 ambiguous potentially native/non-native hybrid cattail species (*Typha sp*) (Table 1). Overall, there was no significant difference in the total mean standing species richness between the native, 3.5 ± 1.4 , and non-native, 3.3 ± 1.3 , plant communities. Shannon diversity indices between the native and non-native plant communities were also not significantly different (Table 3). The native plant community, however, did have significantly more native species richness, 3 ± 1.2 , and significantly less non-native species richness, 0.4 ± 0.6 , on average compared to the standing non-native plant community which hosted an average of 1.6 ± 1.1 native and 1.4 ± 0.9 non-native species per survey plot (Table 3).

In comparison, the seeds directly identified from the seed banks showed significant differences in native and non-native seed relative abundance (%) and native seed richness across the native and non-native seed banks (Table 2 and 3, Appendix C). On average there were significantly more native seeds within the native seed banks, with a mean relative abundance of $47 \pm 33\%$ native seeds compared to only $22 \pm 29\%$ native seeds in the non-native seed bank samples. Although abundant in both the native and non-native seed bank samples, there were significantly more non-native seeds in the non-native seed bank samples with a relative abundance of $76 \pm 29\%$ non-native seeds in the non-native seed bank and $51 \pm 34\%$ non-native seeds in the native seed bank samples. *J. effusus* was the most abundant species found in both the native seed bank followed by *S. lacustris*, *O. sarmetosa*, *C. lyngbyei*, and *P. arundinacea*. *P. arundinacea* was the most abundant species found in the non-native seed bank followed by *J. effusus*, *S. microcarpus*, *S. lacustris*, and *O. sarmetosa* in the non-native seed bank (Table 2). Additionally, the overall native seed bank samples had an average native species richness of 2.7 ± 1.6 compared to the non-native seed bank which had a native species richness of 1.8 ± 1.3 (Table 3). No significant difference in the total and non-native species richness or Shannon Diversity Indices were observed between the native and non-native seed banks (Table 3).

Table 1: Native and non-native plant community field observations: standing species composition. Bonferroni corrected significance level $p < 0.004$, and marginally significant $p < 0.05$ differences highlighted.

| Native and Non-native Plant Community Field Observations: Standing Species Composition – Mean Relative Cover (%; n = 1 m ²) | | | | | | | | | | | |
|---|-------------------------|--------|------|---------|-------------------------|------|------|-----------------------------|------|------|---------------|
| Latin | Common | Status | WIS | Code | Native Seed Bank (n=20) | | | Non-native Seed Bank (n=20) | | | Man-Whitney U |
| | | | | | Mean | SD | SE | Mean | SD | SE | |
| <i>Agrostis sp.</i> | Bentgrass | NN | NI | Ag sp | | | | 1.3 | 5.6 | 1.3 | NA |
| <i>Alisma plantago-aquatica</i> | American water plantain | NAT | OBL | Al pa a | 0.3 | 1.1 | 0.3 | | | | NA |
| <i>Athyrium filix-femina</i> | Ladyfern | NAT | FAC | At fi | | | | 0.1 | 0.2 | 0.1 | NA |
| <i>Carex lyngbyei</i> | Lyngbyes sedge | NAT | OBL | Ca ly | 66.5 | 47.3 | 10.6 | 2.5 | 7.9 | 1.8 | 0.000 |
| <i>Callitriche stagnalis</i> | Starwort | NN | OBL | Ca st | 5.6 | 22.3 | 5.0 | | | | NA |
| <i>Eleocharis palustris</i> | Spike rush | NAT | OBL | El pa | 8.3 | 18.7 | 4.2 | 1.8 | 5.9 | 1.3 | 0.126 |
| <i>Galium triflorum</i> | Bedstraw | NAT | FACU | Ga tr | 0.4 | 1.1 | 0.3 | 0.1 | 0.3 | 0.1 | 0.621 |
| <i>Juncus effusus subsp. effusus</i> | Common rush | NN | FACW | Ju ef | | | | 31.8 | 46.3 | 10.4 | NA |
| <i>Lathyrus nevadensis</i> | Purple peavine | NAT | NI | La ne | 0.3 | 1.1 | 0.3 | 0.1 | 0.2 | 0.1 | 0.554 |
| <i>Lilaeopsis occidentalis</i> | Western grasswort | NAT | OBL | Li oc | 0.6 | 2.2 | 0.5 | 0.1 | 0.2 | 0.1 | 0.299 |
| <i>Lotus corniculatus</i> | Birdsfoot trefoil | NN | FAC | Lo co | 0.1 | 0.2 | 0.1 | 3.3 | 11.3 | 2.5 | 0.144 |
| <i>Oenanthe sarmentosa</i> | Water parsley | NAT | OBL | Oe sa | 12.1 | 14.2 | 3.2 | 3.7 | 8.2 | 1.8 | 0.033 |
| <i>Phalaris arundinacea</i> | Reed canarygrass | NN | FACW | Ph ar | 0.6 | 1.5 | 0.3 | 72.9 | 43.8 | 9.8 | 0.000 |
| <i>Potentilla anserina</i> | Silverweed cinquefoil | NAT | OBL | Po an | 1.9 | 3.7 | 0.8 | 5.7 | 13.4 | 3.0 | 0.899 |
| <i>Scirpus lacustris</i> | Bulrush | NAT | OBL | Sc la | 35.0 | 48.9 | 10.9 | | | | NA |
| <i>Scirpus microcarpus</i> | Panicled bulrush | NAT | OBL | Sc mi | 0.8 | 3.4 | 0.8 | 0.3 | 1.1 | 0.3 | 1.000 |
| <i>Symphotrichum subspicatum</i> | Douglas aster | NAT | FACW | Sy su | 0.1 | 0.2 | 0.1 | | | | NA |
| <i>Typha latifolia x angustifolia</i> | Cattail | Hybrid | OBL | Ty sp | 0.6 | 1.5 | 0.3 | 1.1 | 2.0 | 0.5 | 0.426 |
| <i>Vicia nigricans subsp. gigantea</i> | Giant vetch | NAT | FAC | Vi gi | | | | 2.1 | 5.2 | 1.2 | NA |

Table 2: Native and non-native seed bank composition: frequency of occurrence of species identified through direct seed counts ($n=100$ ml/m² soil each), only species with a relative frequency $\geq 1\%$ shown. Significant, Bonferroni corrected significance level $p < 0.002$, and marginally significant $p < 0.05$ differences highlighted.

| Native and Non-native Seed Bank Composition: Seed Direct Count – Mean Relative Frequency (%) ($n=100$ ml soil each, only species with $\geq 0.1\%$) | | | | | | | | | | | |
|---|-------------------------|--------|---------|---------|-------------------------|------|-----|-----------------------------|------|-----|---------------|
| Latin | Common | Status | WIS | Code | Native Seed Bank (n=20) | | | Non-native Seed Bank (n=20) | | | Man-Whitney U |
| | | | | | Mean | SD | SE | Mean | SD | SE | |
| <i>Agrostis</i> spp. | Bentgrass | N/A | FAC | Ag sp | 0.1 | 0.5 | 0.1 | 1.7 | 7.5 | 0.8 | 0.081 |
| <i>Alisma plantago-aquatica</i> L. | American Water Plantain | NN | OBL | Al pl a | 0.1 | 0.4 | 0.0 | 0.2 | 1.0 | 0.1 | 0.163 |
| <i>Alnus rubra</i> | Red alder | NAT | FAC | Al ru | 0.2 | 0.8 | 0.1 | 0.1 | 0.5 | 0.1 | N/A |
| <i>Alopecurus</i> | Foxtail species | NN | FAC/OBL | Al sp | 1.1 | 4.6 | 0.5 | 1.2 | 3.7 | 0.4 | 0.163 |
| <i>Carex Lyngbyei</i> | Sedge | NAT | OBL | Ca ly | 12.5 | 20.6 | 2.1 | 0.3 | 1.1 | 0.1 | 0.008 |
| <i>Carex obtusa</i> | Slough Sedge | NAT | OBL | Ca ob | 2.9 | 9.1 | 0.9 | | | | N/A |
| <i>Cirsium</i> spp | Thistle | NN | FACU | Ci spp | 1.6 | 5.3 | 0.5 | 0.1 | 0.3 | 0.0 | 0.275 |
| <i>Conium maculatum</i> | Poison hemlock | NN | FAC | Co ma | | | | 0.2 | 1.0 | 0.1 | N/A |
| <i>Eleocharis obtusa</i> | Blunt spike rush | NAT | OBL | El ob | 0.2 | 1.0 | 0.1 | | | | N/A |
| <i>Eleocharis palustris</i> | Spikerush | NAT | OBL | El pa | 4.4 | 12.1 | 1.2 | 0.1 | 0.2 | 0.0 | 0.138 |
| <i>Epilobium ciliatum</i> | Willow herb | NAT | FACW | Ep ci | 0.4 | 1.9 | 0.2 | 0.1 | 0.6 | 0.1 | 1 |
| <i>Eragrostis</i> spp. | Lovegrass | N/A | N/A | Er spp | 0.3 | 1.4 | 0.1 | | | | N/A |
| <i>Fimbristylis</i> spp. | Rush | N/A | N/A | Fi spp | | | | 1.4 | 6.1 | 0.6 | N/A |
| <i>Glyceria</i> spp. | Mannagrass | NAT | OBL | Gl spp | 0.3 | 0.8 | 0.1 | 0.3 | 1.0 | 0.1 | 0.689 |
| <i>Juncus bufonius</i> | Toad rush | NAT | FACW | Ju bu | 0.6 | 2.7 | 0.3 | 0.8 | 2.0 | 0.2 | 0.196 |
| <i>Juncus effusus</i> | Soft rush | NN | FACW | Ju ef | 32.7 | 37.8 | 3.8 | 37.0 | 39.6 | 4.0 | 0.714 |
| <i>Lotus corniculatus</i> | Birdsfoot trefoil | NN | FAC | Lo co | 0.3 | 0.9 | 0.1 | 0.7 | 1.6 | 0.2 | 0.653 |
| <i>Oenanthe Sarmetosa</i> | Water parsley | NAT | OBL | Oe sa | 12.8 | 23.2 | 2.3 | 2.1 | 7.0 | 0.7 | 0.526 |
| <i>Phalaris arundinacea</i> | Reedcanary grass | NAT | FACW | Ph ar | 7.9 | 11.2 | 1.1 | 41.9 | 39.3 | 3.9 | 0.013 |
| <i>Plantago</i> sp. | Plantain | NN | FAC | Pl sp | | | | 0.1 | 0.2 | 0.0 | N/A |
| <i>Poa pratensis</i> L. | Kentucky bluegrass | NN | FAC | Po pr | 0.1 | 0.6 | 0.1 | | | | N/A |
| <i>Poa trivialis</i> | Rough bluegrass | NN | FAC | Po tr | | | | 0.1 | 0.2 | 0.0 | N/A |
| <i>Poa annua</i> | Annual bluegrass | NN | FAC | Poa an | 0.4 | 1.7 | 0.2 | | | | 0.573 |
| <i>Ranunculus repens</i> | Creeping buttercup | NN | FAC | Ra re | 0.3 | 1.2 | 0.1 | 0.3 | 0.9 | 0.1 | 0.554 |
| <i>Rubus armeniacus (fruticosus)</i> | Himalayan blackberry | NN | FACU | Ru ar | | | | 0.4 | 1.1 | 0.1 | N/A |
| <i>Rumex</i> sp. | Dock | N/A | N/A | Ru sp | | | | 0.1 | 0.3 | 0.0 | N/A |
| <i>Scirpus lacustris</i> | Bulrush | NAT | OBL | Sc la | 15.6 | 22.0 | 2.2 | 2.5 | 6.6 | 0.7 | 0.006 |
| <i>Scirpus microcarpus</i> | Panicled | NAT | OBL | Sc mi | 3.0 | 11.2 | 1.1 | 7.3 | 17.0 | 1.7 | 0.256 |
| <i>Trifolium repens</i> | Clover sp. | NN | FAC | Tr re | 0.1 | 0.3 | 0.0 | 0.4 | 0.8 | 0.1 | 0.588 |
| <i>Trifolium</i> sp | Clover | NN | FAC | Tr sp | | | | 0.3 | 1.4 | 0.1 | N/A |
| <i>Unknown seed</i> | Unknown seed | N/A | N/A | UNK1 | 1.7 | 5.8 | 0.6 | 0.4 | 1.5 | 0.1 | 0.621 |
| <i>Vaccinium</i> sp. | Blueberry | NAT | N/A | Va sp | 0.3 | 1.0 | 0.1 | 0.2 | 1.0 | 0.1 | 0.554 |

Table 3: Field and seed bank composition summary by native and non-native dominant plant community status – standing vegetation and seeds identified from the soil, significant, Bonferroni corrected significance level $p < 0.004$, and marginally significant $p < 0.05$ differences highlighted.

| Field and Seed Bank Composition Summary by Native and Non-native Dominant Plant Community Status – Standing Vegetation and Seeds Identified out of the Soil | | | | | | | | | | |
|--|-------------------------------|------------------|------|------|-----|----------------------|------|------|-----|---------------|
| Parameters | | Native Seed Bank | | | | Non-native Seed Bank | | | | Man-Whitney U |
| | | Count | Mean | SD | SE | Count | Mean | SD | SE | |
| Standing Vegetation | Native relative cover | 20 | 96.6 | 9.7 | 2.2 | 20 | 10.7 | 12.4 | 1.2 | 0.000 |
| | Non-native relative cover | 20 | 3.0 | 9.7 | 2.2 | 20 | 87.9 | 14.5 | 1.5 | 0.000 |
| | Total species richness | 20 | 3.5 | 1.4 | 0.3 | 20 | 3.3 | 1.3 | 0.3 | 0.607 |
| | Native species richness | 20 | 3.0 | 1.2 | 0.3 | 20 | 1.6 | 1.1 | 0.3 | 0.002 |
| | Non-native species richness | 20 | 0.4 | 0.6 | 0.1 | 20 | 1.4 | 0.9 | 0.2 | 0.000 |
| | Shannon Diversity Index | 20 | 0.6 | 0.3 | 0.1 | 20 | 0.5 | 0.4 | 0.1 | 0.218 |
| Seed Composition | Native relative abundance | 20 | 53.3 | 33.2 | 3.3 | 20 | 15.4 | 19.5 | 2.0 | 0.000 |
| | Non-native relative abundance | 20 | 44.0 | 33.9 | 3.4 | 20 | 83.1 | 19.9 | 2.0 | 0.000 |
| | Total species richness | 20 | 4.9 | 2.2 | 0.5 | 20 | 4.3 | 2.3 | 0.5 | 0.310 |
| | Native species richness | 20 | 2.7 | 1.6 | 0.4 | 20 | 1.8 | 1.4 | 0.3 | 0.026 |
| | Non-native species richness | 20 | 2.0 | 1.1 | 0.2 | 20 | 2.3 | 1.3 | 0.3 | 0.564 |
| | Shannon Diversity Index | 20 | 1.0 | 0.4 | 0.1 | 20 | 0.6 | 0.4 | 0.1 | 0.075 |

Environmental Conditions by Native and Non-native Seed Bank Sample Locations -

Environmental conditions across the native and non-native standing plant community seed bank sampling locations varied significantly. The non-native plant community was on average significantly higher in elevation than the native plant community, averaging about 0.4 meters (1.1 ft) higher in elevation, with a mean elevation of 2.5 ± 0.2 m (8.1 ± 0.5 ft) compared to the native plant community which had a mean elevation of 2.1 ± 0.4 (7.0 ± 1.3 ft) (Table 4). Given this difference in elevation the native plant community experiences significantly greater tidal flooding frequency (on average flooded 24% more frequently) and duration (on average flooded for 1.5 hours longer per high tide) than the non-native plant community (Table 4, Map 2, Appendix C). This difference in tidal flooding frequency and duration was further highlighted by the difference in soil ORP conditions observed between the plant communities, with the native plant community having significantly lower ORP conditions averaging at 125.9 ± 81.7 mV compared to the non-native plant community average of 234.0 ± 45.3 mV (Table 4). Soil Phosphorus (P, Bray II) levels were also found to be marginally higher (p -value = 0.04) in the native plant community, 56.9 ± 16.7 mg/kg as compared to the non-native average of 45.6 ± 12.6 mg/kg. No significant differences were identified between the overall average native and non-native plant community soil conductivity, salinity, bulk density, organic matter, available nitrogen moisture, pH, texture (% sand, silt, clay), or total exchange capacity (Table 4).

Table 4: Native and non-native plant community field observations: environmental conditions, significant, Bonferroni corrected significance level $p < 0.003$, and marginally significant $p < 0.05$ differences highlighted.

| Native and Non-native Plant Community Field Observations: Environmental Conditions | | | | | | | | | |
|--|------------------|-------|-------|------|----------------------|-------|-------|------|---------------|
| Parameter | Native Seed Bank | | | | Non-native Seed Bank | | | | Man-Whitney U |
| | Count | Mean | SD | SE | Count | Mean | SD | SE | |
| Elevation (ft) | 20 | 7.0 | 1.3 | 0.3 | 20 | 8.1 | 0.5 | 0.1 | 0.002 |
| Elevation (m) | 20 | 2.1 | 0.4 | 0.1 | 20 | 2.5 | 0.2 | 0.0 | |
| Abundance flooded twice a day (% , March 2015) | 20 | 71.7 | 27.3 | 4.3 | 20 | 47.3 | 25.5 | 4.0 | 0.006 |
| Duration of each flooding event (hr, March 2015) | 20 | 2.7 | 1.9 | 0.3 | 20 | 1.2 | 0.8 | 0.1 | 0.010 |
| Bulk density (g/cm ²) | 16 | 0.4 | 0.1 | 0.0 | 12 | 0.4 | 0.1 | 0.0 | 0.693 |
| Organic Matter (%) | 18 | 15.1 | 8.5 | 2.0 | 20 | 15.3 | 7.0 | 1.6 | 0.784 |
| Soil moisture (%) | 18 | 65.4 | 8.4 | 2.0 | 20 | 60.2 | 8.9 | 2.0 | 0.236 |
| Field Conductivity (µS/cm) | 20 | 806.6 | 237.8 | 53.2 | 20 | 795.6 | 341.7 | 76.4 | 0.935 |
| Field ORP (mV) | 20 | 125.9 | 81.7 | 18.3 | 20 | 234.0 | 45.3 | 10.1 | 0.000 |
| Field pH | 20 | 5.9 | 1.2 | 0.3 | 20 | 6.4 | 0.5 | 0.1 | 0.401 |
| Field Salinity (ppm) | 20 | 384.8 | 117.7 | 26.3 | 20 | 390.0 | 172.5 | 38.6 | 0.735 |
| Field Temp (°C) | 20 | 12.6 | 1.3 | 0.3 | 20 | 12.3 | 1.4 | 0.3 | 0.267 |
| Clay (%) | 15 | 4.4 | 3.1 | 0.8 | 17 | 3.1 | 1.9 | 0.4 | 0.165 |
| Sand (%) | 15 | 67.5 | 12.8 | 3.3 | 17 | 73.3 | 8.3 | 2.0 | 0.132 |
| Silt (%) | 15 | 28.2 | 10.5 | 2.7 | 17 | 23.6 | 7.5 | 1.8 | 0.246 |
| Bray II P (mg/kg) | 18 | 56.9 | 16.7 | 3.9 | 20 | 45.6 | 12.6 | 2.8 | 0.038 |
| Estimated Nitrogen Release (N/acre) | 18 | 125.3 | 11.2 | 2.7 | 20 | 126.7 | 9.3 | 2.1 | 0.768 |
| Total Exchange Capacity (meq/100 g) | 18 | 29.7 | 9.7 | 2.3 | 20 | 26.7 | 7.8 | 1.7 | 0.497 |

FIELD OBSERVATIONS: FIELD AND SEED COUNT DATA MULTIVARIATE ANALYSIS

Standing Plant Community - The Bray-Curtis similarity NMDS of the standing plant community composition similarity shows clear grouping of the dominant plant communities sampled (Figure 3). The *P. arundinacea*, *J. effusus*, *C. lyngbyei*, and *S. lacustris* plant community samples are tightly clustered and arranged in the NMDS space with *J. effusus* and *S. lacustris* on the bottom and *P. arundinacea* and *C. lyngbyei* on the top (Figure 3). Plant community groupings were found to be significantly different from one another (ANOSIM, sig level $p < 0.01$), and additionally no significant difference was found between samples from the same plant community collected from different sites (ANOSIM, sig level $p < 0.01$). Overall, the standing plant community composition appears to be more heavily influenced by the dominant species present than by the site from which it was taken (Figure 3).

Environmental variables (vectors) were significantly associated with the standing plant community groupings in the NMDS space (Figure 3, Table 5). These standing plant community groupings and associated significant vectors echo many of the similarities and differences among the overall native and non-native plant communities (described in the sections above) and differences observed between the sites (described in Appendix D). Specifically, the clustering of groups is reflective of the overall native and non-native plant community groupings, with the

non-native plant communities *P. arundinacea* and *J. effusus* occupying the right-hand side of the NMDS space and the native plant communities *C. lyngbyei* and *S. lacustris* occupying the left-hand side of the space. The non-native side of the NMDS space is significantly associated with relative non-native plant abundance and non-native seed abundance vectors, which were also found to be greatest within these *P. arundinacea* and *J. effusus* plant community samples. Similarly, the native side of the NMDS space is significantly associated with relative native plant abundance and native seed abundance vectors, which were also found to be greatest within these *C. lyngbyei* and *S. lacustris* plant community samples. Additionally, the top portion of the NMDS space is occupied by both *C. lyngbyei* and *P. arundinacea* sample groupings and these were both primarily taken from the 1959 site. The 1959 site was shown to have greater soil estimated N release, and this is highlighted as a significant vector in the NMDS space pointing right between these two plant communities (Figure 3, Appendix D).

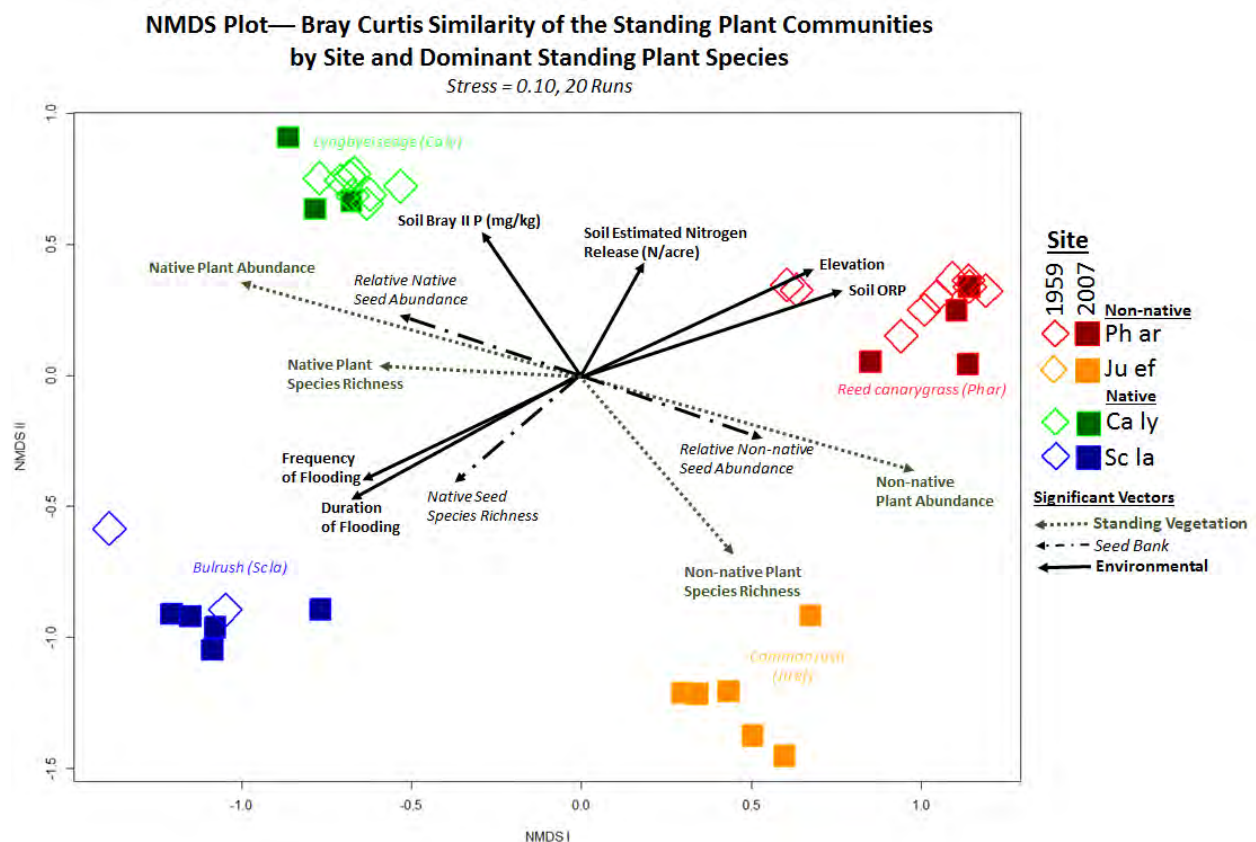


Figure 4: NMDS plot of Bray-Curtis Similarity of the standing plant communities by site and dominant standing plant species Reed canarygrass (*P. arundinacea*, Ph ar), Common rush (*J. effusus*, Ju ef), Lyngbye's sedge (*C. lyngbyei*, Ca ly), Bulrush (*S. lacustris*, Sc la). Significant vectors ($p < 0.05$) included for environmental and vegetation summary metrics.

Plant community specific significant vectors are also highlighted in the NMDS space (Table 5, Figure 4). Greater soil P levels are significantly associated with the *C. lyngbyei* plant

community grouping, and were found to be significantly higher in the *C. lyngbyei* plant community than the *P. arundinacea* and *J. effusus* plant communities (Appendix D). Elevation and soil ORP levels were found to be significantly associated with the *P. arundinacea* plant community grouping, and the *P. arundinacea* plant community samples were found to have the highest elevation and soil ORP values among the different plant communities (Table 5, Figure 4, Appendix D). Similarly, higher levels of flooding duration and frequency were significantly associated with the *S. lacustris* plant community grouping in the NMDS space, and the *S. lacustris* plant community samples were found to have the lowest elevation and highest flooding compared to all of the other plant communities (Appendix D). Additionally, native seed species richness was significantly associated with the *S. lacustris* plant community and was also found to be higher in the *S. lacustris* seed bank samples than the other plant communities. Non-native standing plant community species richness was found to be significantly associated with the *J. effusus* plant community grouping and also found to be greatest in the *J. effusus* samples compared to the other plant communities (Table 5, Figure 4, Appendix D).

Seed Bank - Using the multivariate Bray-Curtis Similarity Index to evaluate and compare the species composition of the seed bank (% relative species seed abundance) it was found that the dominant plant community seed bank samples were significantly different from one another, however the *C. lyngbyei* seed bank samples collected from the 2007 site were also significantly different from the *C. lyngbyei* samples taken from the 1959 site (ANOSIM, p-value <0.01, Figure 4, Appendix D). Overall, it appears that there was a greater similarity in seed bank composition among plant communities sampled within the same site than on their own as seen in the standing plant community composition similarity (Figure 5). This is clearly depicted in the NMDS space with the 1959 seed bank samples falling onto the right-hand side of the figure and the 2007 seed bank samples falling onto the left-hand side of the figure (Figure 5). Overall there was more similarity among the 2007 seed bank samples than the 1959 site seed bank samples, and this could be partially due to the high number of *J. effusus* seeds found across all of the samples and plant communities sampled in the 2007 site, while very few *J. effusus* seeds were found in the 1959 site samples (Appendix D). Additionally, the NMDS space is also organized loosely by native and non-native plant community groupings with the *P. arundinacea* and *J. effusus* samples grouping on the bottom portion of the figure and the *S. lacustris* and *C. lyngbyei* samples grouping themselves on the upper portion of the figure (Figure 5).

Many of the environmental vectors were found to be significantly associated with the seed bank composition similarity (Table 5, Figure 5). Soil salinity, conductivity, organic matter,

and soil moisture were significantly associated with the 1959 site sample side of the figure, with the 1959 site samples having overall greater levels of soil salinity, conductivity, and organic matter compared to the 2007 site samples (Appendix D). Soil bulk density, non-native seed species richness, and total species richness were all associated with the 2007 site seed bank sample groupings, and this is also reflected in the site data (Appendix D). Relative native seed abundance was found to be significantly associated with both the 1959 site *C. lyngbyei* and *S. lacustris* samples in the upper native region of NMDS space, with greater native seed abundance found, overall, in the 1959 site samples and within the *S. lacustris* and *C. lyngbyei* plant communities. Relative native plant abundance was significantly associated with the upper native portion of the NMDS space, with the native plant community samples having greater levels of native abundance. Similarly, relative non-native plant abundance was found to be significantly associated with the lower non-native portion of the NMDS space, with the non-native plant community samples having greater levels of non-native abundance overall. Elevation, soil ORP, relative non-native plant abundance, and relative non-native seed abundance were all significantly associated with the overall *P. arundinacea* plant community grouping, which included samples from both sites, and the lower non-native portion of the NMDS space. Additionally, duration and frequency of flooding were associated with the *C. lyngbyei* and *S. lacustris* samples from the 2007 site and overall also associated with the upper native portion of the NMDS space.

**NMDS Plot— Bray Curtis Similarity of the Seed Bank Composition
by Site and Dominant Standing Plant Species**

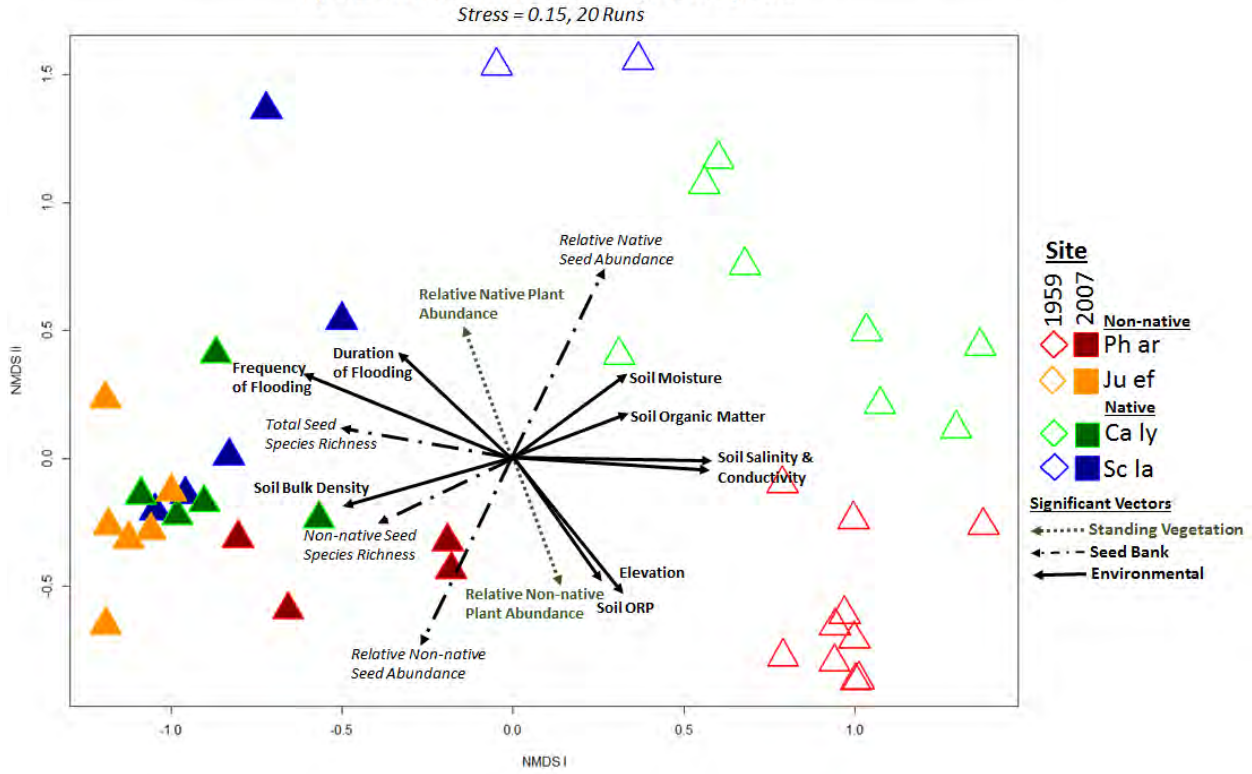


Figure 5: NMDS plot of Bray-Curtis Similarity of the seed bank species composition by site and dominant standing plant species *Reed canarygrass* (*P. arundinacea*, Ph ar), *Common rush* (*J. effusus*, Ju ef), *Lynngbye's sedge* (*C. lyngbyei*, Ca ly), *Bulrush* (*S. lacustris*, Sc la). Significant vectors ($p < 0.05$) included for environmental and vegetation summary metrics.

Standing Plant Community vs. Seed Bank - When compared separately as independent samples the standing plant community and seed bank seed composition were found to be significantly different across sites and native and non-native plant community groupings (ANOSIM p -value = 0.01, Figure 6, Appendix D). The *P. arundinacea* standing vs. seed bank composition and the *J. effusus* standing vs. seed bank composition were not found significantly different. However, the *C. lyngbyei* standing vs. seed bank composition and the *S. lacustris* standing vs. seed bank composition were found significantly different (ANOSIM p -value = 0.01, Figure 6, Appendix D). Within the NMDS space, the standing plant communities and seed banks are clearly clustered by dominant plant species and by site. The 2007 site seed bank samples are more closely clustered than the 1959 site samples, likely due to the ubiquitous *J. effusus* seed abundance throughout that site; these seed bank samples were also similar to and cluster around

the *J. effusus* standing plant community samples in the NMDS space.

NMDS Plot— Bray Curtis Similarity of the Seed Bank vs. Standing Plant Community Compositions by Site and Dominant Plant Communities

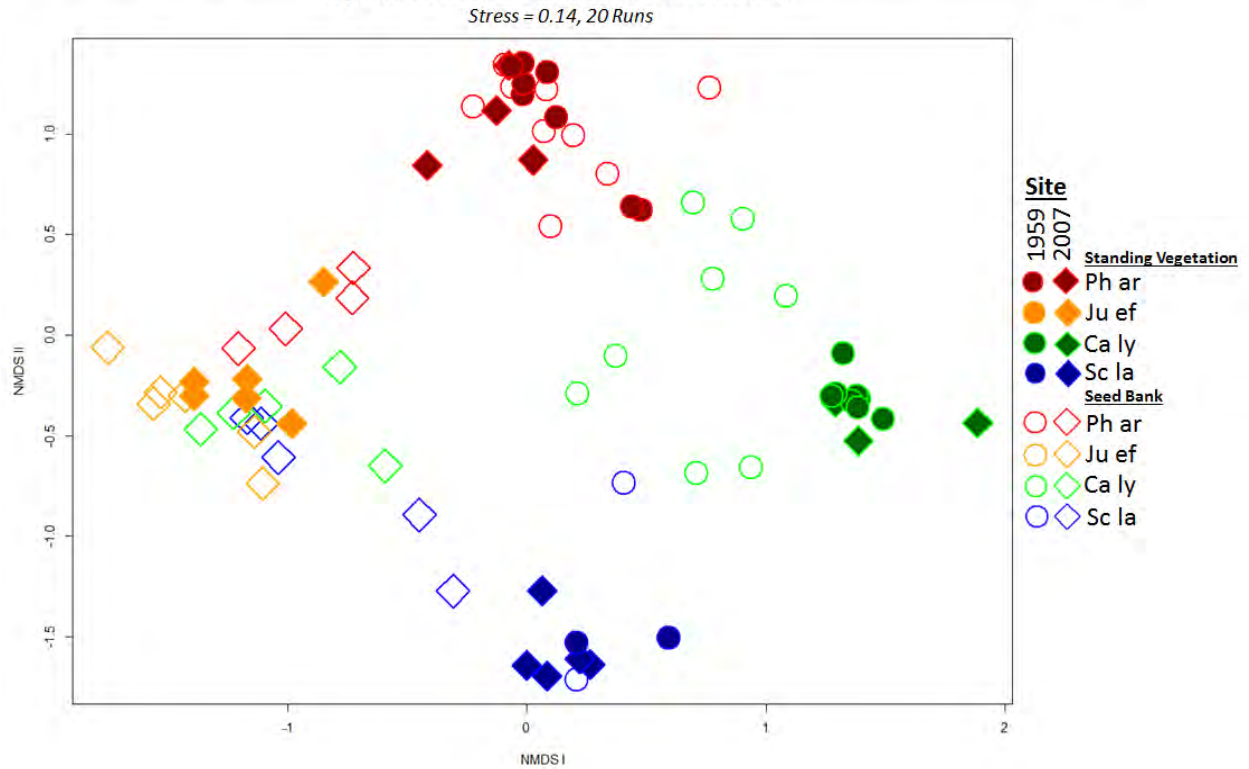


Figure 6: NMDS plot of Bray-Curtis Similarity of the standing plant community vs. seed bank species composition by site and dominant standing plant species Reed canarygrass (*P. arundinacea*, Ph ar), Common rush (*J. effusus*, Ju ef), Lyngbye's sedge (*C. lyngbyei*, Ca ly), Bulrush (*S. lacustris*, Sc la).

Table 5: NMDS Vector analysis R² and p-values of the environmental, standing plant community, and seed bank composition metrics with the NMDS similarity evaluations.

| NMDS Vector Analysis | | | | |
|--|----------------------------|----------------|----------------------|----------------|
| Parameters | Standing Vegetation | | Seed Bank | |
| | R² | P-Value | R² | P-Value |
| Elevation | 0.64 | 0.001 | 0.59 | 0.001 |
| Frequency flooded twice a day (% , March 2015) | 0.56 | 0.001 | 0.83 | 0.001 |
| Duration of each flooding event (hr, March 2015) | 0.64 | 0.001 | 0.59 | 0.001 |
| Bulk density (g/cm ²) | 0.19 | 0.107 | 0.40 | 0.004 |
| Organic Matter (%) | 0.15 | 0.165 | 0.37 | 0.014 |
| Soil moisture (%) | 0.06 | 0.474 | 0.38 | 0.010 |
| Field Conductivity (µS/cm) | 0.09 | 0.332 | 0.50 | 0.001 |
| Field ORP (mV) | 0.64 | 0.002 | 0.59 | 0.001 |
| Field pH | 0.07 | 0.439 | 0.00 | 0.978 |
| Field Salinity (ppm) | 0.08 | 0.409 | 0.49 | 0.001 |
| Field Temp (°C) | 0.05 | 0.568 | 0.01 | 0.888 |
| Clay (%) | 0.25 | 0.038 | 0.08 | 0.444 |
| Sand (%) | 0.17 | 0.129 | 0.01 | 0.866 |
| Silt (%) | 0.11 | 0.281 | 0.02 | 0.811 |
| Bray II P (mg/kg) | 0.25 | 0.057 | 0.01 | 0.925 |
| Estimated Nitrogen Release (N/acre) | 0.22 | 0.072 | 0.30 | 0.035 |
| Total Exchange Capacity (meq/100 g) | 0.10 | 0.332 | 0.07 | 0.464 |
| Standing Plant Community Composition | R² | P-Value | R² | P-Value |
| Native relative cover (%) | 0.90 | 0.001 | 0.29 | 0.015 |
| Non-native relative cover (%) | 0.89 | 0.001 | 0.29 | 0.006 |
| Total species richness | 0.22 | 0.075 | 0.16 | 0.136 |
| Native species richness | 0.29 | 0.035 | 0.25 | 0.054 |
| Non-native species richness | 0.38 | 0.009 | 0.051 | 0.596 |
| Shannon Diversity Index | 0.15 | 0.174 | 0.21 | 0.099 |
| Seed Bank Composition | R² | P-Value | R² | P-Value |
| Native relative frequency (%) | 0.25 | 0.057 | 0.91 | 0.001 |
| Non-native relative frequency (%) | 0.25 | 0.054 | 0.9 | 0.001 |
| Total species richness | 0.07 | 0.475 | 0.36 | 0.006 |
| Native species richness | 0.24 | 0.049 | 0.26 | 0.035 |
| Non-native species richness | 0.03 | 0.702 | 0.37 | 0.009 |
| Shannon Diversity Index | 0.09 | 0.336 | 0.14 | 0.197 |

SEED BANK: GERMINATION OVERVIEW

Total Germination Seedling Counts and Species Richness - Overall a total of 23,920 seedlings from 43 different plant species were identified during the 5-month duration of the

experiment. The total seedling count was composed of 20 native species (2,176 seedlings), 14 non-native species (20,087 seedlings), 1 native/non-native ambiguous species (*Typha sp*, 1,443 seedlings), and 7 unknown species (214 seedlings) (Appendix C). The majority of these species were perennial in life duration with a total of 34 perennial species (2,173 native and 20,037 non-native, and 1,443 *Typha sp* seedlings), with only 3 annual species (3 native and 50 non-native seedlings), and 6 unknown duration species (164 unknown status seedlings) identified. Wetland indicator status of these seedlings varied with a total of 1 FACU species (*G. triflorum*, 4 native seedlings), 9 FAC species (1,391 native and 308 non-native seedlings), 7 FACW species (1,104 native and 18,109 non-native seedlings), and 18 OBL species (737 native and 606 non-native, 1,433 *Typha sp* seedlings) identified. The overall most abundant species found germinating out of the seed bank samples was *J. effusus* (non-native, FACW), with a total seedling count of 18,085. The second most abundant species by total seedling count was *Typha sp* (native/non-native ambiguous, OBL) with 1,443 seedlings, followed by *A. filix-femina* (native, FAC) with 1,389 seedlings, and *P. arundinacea* (non-native, FACW) with 1,060 seedlings.

The non-native seed bank samples produced more seedlings than the native seed bank, with a total of 15,584 seedlings composed of 36 species, 15 native species (1,067 seedlings), 12 non-native species (14,318 seedlings), 1 native/non-native ambiguous species (*Typha sp*, 79 seedlings), and 8 unknown species (120 seedlings). The most abundant species that germinated out of the non-native seed bank were *J. effusus* (non-native, FACW) with 12,867 seedlings, *P. arundinacea* (non-native, FACW) with 940 seedlings, and *A. filix-femina* (native, FAC) with 757 seedlings. The native seed bank samples produced a total of 8,336 seedlings composed of 37 species, 17 native (1,174 seedlings), 11 non-native (5,704 seedlings), 1 native/non-native ambiguous species (*Typha sp*, 1,364 seedlings), and 8 unknown species (94 seedlings). The greatest seedling counts in the native seed bank were contributed by *J. effusus* with 5,218 seedlings, *Typha sp* with 1,364 seedlings, and *A. filix-femina* with 632 seedlings.

SEED BANK: GERMINATION RESULTS ACROSS TIDAL FLOODING AND SALINITY TREATMENTS

Mean Species Richness Across Treatments - Across all tidal flooding and salinity treatment combinations (n= 40 over 9 treatments, Figure 3), the greatest total species richness was found in the freshwater high to low marsh treatments ranging from 5.2 – 4.1 (± 2.1 -1.8), with the lowest total species richness occurring in the brackish low to high marsh conditions ranging from 1.2-1.5 (± 1.2 -1.0) (Table 6, Figure 7). Native species richness was significantly greater in the freshwater high marsh treatment, 2.4 (± 1.2) compared to the freshwater low marsh treatment,

1.2 (± 1.0), and continued to decline along the salinity and flooding treatment gradient with brackish low to high marsh treatments having the lowest native species richness ranging from 0.2-0.3 ($\pm 0.5 - 0.5$). Non-native species richness also declined with increased salinity and increased flooding, the highest non-native species richness occurring in the freshwater high to low marsh treatments ranging from 2.3-2.4 ($\pm 1.1-1.5$) and declining significantly in the oligohaline mid to low marsh treatments, and further declining in the brackish high-low marsh treatments ranging from 0.7-0.8 ($\pm 0.8-1.0$). Correspondingly, the mean Shannon Diversity Index was greatest in the freshwater mid and low marsh treatments, 0.8-0.9 (± 0.4), less in the oligohaline mid and low marsh treatments both at 0.5 (± 0.4), and lowest in the high to low brackish marsh treatments, 0.2-0.3 ($\pm 0.4-0.4$) (Table 6).

In summary, mean total, native, and non-native species richness were all significantly less in the brackish low marsh treatment conditions compared to the freshwater high marsh treatment conditions. Increases in salinity appeared to have a greater impact on species richness than increases in flooding duration and frequency across treatments. Generally, all brackish (10ppt) treatments had significantly lower species richness when compared to all other tidal flooding and salinity treatments (Table 6, Figure 7). A clear interaction effect from an increase in salinity and flooding was evident along the treatment gradient with a significant drop in species richness occurring in the mid to low marsh oligohaline treatments compared to the freshwater high to low marsh and oligohaline high marsh treatments (Figure 7). Seedling native species richness was significantly lower than non-native species richness across all tidal flooding and salinity treatments except the freshwater high and mid-marsh treatments (Table 6, Figure 7).

Shannon Diversity Index Across Treatments – Mean Shannon Diversity Indices followed a significant trend of declining across the salinity treatment gradient, the highest diversity values were found in the freshwater treatment combinations, 0.7-0.9 ($\pm 0.3-0.4$) and the lowest in the brackish treatment combinations, 0.2-0.3 (± 0.4) (Table 6). No significant differences, however, were observed among the different flooding levels within each salinity treatment category (Table 6).

Table 6: Germination Experiment: Overall Species Summary Mean Count and Relative Abundance (%) Across Flooding and Salinity Treatments (n=40 each, 100 ml soil per sample). Coloration indicates higher numbers in red and lower numbers in green.

| Germination Experiment: Species Summary Mean Count and Relative Abundance (%) Across Flooding and Salinity Treatments (n=40 each) | | | | | | | | | | | | | | | | | | | |
|---|-------------------------|-----------------------------------|-------|----------------------------------|-------|----------------------------------|-------|-----------------------------------|-------|----------------------------------|------|----------------------------------|------|-----------------------------------|------|----------------------------------|------|----------------------------------|------|
| Salinity Treatments | | Fresh (<1 ppt) | | | | | | Oligohaline (3 ppt) | | | | | | Brackish (10 ppt) | | | | | |
| Flooding Treatments | | High Marsh (Flooded 1 hr x 1 day) | | Mid-Marsh (Flooded 3 hr x 2 day) | | Low Marsh (Flooded 6 hr x 2 day) | | High Marsh (Flooded 1 hr x 1 day) | | Mid-Marsh (Flooded 3 hr x 2 day) | | Low Marsh (Flooded 6 hr x 2 day) | | High Marsh (Flooded 1 hr x 1 day) | | Mid-Marsh (Flooded 3 hr x 2 day) | | Low Marsh (Flooded 6 hr x 2 day) | |
| Parameters | | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| Total Species Richness | | 5.2 ^a | 2.1 | 4.8 ^a | 1.9 | 4.1 ^a | 1.8 | 3.9 ^a | 1.5 | 2.3 ^b | 1.3 | 2.8 ^b | 1.6 | 1.2 ^c | 1.2 | 1.5 ^c | 1.2 | 1.5 ^c | 1.0 |
| Native Species Richness | | 2.4 ^a | 1.2 | 1.8 ^a | 1.3 | 1.2 ^b | 1.0 | 1.1 ^b | 1.0 | 0.5 ^{cd} | 0.6 | 0.7 ^{bc} | 0.8 | 0.3 ^d | 0.5 | 0.2 ^d | 0.4 | 0.2 ^d | 0.5 |
| Non-native Species Richness | | 2.3 ^{ac} | 1.5 | 2.2 ^a | 1.3 | 2.4 ^a | 1.1 | 2.1 ^a | 1.2 | 1.3 ^{ab} | 1.0 | 1.5 ^{ab} | 1.2 | 0.7 ^d | 0.8 | 0.7 ^d | 0.8 | 0.8 ^{de} | 1.0 |
| Shannon Diversity Index | | 0.7 ^{ab} | 0.3 | 0.9 ^a | 0.4 | 0.8 ^a | 0.4 | 0.7 ^{ab} | 0.4 | 0.5 ^b | 0.4 | 0.5 ^b | 0.4 | 0.2 ^c | 0.4 | 0.3 ^c | 0.4 | 0.3 ^c | 0.4 |
| Abundance - count | Native Species | 34.4 | 30.9 | 8.8 | 9.7 | 2.3 | 2.4 | 2.6 | 3.0 | 1.2 | 2.6 | 2.2 | 3.2 | 0.5 | 1.1 | 0.6 | 2.2 | 0.7 | 2.2 |
| | Non-native Species | 138.4 | 200.4 | 75.3 | 100.0 | 61.8 | 106.4 | 122.4 | 199.0 | 22.3 | 54.9 | 37.6 | 71.2 | 22.8 | 68.0 | 9.0 | 26.9 | 5.2 | 11.6 |
| | Reed canarygrass, Ph ar | 7.2 | 9.6 | 4.7 | 6.3 | 3.0 | 3.7 | 5.0 | 7.8 | 2.1 | 3.2 | 2.2 | 3.0 | 1.2 | 2.3 | 0.6 | 1.2 | 0.6 | 1.2 |
| | Common rush, Ju ef | 127.7 | 197.1 | 67.1 | 97.9 | 54.9 | 104.4 | 115.0 | 198.1 | 19.0 | 53.9 | 34.3 | 71.3 | 21.6 | 68.2 | 8.2 | 26.8 | 4.4 | 11.4 |
| | Lyngbye's sedge, Ca ly | 0.9 | 1.9 | 0.3 | 0.8 | 0.4 | 1.1 | 0.3 | 1.0 | 0.2 | 0.7 | 0.4 | 1.2 | 0.2 | 0.6 | 0.0 | 0.2 | 0.6 | 2.1 |
| | Bulrush, Sc la | 1.0 | 2.4 | 0.9 | 2.1 | 0.7 | 1.4 | 1.1 | 2.3 | 0.8 | 2.5 | 1.2 | 2.8 | 0.2 | 0.9 | 0.6 | 2.2 | 0.1 | 0.6 |
| Relative Germination (%) | Native Species | 22.5 ^a | 23.5 | 4.4 ^b | 5.7 | 1.0 ^c | 1.4 | 1.0 ^c | 1.5 | 0.5 ^{df} | 1.0 | 0.7 ^{cd} | 1.4 | 0.2 ^f | 0.6 | 0.1 ^f | 0.3 | 0.2 ^f | 0.7 |
| | Non-native Species | 16.9 ^a | 11.7 | 10.3 ^a | 7.1 | 8.1 ^a | 6.7 | 12.4 ^a | 11.6 | 2.6 ^{bc} | 3.0 | 4.8 ^b | 7.2 | 1.7 ^c | 3.3 | 1.3 ^c | 2.3 | 0.7 ^c | 0.9 |
| | Reed canarygrass, Ph ar | 22.5 ^a | 24.9 | 14.0 ^{ab} | 16.2 | 12.7 ^{ab} | 20.4 | 16.8 ^b | 24.4 | 5.8 ^b | 8.4 | 5.4 ^b | 7.6 | 5.8 ^{bc} | 17.5 | 4.7 ^{bc} | 17.4 | 2.3 ^c | 6.9 |
| | Common rush, Ju ef | 24.0 ^a | 24.5 | 15.5 ^a | 16.8 | 18.0 ^a | 20.7 | 18.6 ^a | 20.2 | 2.5 ^{bc} | 4.7 | 9.7 ^c | 22.8 | 1.5 ^{cd} | 3.9 | 4.6 ^{cd} | 12.3 | 0.5 ^{cd} | 0.9 |
| | Lyngbye's sedge, Ca ly | 17.4 | 32.7 | 5.9 | 19.3 | 9.3 | 24.5 | 8.0 | 23.0 | 1.7 | 6.0 | 6.6 | 17.0 | 2.8 | 10.7 | 0.1 | 0.7 | 10.6 | 28.5 |
| | Bulrush, Sc la | 10.3 | 22.2 | 11.1 | 24.3 | 12.4 | 27.5 | 13.6 | 28.9 | 9.1 | 24.4 | 9.7 | 21.5 | 0.6 | 2.2 | 2.2 | 9.2 | 3.4 | 16.7 |

Dunn Test Post Hoc Pairwise Comparisons – Sig p<0.05 Corrected for Multiple Comparisons, Shared Letters Indicate Not Sig Different Across Treatments

Total, Native, and Non-native Mean (±SE) Germination Species Richness Across Tidal Flooding and Salinity Treatments

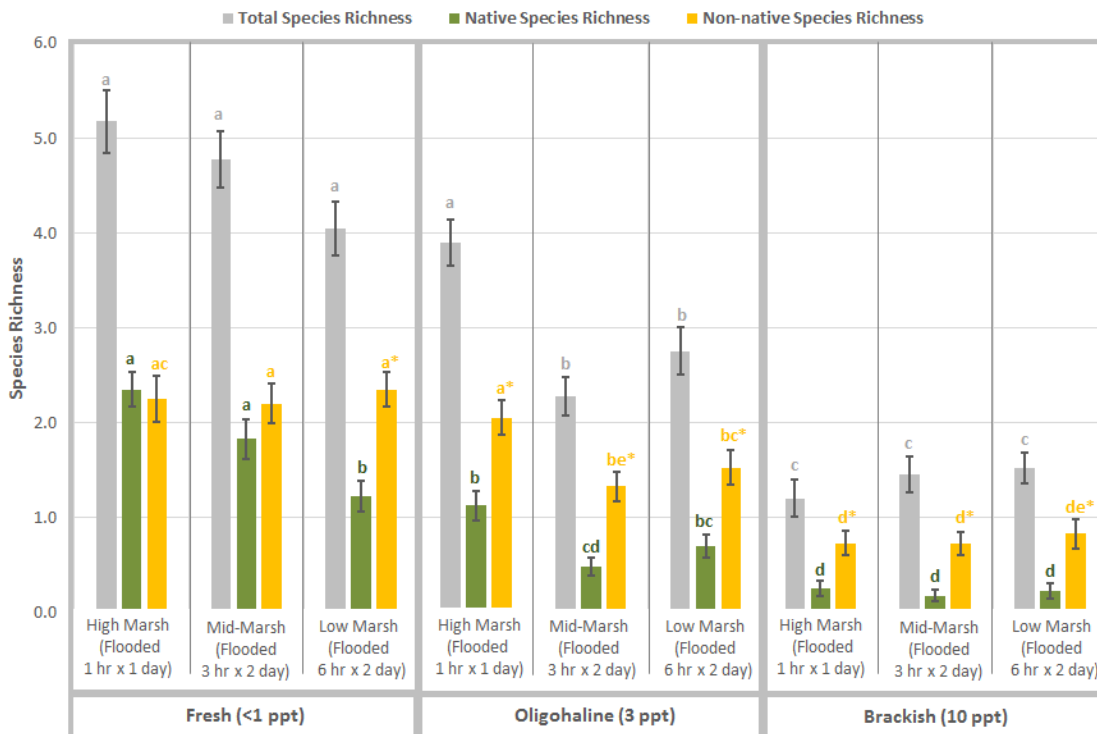


Figure 7: Total, Native, and Non-native Germination Species Richness across Tidal Flooding and Salinity Treatments. Dunn's posthoc analysis comparisons made among treatment levels for each species richness type– shared letters within a species richness type among the treatment combinations indicate no significant difference (significance level p < 0.5). *Indicates non-native species richness significantly greater than native species richness within that treatment group.

Mean Relative Germination Frequency Across Treatments - Across tidal flooding and salinity treatment combinations (n= 40 over 9 treatments, Figure 3), the high marsh freshwater treatment had significantly greater native RGF, 22.5% ($\pm 23.5\%$), than the other treatment combinations (Table 6, Figure 8). The lowest native RGF occurred across the brackish treatment combinations, with no significant difference in native RGF occurring among the brackish high-low marsh flooding treatments, high 0.2% ($\pm 0.6\%$), mid 0.1% ($\pm 0.3\%$), low 0.2% ($\pm 0.7\%$) (Table 6, Figure 8). These trends were mirrored by the non-native RGF across the treatment combinations, overall mean non-native RGF being highest in the freshwater treatments with 16.9-8.1% ($\pm 6.7-11.7\%$) RGF, and dropping significantly in the oligohaline mid and low marsh treatments at 4.8-2.6% ($\pm 7.2-3.0\%$) RGF, and then dropping further across the brackish treatments 1.7-0.7 ($\pm 3.3-0.9\%$). Overall, non-native RGF was also significantly greater than the native RGF across all treatment combinations (Table 6, Figure 8).

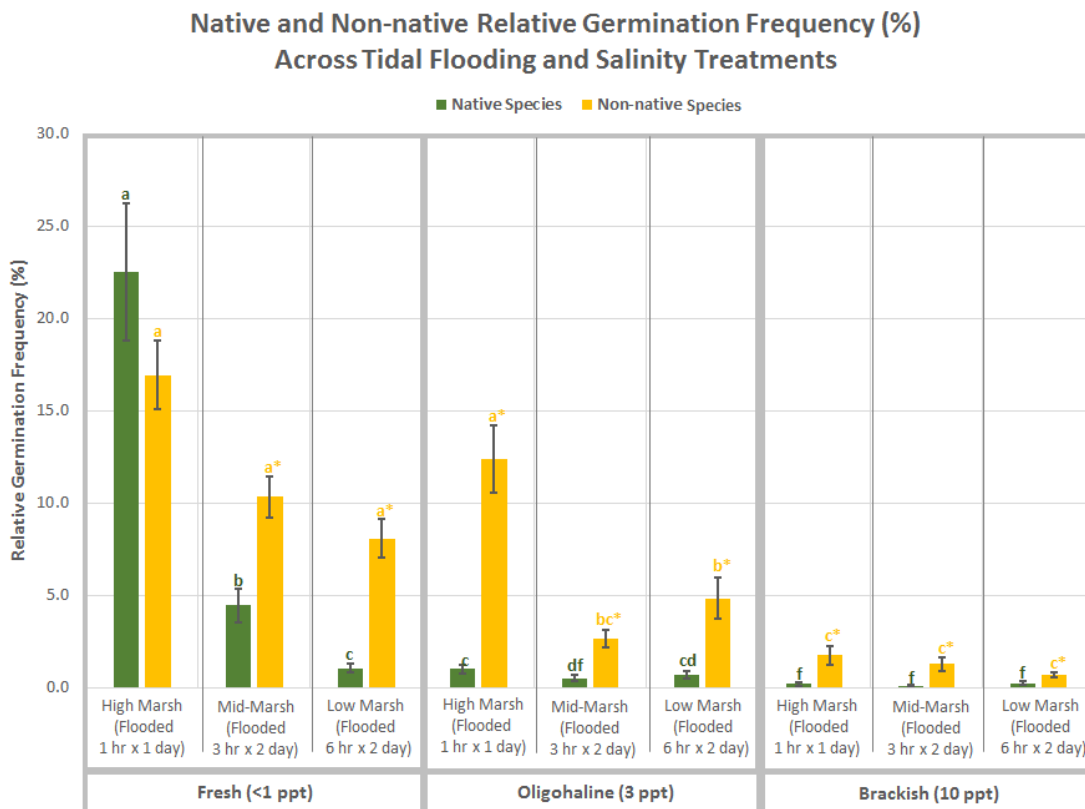


Figure 8: Native, and Non-native relative germination frequency across Tidal Flooding and Salinity Treatments. Dunn's post-hoc analysis comparisons made among treatment levels Shared letters among the treatment combinations indicate no significant difference (Significance level $p < 0.5$) within the native and non-native categories. *Indicates relative germination frequency significantly greater within that treatment group.

Relative Germination Frequency: Across Treatments - Species germination across all seed bank samples and treatment combinations revealed species-specific variability (Table 6 and

7, Figure 9 and 10). As hypothesized, dominant non-native species *P. arundinacea* and *J. effusus* exhibited similar trends in germination suppression under increased flooding and salinity treatment combinations, with significantly less germination occurring under brackish low marsh conditions as compared to freshwater high marsh conditions (Table 6, Graph 9). It was also hypothesized that the dominant native species *C. lyngbyei* and *S. lacustris* would exhibit an increase in germination in response to increased flooding and salinity, mirroring their abundance along this gradient in the field (Table 6, Figure 9). This trend was not observed; instead, both *C. lyngbyei* and *S. lacustris* showed minimal flooding and salinity treatment effects, germinating at similar (no significant differences detected) relative frequencies across most treatment combinations (Table 6, Figure 9).

The greatest native species RGF occurred in the high marsh freshwater treatment (Table 6). The species contributing the greatest RGF in this treatment were *A. filix-femina* (native, FAC), followed by *C. lyngbyei* (native, OBL), and *O. sarmetosa* (native, OBL). *A. filix-femina* showed a clear decrease in RGF under increased flooding frequency and duration and salinity, with significantly greater RGF in the freshwater high marsh treatment, 78.7% ($\pm 34.3\%$), compared to the freshwater mid, 13.6% ($\pm 26.0\%$), and low 0.2% ($\pm 0.5\%$) marsh treatments, and no germination under the oligohaline and brackish treatment combinations (Table 21, Figure 15). *O. sarmetosa* also showed a clear decrease in germination under increased flooding and salinity, with a significant drop in germination under the oligohaline high, 10.6% ($\pm 23.9\%$), mid, 4.2% ($\pm 15.0\%$), and low, 0.8% ($\pm 3.7\%$) marsh treatments, and very little germination under the brackish treatment combinations, 0.0-2.5% ($\pm 0-15.8\%$) (Table 7, Figure 10). *C. lyngbyei*, on the other hand, did not vary significantly across the treatment combinations. The only native species which did germinate across all of the brackish water treatment combinations were *C. lyngbyei*, 2.8-10.6% ($\pm 10.7-28.5\%$), and *S. lacustris*, 0.6-3.4% ($\pm 2.2-16.7\%$) (Table 6, Figure 9 and 10). *S. lacustris* RGF was similar across high to low marsh flooding treatments under fresh and oligohaline conditions, 10.3-9.7% ($\pm 22.2-21.5$), and marginally reduced under brackish conditions, 0.6-3.4% ($\pm 2.2-16.7$) (Table 6, Figure 9 and 10).

The greatest non-native species RGF also occurred in the high marsh freshwater treatment. The species contributing the greatest RGF were *J. effusus* (non-native, FACW), *P. arundinacea* (non-native, FACW), and *C. stagnalis* (non-native, OBL) (Table 7, Figure 10). *P. arundinacea* and *J. effusus* showed a similar trend of RGF decline when exposed to increased flooding and salinity treatments with significantly higher RGF in the freshwater high marsh treatment, *P. arundinacea*, 22.5% (± 24.9), and *J. effusus* 24.0% (± 24.5), compared to the

brackish low marsh treatment, *P. arundinacea* 2.3% (± 6.9) and *J. effusus* 0.5% (± 0.9). Under the oligohaline (3 ppt) treatment combinations both *P. arundinacea* and *J. effusus* germination frequency were reduced significantly in the mid to low, *P. arundinacea* 5.8-5.4% ($\pm 8.4-7.6$) and *J. effusus* 2.5-9.7% ($\pm 4.7-22.8$), marsh flooding treatments relative to the high, *P. arundinacea* 16.8% (± 24.4) and *J. effusus* 18.6% (± 20.2), marsh flooding treatments. *P. arundinacea* and *J. effusus* germination were also significantly reduced under the brackish (10 ppt) treatments relative to the freshwater and oligohaline treatments, however no significant difference in germination frequency was observed within the brackish low to high marsh flooding treatments, *P. arundinacea* 5.8-2.3% ($\pm 17.5-6.9$) and *J. effusus* 1.5-0.5% ($\pm 3.9-0.9$) (Table 6, Figure 9 and 10).

Overall, *J. effusus* germination frequency was significantly greater than the other species compared within the freshwater low to high marsh treatments and the oligohaline high marsh treatment (Table 7, Figure 10). *C. stagnalis* germination was also significantly reduced under increased salinity, with the greatest RGF occurring under the freshwater treatment combinations, 14.5-14.1% ($\pm 26.4-25.1$ %), and the lowest in the brackish treatment combinations, 0.0-3.2% ($\pm 0.0-15.9$ %). There were, however, no significant differences in *C. stagnalis* RGF among tidal flooding treatments within each salinity treatment combination (Table 7, Figure 10). The only non-native species that germinated across all of the brackish water treatment combinations were *J. effusus*, 1.5-0.5% ($\pm 3.9-0.9$ %), and *P. arundinacea*, 5.8-2.3% ($\pm 17.5-6.9$ %) (Table 7, Figure 9). *Typha sp* (unknown, OBL) also germinated across all treatment combinations and although there was variability in the *Typha sp* RGF across the flooding and salinity treatments, no significant differences were detected (Table 7, Figure 10).

Dominant Species Relative Germination Frequency (%) Across Tidal Flooding and Salinity Treatments

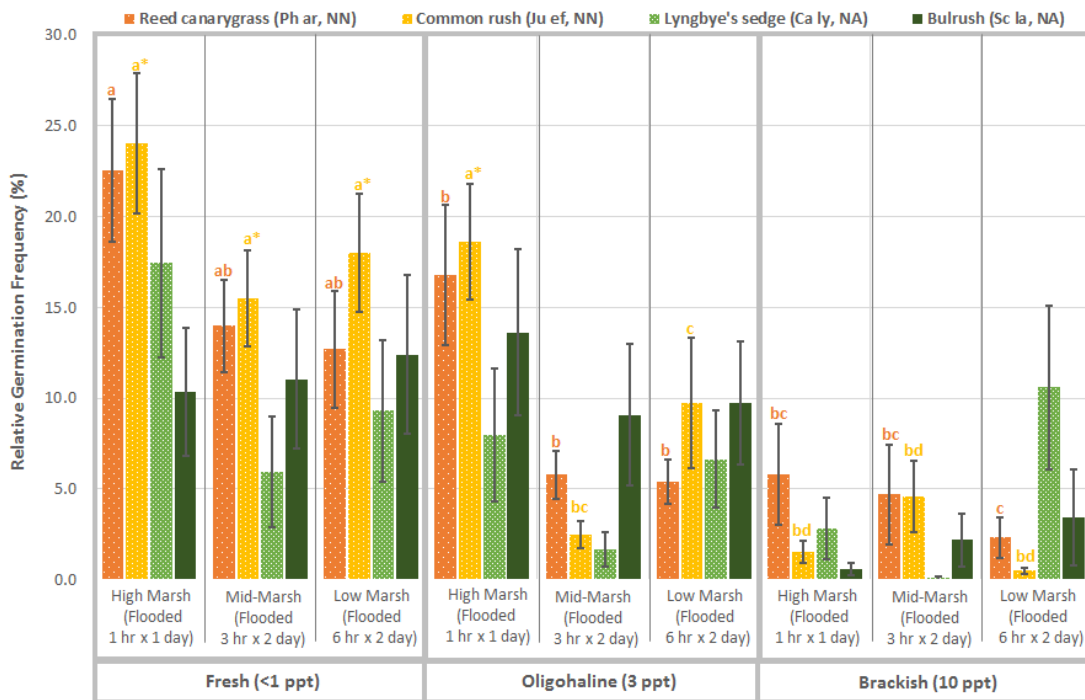


Figure 9: Dominant species germination frequency across tidal flooding and salinity treatments. Dunn's post-hoc analysis comparisons made among treatment levels for each species. Shared letters within a species category among the treatment combinations indicate no significant difference (significance level $p < 0.5$). *Indicates significantly greater than all other species within that treatment group.

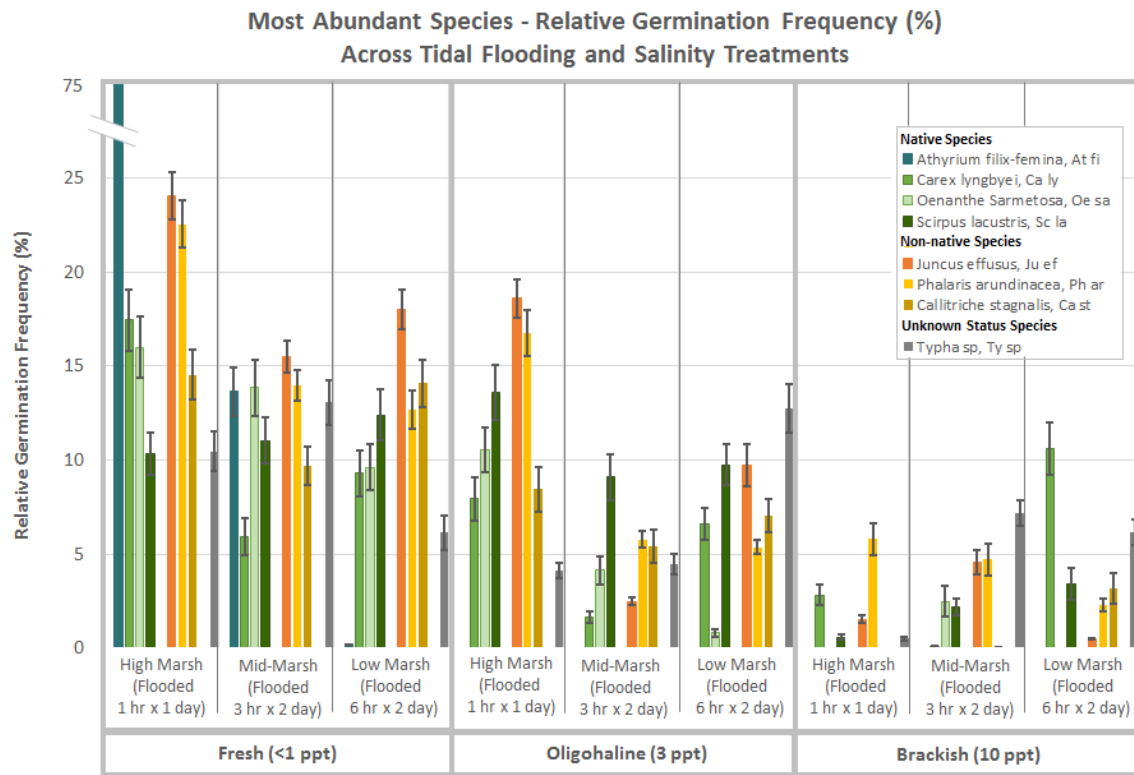


Figure 10: Most abundant species relative germination frequency (%) across tidal flooding and salinity treatments.

Table 7: Germination experiment: individual species mean relative abundance (%) across flooding and salinity treatments (n=40 each). Dominant standing species highlighted in gray on the left, germination data heat mapped from red representing higher levels of germination to green representing lower levels of germination across the treatment gradient.

| Germination Experiment: Individual Species Mean Relative Abundance (%) Across Flooding and Salinity Treatments (n=40 each) | | | | | | | | | | | | | | | | | | | | | |
|--|---------------|-------------------|-----------------------------------|------|----------------------------------|------|----------------------------------|------|-----------------------------------|------|----------------------------------|------|----------------------------------|------|-----------------------------------|------|----------------------------------|------|----------------------------------|------|------|
| Salinity Treatments | | | Fresh (<1 ppt) | | | | | | Oligohaline (3 ppt) | | | | | | Brackish (10 ppt) | | | | | | |
| Flooding Treatments | | | High Marsh (Flooded 1 hr x 1 day) | | Mid-Marsh (Flooded 3 hr x 2 day) | | Low Marsh (Flooded 6 hr x 2 day) | | High Marsh (Flooded 1 hr x 1 day) | | Mid-Marsh (Flooded 3 hr x 2 day) | | Low Marsh (Flooded 6 hr x 2 day) | | High Marsh (Flooded 1 hr x 1 day) | | Mid-Marsh (Flooded 3 hr x 2 day) | | Low Marsh (Flooded 6 hr x 2 day) | | |
| Species Code | Native Status | Wetland Indicator | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD | |
| Ag sp | N/A | FAC | 0.0 | 0.0 | 3.0 | 16.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 2.0 | 12.7 | 0.0 | 0.0 |
| Ag st | Non-native | FAC | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Al ge | Non-native | OBL | 9.3 | 27.0 | 8.9 | 22.1 | 4.8 | 10.9 | 6.1 | 18.9 | 0.7 | 2.4 | 3.8 | 14.2 | 0.3 | 1.2 | 0.0 | 0.0 | 1.2 | 6.5 | 6.5 |
| Al pl a | Native | OBL | 6.0 | 20.8 | 2.7 | 9.6 | 12.0 | 31.6 | 1.3 | 4.9 | 0.4 | 2.3 | 0.2 | 1.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Al pr | Non-native | FAC | 12.0 | 30.5 | 8.5 | 25.7 | 3.1 | 16.0 | 6.1 | 22.9 | 0.0 | 0.0 | 1.4 | 7.1 | 0.9 | 4.4 | 0.4 | 2.6 | 0.0 | 0.0 | 0.0 |
| Al ru | Native | FAC | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Al sp | Non-native | FAC/OBL | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| At fi | Native | FAC | 78.7 | 34.3 | 13.6 | 26.0 | 0.2 | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ca ly | Native | OBL | 17.4 | 32.7 | 5.9 | 19.3 | 9.3 | 24.5 | 8.0 | 23.0 | 1.7 | 6.0 | 6.6 | 17.0 | 2.8 | 10.7 | 0.1 | 0.7 | 10.6 | 28.5 | 28.5 |
| Ca st | Non-native | OBL | 14.5 | 26.4 | 9.7 | 19.8 | 14.1 | 25.1 | 8.5 | 23.2 | 5.4 | 17.2 | 7.1 | 17.5 | 0.0 | 0.0 | 0.0 | 0.3 | 3.2 | 15.9 | 15.9 |
| El pa | Native | OBL | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ep ci | Native | FACW | 10.1 | 28.5 | 12.5 | 31.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ga tr | Native | FACU | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Gl sp | Native | OBL | 3.5 | 16.8 | 8.6 | 27.3 | 2.9 | 16.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Gn ul | Non-native | FAC | 4.5 | 17.5 | 8.1 | 22.7 | 2.3 | 9.1 | 0.2 | 1.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ju ba | Native | OBL | 7.5 | 21.7 | 5.0 | 15.5 | 7.2 | 23.0 | 6.7 | 19.8 | 2.4 | 11.2 | 0.8 | 5.3 | 0.0 | 0.0 | 0.3 | 2.0 | 2.5 | 15.8 | 15.8 |
| Ju bu | Native | FACW | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ju ef | Non-native | FACW | 24.0 | 24.5 | 15.5 | 16.8 | 18.0 | 20.7 | 18.6 | 20.2 | 2.5 | 4.7 | 9.7 | 22.8 | 1.5 | 3.9 | 4.6 | 12.3 | 0.5 | 0.9 | 0.9 |
| Ju sp | N/A | FACW | 0.0 | 0.0 | 9.2 | 28.2 | 0.8 | 5.3 | 0.0 | 0.0 | 2.5 | 15.8 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Li oc | Native | OBL | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Li sc | Native | OBL | 0.0 | 0.0 | 1.7 | 10.5 | 0.0 | 0.0 | 0.8 | 5.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Lo co | Non-native | FAC | 7.6 | 23.5 | 6.6 | 23.2 | 5.1 | 18.2 | 7.0 | 22.7 | 1.3 | 7.9 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ly nu | Non-native | FACW | 5.0 | 22.1 | 0.0 | 0.0 | 0.0 | 0.0 | 1.7 | 10.5 | 0.8 | 5.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| My la | Native | OBL | 3.5 | 16.9 | 2.7 | 12.2 | 0.0 | 0.0 | 5.0 | 22.1 | 0.0 | 0.0 | 0.5 | 3.2 | 0.8 | 5.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| My sy | Non-native | OBL | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Oe sa | Native | OBL | 16.0 | 32.6 | 13.9 | 29.7 | 9.6 | 24.7 | 10.6 | 23.9 | 4.2 | 15.0 | 0.8 | 3.7 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 |
| Ph ar | Native | FACW | 22.6 | 24.9 | 14.0 | 16.2 | 12.7 | 20.4 | 16.8 | 24.4 | 5.8 | 8.4 | 5.4 | 7.6 | 5.8 | 17.5 | 4.7 | 17.4 | 2.3 | 6.9 | 6.9 |
| Po an | Native | OBL | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 15.8 | 2.5 | 15.8 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Po pe | Non-native | FACW | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Poa an | Non-native | FAC | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ra re | Non-native | FAC | 7.1 | 24.1 | 3.5 | 17.0 | 6.7 | 22.9 | 5.2 | 22.1 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ru sp | N/A | N/A | 2.5 | 15.8 | 2.5 | 15.8 | 5.0 | 22.1 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Sc la | Native | OBL | 10.3 | 22.2 | 11.1 | 24.3 | 12.4 | 27.5 | 13.6 | 28.9 | 9.1 | 24.4 | 9.7 | 21.6 | 0.6 | 2.2 | 2.2 | 9.2 | 3.4 | 16.7 | 16.7 |
| Sc mi | Native | OBL | 5.1 | 18.2 | 3.6 | 12.7 | 1.7 | 10.5 | 2.8 | 15.9 | 0.0 | 0.0 | 3.8 | 15.4 | 0.6 | 2.6 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| St hu | Native | OBL | 5.0 | 22.1 | 5.0 | 22.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ty sp | Unknown | OBL | 10.5 | 21.1 | 13.1 | 23.5 | 6.1 | 18.0 | 4.2 | 7.8 | 4.5 | 10.6 | 12.8 | 26.0 | 0.5 | 2.0 | 7.2 | 13.3 | 6.2 | 13.7 | 13.7 |
| UNK2 | Unknown | N/A | 7.1 | 23.9 | 9.3 | 25.5 | 6.5 | 22.7 | 12.9 | 26.6 | 4.4 | 16.8 | 3.5 | 11.3 | 8.3 | 23.2 | 8.0 | 23.5 | 5.0 | 18.2 | 18.2 |
| UNK3 | Unknown | N/A | 1.3 | 5.5 | 0.0 | 0.0 | 0.0 | 0.0 | 5.6 | 20.8 | 0.6 | 4.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| UNK4 | Unknown | N/A | 5.0 | 19.0 | 0.0 | 0.0 | 3.3 | 16.5 | 2.5 | 11.0 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 2.5 | 15.8 | 1.7 | 10.5 | 10.5 |
| UNK5 | Unknown | N/A | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| UNK6 | Unknown | N/A | 0.0 | 0.0 | 1.3 | 7.9 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 5.0 | 22.1 | 6.3 | 23.2 | 23.2 |
| UNK7 | Unknown | N/A | 0.0 | 0.0 | 0.5 | 3.2 | 2.5 | 15.8 | 3.9 | 17.1 | 3.1 | 16.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ve am | Native | OBL | 0.0 | 0.0 | 0.0 | 0.0 | 2.5 | 15.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

SEED BANK: GERMINATION MULTIVARIATE ANALYSIS - NMDS AND PERMANOVA

The Bray-Curtis similarity NMDS of the germination composition data clearly shows a shift in germinating species composition across the tidal flooding and salinity gradient tested in the greenhouse experiment (Figures 11 and 12). Vector and PERMANOVA analysis both

indicated that salinity and flooding treatments significantly affected the seed bank germination composition across the gradient of treatments. Low marsh and brackish salinity treatment combinations had much greater similarity to each other than to the high marsh and freshwater treatments (Figures 11 and 12). Germination species composition was also heavily influenced by the site and the dominant native and non-native standing plant communities from which the seed bank samples were taken (Tables 8 and 9, Figures 11 and 12). Germination composition was more similar among samples taken from the same site and/or native/non-native seed bank, both site and seed bank type being significant NMDS vectors (Tables 8 and 9, Figures 11 and 12). This similarity among samples is to be expected given the differences in seed bank compositions that were found between sites and seed banks identified through the direct seed count analysis (Table 3). Native and non-native species richness and RGF were also significantly correlated with germination composition along the treatment gradient, with both native and non-native species richness and RGF increasing in opposition of increased flooding and salinity (Figures 11 and 12). Native species richness and RGF were both associated more closely with the 1959 site samples, and non-native species richness and RGF more closely related to the 2007 site samples. These results also mirror the differences in the site seed banks found through the direct seed count analysis (Table 3). Overall, the NMDS of the experimental germination data shows similar trends along the tidal flooding and salinity gradient to those observed in the standing plant community distributions, a significant shift in species composition and abundance occurring along the restored wetland elevation gradient. The experimental germination plant community compositions also exhibit a heavy influence from the seed bank composition of each sample tested, reflecting the influence of the standing plant community composition from which it was taken (Tables 8 and 9, Figure 12).

Table 8: NMDS Vector Analysis of Germination Data

| NMDS Vector Analysis Germination Data | | |
|---|----------------|---------|
| Parameters | R ² | P-Value |
| Salinity Treatment Level | 0.47 | 0.001 |
| Flooding Treatment Level | 0.04 | 0.001 |
| Site | 0.43 | 0.001 |
| Dominant Seed Bank Types (<i>P. arundinacea</i> , <i>J. effusus</i> , <i>C. lyngbyei</i> , <i>S. lacustris</i>) | 0.10 | 0.001 |
| Total Species Richness (SR) | 0.79 | 0.001 |
| Non-native Species Richness (SR) | 0.66 | 0.001 |
| Native Species Richness (SR) | 0.50 | 0.001 |
| Shannon Diversity Index | 0.44 | 0.001 |
| Non-native Relative Germination Frequency (RGF) | 0.53 | 0.001 |
| Native Relative Germination Frequency (RGF) | 0.31 | 0.001 |

Table 9: Permutational Multivariate Analysis of Variance (PERMANOVA) of the germination species community data, significant parameters highlighted.

| Germination Community Data – PERMANOVA | | | | | | |
|--|-----------|-------|--------|-------|----------------|---------|
| Parameters | Df | SS | MS | F | R ² | P-Value |
| Flooding Treatment | 2 | 3.21 | 1.61 | 6.65 | 0.02 | 0.000 |
| Salinity Treatment | 2 | 11.99 | 5.99 | 24.81 | 0.09 | 0.000 |
| Site | 1 | 17.29 | 17.29 | 71.59 | 0.12 | 0.000 |
| Seed Bank Status | 1 | 4.67 | 4.67 | 19.34 | 0.03 | 0.000 |
| Dominant Standing Species Seed Bank | 2 | 3.57 | 1.78 | 7.39 | 0.03 | 0.000 |
| Flooding x Salinity Treatments | 4 | 4.51 | 1.13 | 4.67 | 0.03 | 0.000 |
| Flooding x Salinity Treatments x Sites | 8 | 11.65 | 1.46 | 6.03 | 0.08 | 0.000 |
| Flooding x Salinity Treatments x Seed Bank Status | 8 | 2.97 | 0.37 | 1.54 | 0.02 | 0.004 |
| Flooding x Salinity Treatments x Dominant Standing Species Seed Bank | 16 | 3.73 | 0.23 | 0.97 | 0.03 | 0.587 |
| Flooding x Salinity Treatments x Site x Seed Bank Status | 9 | 2.68 | 0.30 | 1.23 | 0.02 | 0.076 |
| | Residuals | 306 | 73.91 | 0.24 | 0.53 | |
| | Total | 359 | 140.17 | 1.00 | | |

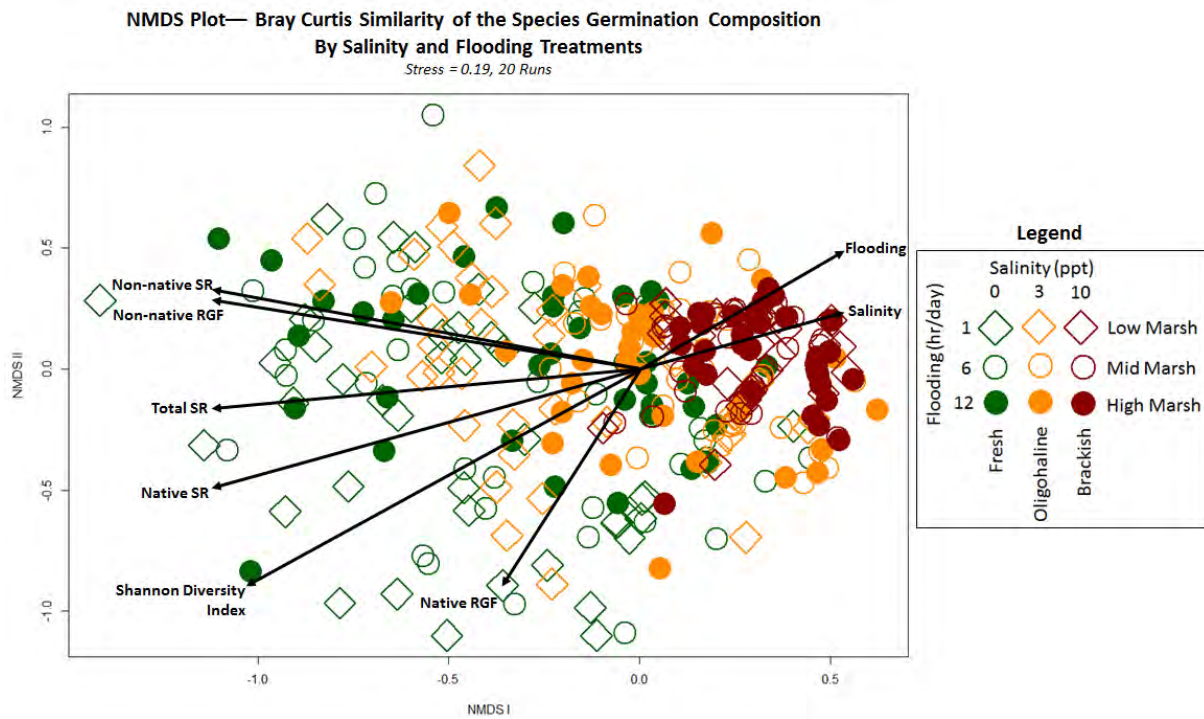


Figure 11: NMDS plot Bray-Curtis similarity of the species germination composition by salinity and flooding treatments with significant vectors.

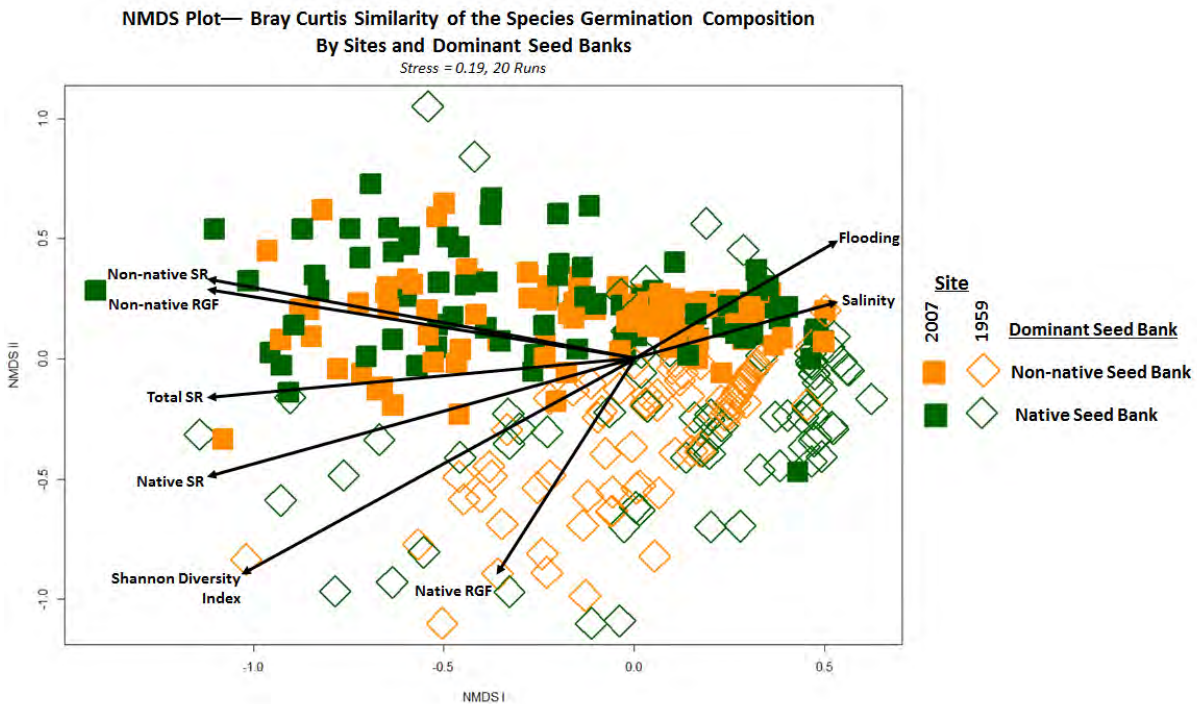


Figure 12: NMDS plot Bray-Curtis similarity of the species germination composition with symbols indicating the site and dominant seed bank from which the samples were taken with significant vectors.

DISCUSSION

OVERVIEW

This study has provided clear evidence linking restored *in situ* environmental conditions of flooding frequency and duration, soil redox (ORP), and salinity, to patterns of seed bank expression and standing plant community composition. The field survey data clearly identified significant differences in elevations, flooding conditions, soil ORP, and soil salinity among the dominant native and non-native standing plant communities. Additionally, seed bank composition survey data supported the hypotheses that these plant communities are self-seeding which promotes their own resilience. The seeds of the dominant non-native species, *P. arundinacea* and *J. effusus*, were also found to be more abundant and ubiquitously distributed throughout all of the seed bank samples (native and non-native) than the dominant native species, *C. lyngbyei* and *S. lacustris*. Although found throughout all of the seed bank samples the non-native species' expression out of the seed bank was not uniform, showing significant variability under the experimental gradient of tidal flooding salinity conditions.

As hypothesized, the non-native species' germination echoed their *in situ* abundance, with greater germination in the high marsh freshwater treatments than in the low marsh oligohaline and brackish treatments. Given the non-native species' ubiquitous presence in the seed bank, these germination results highlight the importance of the restored flooding and salinity gradients in preventing these species from becoming dominant in the lower marsh zones (Figure 1). The dominant native species, on the other hand, did not follow their hypothesized and observed in-situ trends, with their germination being similar across of all of the high-low marsh and fresh-brackish treatments. This native germination response highlights the ability of these native species, unlike the dominant non-native species, to develop without germination suppression across the entire potential environmental gradient. Dominant native species' seed densities and germination were, however, significantly less, overall, than the non-native species', especially in the high marsh zone where the non-natives were most successful (both in terms of germination and established in-situ dominance). These results suggest seed bank abundance and plant competition likely play an important role in the observed exclusion of these native species in the restored high marsh zone (Figure 1).

In oligohaline tidal reference wetlands, which have never experienced the complete vegetational and environmental shift that occurs with draining and intense agricultural land use, *J. effusus* and *P. arundinacea* abundance is very low and *C. lyngbyei* and *S. lacustris* are

commonly found growing as co-dominants with the native fern *A. filix-femina* in the high marsh elevation zone (Kidd and Yeakley, in prep). These observed high to low marsh distributions of *C. lyngbyei* and *S. lacustris* in the reference marshes were clearly echoed by the wide germination tolerance these species exhibited in the greenhouse, further highlighting the potential for these species to dominate the high marsh in the absence of *J. effusus* and *P. arundinacea*. Although present in reference marshes, *A. filix-femina*, on the other hand, was not included as a dominant native species in this study because it was not found growing in any great abundance within either of the restoration sites surveyed, with only trace abundances recorded within the 1959 site. It was, therefore, quite surprising to see it germinate in great abundance out of the seed bank in the greenhouse experiment. *A. filix-femina* exhibited high germination abundance in the freshwater high marsh treatments, with germination dropping off significantly under increased flooding and salinity conditions. These germination responses mirror observations of *A. filix-femina* abundance in the field, with *A. filix-femina* being primarily found only in the high marsh zone of reference wetlands located in the same watershed (Kidd and Yeakley, in prep). Other researchers have found *A. filix-femina* spores have longevity in the seed bank and that their germination is suppressed by low light levels (Dyer and Lindsay 1992). Given *A. filix-femina* was found abundant in the seed bank but not well represented in the standing plant communities these results suggest that *J. effusus* and *P. arundinacea* are also growing at the exclusion of *A. filix-femina* in these high marsh areas.

In a study of plant competition between *C. lyngbyei* and *S. lacustris* in a brackish tidal marsh, Pidwirny (1990) found that the availability and competition for light was a primary mechanism driving plant species' distributions. Experimentally, Pidwirny (1990) tested the effect of light reduction on mature specimens of both species and found that shading significantly reduced the growth of both *S. lacustris* and *C. lyngbyei*. *S. lacustris* growth was, however, significantly more sensitive to reduced light conditions than *C. lyngbyei*. Pidwirny (1990) concluded that *C. lyngbyei*'s earlier emergence (than *S. lacustris*) in the spring and greater above ground biomass production worked to, effectively, shade out *S. lacustris* in the high marsh zones. Through transplant and nutrient enrichment experiments Pidwirny's (1990) found that *S. lacustris* was, on the other hand, better adapted to compete for resources in the more stressful low marsh zone, outcompeting *C. lyngbyei* through below ground competition for nutrients and biomass production. When considered alongside this study's greenhouse experimental germination findings, Pidwirny's (1990) results further highlight the importance of restored abiotic gradients, in addition to species competition in determining native plant community

development. Although [Pidwirny \(1990\)](#) did not observe non-native species invasions in his study site, these results highlight the potential for both *S. lacustris* and *C. lyngbyei* germination and growth to be effectively suppressed through shading by each other and by the non-native species *P. arundinacea* and *J. effusus* (Appendix A).

In newly restored sites *P. arundinacea* and *J. effusus* may be getting a competitive jump start both in the standing plant community and seed bank, as both are commonly found in the agricultural pastures slated for restoration (Suding 2004, Kidd and Yeakley, in prep, Figure 13). Other researchers have also noted the copious seed production and seed bank longevity of both *J. effusus* (Leck and Simpson 1994) and *P. arundinacea* (Budelsky and Galawitsch 2000) in wetland environments, suggesting this is an important biological mechanism promoting these non-native species' continued spread and persistence (Suding et al. 2004, Figure 1 and 13, Appendix A). Given *S. lacustris* and *C. lyngbyei* growth and development can be suppressed by shading and *P. arundinacea* and *J. effusus* are potentially present both in the standing plant community and seed bank at the time of tidal reconnection, this would effectively reduce the potential for these native species to successfully germinate and/or establish in the invaded high marsh zones. Additionally, researchers have noted that both *J. effusus* (Ervin and Wetzel. 2000) and *P. arundinacea* (Lavergne and Molosky 2006) may release allelopathic chemicals that suppress nearby seedling germination which would also reduce native recruitment in areas where they become established (Figure 13).

The results from this study support van der Valk's (1981) adapted Gleasonian theory for ecological succession in wetlands, with the restored environmental gradients and existing seed bank providing a template for wetland plant community development within these sites (Figure 13). Researchers have observed a similar pattern in *P. arundinacea* wetland dominance at the exclusion of *C. lyngbyei* and other native species throughout both the high to low marsh zones in freshwater wetlands (Christy 2004, Diefenderfer et al. 2013, Hanson et al. 2016). Comparing known distributions of these species in fresh water (Christy 2004, Diefenderfer et al. 2013), oligohaline (this study, Kidd and Yeakley, in press), and brackish ([Pidwirny 1990](#)) tidal wetlands with the germination responses observed under similar conditions in the greenhouse, it is clear that the tidal flooding and salinity stress gradients are especially key in determining the *J. effusus* and *P. arundinacea* plant community distributions within these sites (Figure 13).

Clearly, abiotic thresholds, seed bank abundance, timing of emergence, and rate of development are key factors underlying plant community development within these restored tidal wetlands (Figure 13). In less stressful wetland environments (freshwater tidal wetlands and low

salinity high marsh zones), where these non-native species, *P. arundinacea* and *J. effusus*, are found most abundant, pre-restoration plant community and seed bank compositions may be providing a competitive advantage to these species post-restoration through both shading and allelopathy (Suding 2004, Figure 13). If these species are able to establish first, native species' germination and growth could be suppressed. Then, continued growth and seed bank enrichment from the non-native species over time may prevent the native plant communities from establishing. If, however, native plant communities establish before the non-native, such as those found in the high marshes of reference wetlands, it may be possible for these native plant communities to remain resilient through similar means, *P. arundinacea* and *J. effusus* germination and growth also being effectively reduced from shading (Ervin and Wetzel. 2000 and Lavergne and Molosky 2006). These potential feedback loops provide plant communities with long-term resilience, making the non-native species especially difficult to eradicate once established and the reference high marsh resilient to invasion (Suding 2004). For a fuller understanding of these plant community dynamics (Figure 13) further research is needed to determine 1) how these mature native and non-native plant communities respond to changes in the abiotic environment post-tidal reconnection, 2) evaluate their competitive interactions in the field including early emergence, allelopathy, and rates of biomass development, and 3) evaluate the potential feedback loops and plant community resilience stemming from these outcomes and interactions.

Key Factors and Interactions: Restored Wetland Plant Community Development

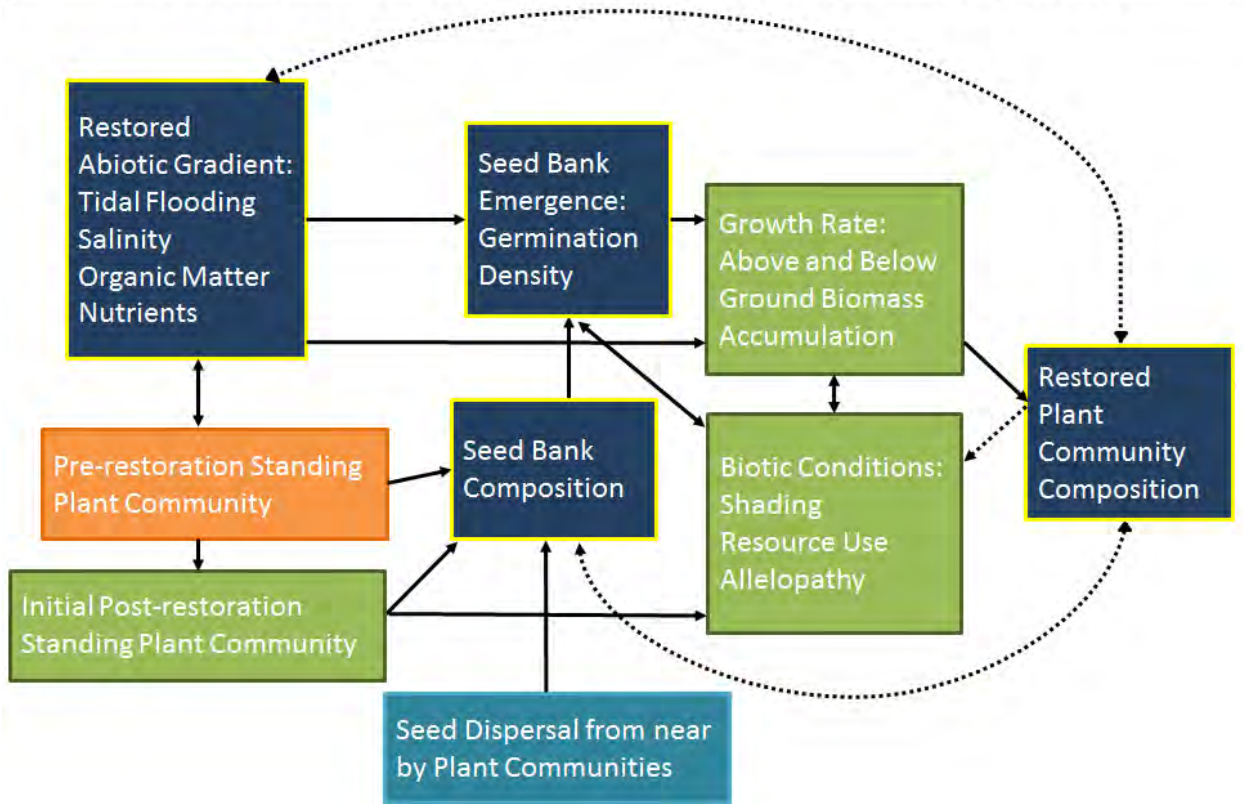


Figure 13: Conceptual figure highlighting the major factors and interactions of wetland plant community restoration in tidal wetlands. Solid lines indicate immediate feedback mechanism and dotted lines indicate mechanisms and feedback loops responsible for wetland plant community resilience over time. Dark blue boxes are those mechanisms examined in this study.

CONCLUSIONS

The results from this study provide a unique look at plant community dynamics and seed bank distributions within restored oligohaline wetlands of the Columbia River Estuary. Many researchers in other regions have tied wetland elevation and salinity to plant community zonation (e.g., Eicher 1987, Zedler et al. 1999, Weihoefer et al. 2012, Janousek and Folger 2013b), but only a few have tested these relationships experimentally and with care to simulate tidal wetland flooding dynamics (Baldwin et al. 2001, Sharp and Baldwin 2012, Janousek and Folger 2013a). The novel approach taken in this experiment provided strong evidence tying seed bank composition and restored environmental flooding and salinity gradients to the development of mature and resilient native and non-native plant communities, further supporting van der Valk's (1981) adapted Gleasonian theory of wetland succession and highlighting the importance of seed bank composition and recreated environmental gradients in plant community restoration and

resilience (Figure 1). These results have significant implications for ongoing wetland restoration efforts and for anticipating the ecological changes induced by sea level rise, both from increases in salinity and flooding. Depending on the degree of which water levels and salinities increase, non-native dominants like *P. arundinacea* and *J. effusus*, may be flooded out of the current high marsh zone, providing an opportunity for more salt and flood tolerant natives such as *C. lyngbyei* and *S. lacustris* to move in. While potentially helping to limit the extent of *P. arundinacea* and *J. effusus*, these increases in flooding and salinity may be detrimental to species' richness, with increases in flooding and/or salinity significantly reducing both wetland native and non-native species richness germination, in addition to Shannon Diversity Index. In support of these findings, Sharpe (2009), Baldwin et al. (2001), and Janousek and Folger (2013b) also found a decline in species' richness and diversity when experimentally exposing wetland seed banks to increases in flooding and salinity. These results provide insight into the plant community dynamics that could be expected along the elevation gradient of newly created tidal wetlands and/or within wetlands exposed to increases in flooding and water salinity conditions.

MANAGEMENT IMPLICATIONS: RESTORATION

Through examining seed bank composition and germination flooding and salinity thresholds, this research has provided insight into the potential competitive dynamics occurring between dominant wetland species, *J. effusus*, *P. arundinacea*, *C. lyngbyei*, and *S. lacustris*. Future wetland restoration and management efforts should pay close attention to the anticipated flooding conditions that will be restored to a site post tidal reconnection, small differences in flooding frequency and duration could have significant implications for the successful restoration of native plant communities. Results of this study suggest that the spread of *J. effusus* and *P. arundinacea* plant communities may be controlled by lowering the wetland elevation gradient and removing their existing seed banks. Depending on the local hydrology and seed bank composition, a flooding/elevation change as little as 0.1 m (0.32 ft) could result in a complete shift in plant community dominance (Figure 10 and 11). Lowering the wetland elevations will, however, also come at the cost of losing potential high marsh habitat. It may be necessary to both remove the existing non-native dominant seed bank while maintaining high marsh elevations and supplement the high marsh seed banks with native seeds (and/or plantings) to promote native high marsh plant community establishment. Due to the ubiquitous abundance of *J. effusus* and *P. arundinacea* both as seeds and rhizomes, small increases in flooding and salinity, either from changes in management or sea level rise, may only serve to migrate these species' distributions

further up the elevation gradient. Given this study focused primarily on seed germination, further research is needed to evaluate how standing wetland plant communities with deep rhizome mats will respond to small shifts in flooding and salinity conditions. Due to the aggressive growth of these common non-native species, active adaptive management, and long-term monitoring should be used to improve tidal wetland restoration outcomes, especially of native high marsh plant communities.

MANAGEMENT IMPLICATIONS: SEA LEVEL RISE

Climate change and sea level rise scenarios and their impacts on estuary water levels and salinities are uncertain for the Columbia River Estuary (Glick et al. 2007, Jay et al. 2011, Tebaldi et al. 2012). Some of this uncertainty is tied to river flow management provided by the Columbia River Basin Dam complex and to the continued development and dredging of the river (Jay et al. 2011). If river flows and tidal amplitudes drop throughout the estuary as a result of shifts in regional climate, local glacial uplift, development, and dam management (Jay et al. 2011, Tebaldi et al. 2012) then there could be a shift in the current non-native, *P. arundinacea* and *J. effusus*, plant communities down the wetland elevation gradient into the current mid to low marsh plant community zones. If on the other hand, sea level rise outpaces glacial uplift and compensates for potentially low river flows (Glick et al. 2007, Jay et al. 2011), then this non-native zone may be reduced or migrate up the elevation gradient. The specific changes in plant community zonation will be heavily dependent on the exact shifts in flooding and salinity conditions experienced throughout the estuary. Careful observation and management will be needed as other more salt and flood tolerant invasive species such as Narrow leaf cattail, *Typha angustifolia*, and Common reed, *Phragmites australis*, which are currently present but not dominant in the watershed, may spread.

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APPENDIX A: COMMON PNW TIDAL WETLAND PLANT SPECIES AND THEIR GERMINATION REQUIREMENTS

Table A1: Part 1- A collection of biological data on the growth and germination requirements of common tidal wetland plant species, all basic data collected from the USDA plants database- data from all other references are numbered and listed below the table.

| Code | Latin | Common | Group | Family | Duration | Growth Habit | Native Status | Wetland Indicator | Classification |
|--------|--|-------------------------------------|---------|---------------|-----------|----------------|--------------------------------|-------------------|----------------|
| Ag st | <i>Agrostis stolonifera</i> | Creeping bent grass | Monocot | Poaceae | Perennial | Graminoid | Non-native | FAC | Subdominant |
| Al ge | <i>Alopecurus geniculatus</i> | Water foxtail | Monocot | Poaceae | Perennial | Graminoid | Unclear | OBL | Subdominant |
| Al pl | <i>Alisma plantago-aquatica</i> L. | American water plantain | Monocot | Alismataceae | Perennial | Forb/herb | Native | OBL | Subdominant |
| Al pr | <i>Alopecurus pratensis</i> | Short awned foxtail, meadow foxtail | Monocot | Poaceae | Perennial | Graminoid | Native | OBL | Subdominant |
| Ca ly | <i>Carex Lyngbyei</i> | Sedge | Monocot | Cyperaceae | Perennial | Graminoid | Native | OBL | Dominant |
| El pa | <i>Eleocharis palustris</i> | Creeping spike rush | Monocot | Cyperaceae | Perennial | Graminoid | Native | OBL | Subdominant |
| Ga tr | <i>Galium triflorum</i> | Sweet smelling bedstraw | Dicot | Rubiaceae | Perennial | Forb/herb/vine | Native | FACU, FACW + | Subdominant |
| Ho spp | <i>Holcus spp</i> | Velvet grass species | Monocot | Poaceae | Perennial | Graminoid | Non-native | FACW | Subdominant |
| Ju ef | <i>Juncus effusus</i> subsp <i>effusus</i> | Common rush | Monocot | Juncaceae | Perennial | Graminoid | Native | FACW | Dominant |
| Li oc | <i>Lilaeopsis occidentalis</i> | Western grasswort | Dicot | Apiaceae | Perennial | Forb/herb | Native | OBL | Subdominant |
| Lo co | <i>Lotus corniculatus</i> | Birdsfoot trefoil | Dicot | Fabaceae | Perennial | Forb/herb | Non-native | FAC | Subdominant |
| Oe sa | <i>Oenanthe Sarmetosa</i> | Water parsley | Dicot | Apiaceae | Perennial | Forb/herb | Native | OBL | Subdominant |
| Ph ar | <i>Phalaris arundinacea</i> | Reedcanary grass | Monocot | Poaceae | Perennial | Graminoid | Non-native | FACW | Dominant |
| Po an | <i>Potentilla anserina</i> | Silverweed cinquefoil | Dicot | Rosaceae | Perennial | Forb/herb | Native | OBL, FAC- | Subdominant |
| Ra re | <i>Ranunculus repens</i> | Creeping buttercup | Dicot | Ranunculaceae | Perennial | Forb/herb | Non-native | FAC, FACW | Subdominant |
| Sc la | <i>Schoenoplectus lacustris</i> (L.) Palla | Hardstem bulrush | Monocot | Cyperaceae | Perennial | Graminoid | Native | OBL | Dominant |
| | | Softstem bulrush | Monocot | Cyperaceae | Perennial | Graminoid | Native | OBL | Dominant |
| Sc mi | <i>Scirpus microcarpus</i> | Small-fruited bulrush | Monocot | Cyperaceae | Perennial | Graminoid | Native | OBL | Subdominant |
| Sy su | <i>Symphotrichum subspicatum</i> | Douglas Aster | Dicot | Asteraceae | Perennial | Forb/herb | Native | FACW | Subdominant |
| Ty spp | <i>Typha latifolia</i> and <i>Typha Angastofolia</i> | Broadleaf and Narrowleaf Cattail | Monocot | Typhaceae | Perennial | Forb/herb | Native, Non-native, and Hybrid | OBL | Subdominant |
| Vi am | <i>Vicia americana</i> | American vetch | Dicot | Fabaceae | Perennial | Vine/forb/herb | Native | FAC | Subdominant |

Appendix A: Common PNW Tidal Wetland Plant Species and their Germination Requirements

Table A1: Part 2 - A collection of biological data on the growth and germination requirements of common tidal wetland plant species, all basic data collected from the USDA plants database- data from all other references are numbered and listed below the table.

| Code | Germination Requires Dormancy | Length | Germination Requires Light | Germination Moisture Conditions | Germination Temperature Requirements | Soil Requirements |
|--------|---|------------------------------|--|---|---|--|
| Ag st | Unclear (13, 14) | | Requires light (1) | Moist, drained (1) | | Tolerant of frequently saturated, flooded soil conditions (12, 13), grows best in loam, clay-loam, and sandy soils - can also grow in gravelly and rocky substrates (13) |
| Al ge | None (potentially cold, moist stratification) (2) | 20 Weeks (2) | | Moist, saturated (2) | | Germinates best in sandy soil saturated with water (2) |
| Al pl | Cold wet stratification, require scarification (3) | 4-8 Weeks (3) | Requires light (1,3) | Moist, saturated (1, 3) | | Germinates best in finely textured soils and adequate sustained soil moisture (1). |
| Al pr | Cold, moist stratification (4) | 4-40 Weeks (4) | Somewhat shade tolerant (4) | Moist, saturated (4) | | Grows best in moist, nutrient rich, fine to medium texture soils, pH between 5.6-8 (4), adapted to wet soils that are subject to frequent and/or prolonged flooding (4) |
| Ca ly | Cold, moist stratification (2) | 4 Weeks (1) | Requires light (2) | Moist/Saturated, will not grow in standing water (3) | Similar species Carex obruata, germinates best 21-24°C (1) | Can grow in fine grained silt or sand but can also grow in silt/gravel with pH between 5.0 to 6.0 (2) |
| El pa | Cold moist stratification (3), no dormancy (2) | 8 Weeks (2) | Requires light (2, 17) | Moist-saturated (2), can germinate in inundated soil (17) | | High clay, low sand content (9), grows well in fine textured soils (17) |
| Ga tr | None, or not shown to improve germination (19) | | Dappled light (19) | Moist, well drained (19) | | |
| Ho spp | None | | Requires light (20) | Moist (20) | Fluctating temperatures increase germination, best germinating temperature 8-20 °C (20), 7-8 °C (21) | Grows best in moist soil conditions, prolonged drought or inundation suppresses growth, can grow in soils with pH of 3.5 - 8, best in pH 5-6 (20), grows in sandy, silt, and clay soils with |
| Ju ef | None, however seeds require soaking for up to 7 days for best germination (1) | | Requires light (3) | Moist (1,2,3) | Germinates best in spring - when exposed to fluctuating temperatures (3), greenhouse temperature best between 32- | Can grow in compacted mineral soils (2) with pH between 4.0-6.0 (1), thrives in fine textured soil with water <6 inches deep (1) with medium nitrogen levels (2) |
| Li oc | | | | Moist, saturated, inundated (22) | | |
| Lo co | None, however seeds require soaking/scarification (5) | | Requires light (5) | Moist (1) | Optimum temperature for growth is 24°C (5) | Can grow in wet, acid, and infertile soil conditions, also considered drought resistant (5) |
| Oe sa | Cold moist stratification (2, 23) | 2-4 Weeks (2, 23) | Can grow in shade (2) | Moist/saturated (2, 23) | | Grows best in water less than 1.5 ft deep (24) |
| Ph ar | None (2) | | Light increases germination rates (2) | Saturated (but will also germinate under moist and flooded conditions - but less) (25), germinates best in moist, well drained conditions (2) | Can germinate in temperatures ranging from 7-27°C (25) | Grows in saturated clay and clay loam soils, tolerates soil pH ranging from 6.0 - 8.1 (25) |
| Po an | Not required (1) | | Best in full light (1) | Moist, well drained (26) | | Grows best in alkaline soil conditions, tolerates slightly acidic soil (26), can grow in sandy, loam, and clay soil conditions (28) |
| Ra re | Yes (requires warmth >1C and moisture to break dormancy) (29), an increase in soil oxygen | Not identified | None (31) | Moist or saturated (30) | >1C (30) | Grows best in neutral pH clay soils, tolerant of compact soil conditions, can withstand water logging and short periods of drought (30), can grow in sand and gravel (4) |
| Sc la | Cold wet storage (1) | 4-10 Weeks (1) | Light increases germination rate (1) | Best under moist, not saturated (1) | Germinates quickly in warm temperatures - 35 to 38° C (1) | Can grow in silt loam, clay, sandy loam, gravel, organic soils, in areas flooded with up to 1.5 m of water, can tolerate moderate drought (36) |
| | Cold wet storage (3) | 12 Weeks (3), 5-7 Months (2) | Requires light (3), light increases germination rate (2) | Best under moist and saturated conditions (1, 2), will emerge under 1 m of water (2) | Germinates best under alternating temperatures 30°/5° C (2) | Grows best in saturated organic silty and clay soils, also grows in sandy soils (2, 34) |
| Sc mi | Cold moist stratification (2) | 8-12 Weeks (2) | Can grow in shade (2, 37) | Flooded: under 3 cm of water, planted 2-5 cm into moist to wet soil (2), this species does not tolerate long periods of flooding (37) | | Grows in silty-mucky soil with high water holding capacity, has a wide pH tolerance (2), <15 cm standing water (38) |
| Sy su | Yes (3) | 4 Weeks (3) | Requires light (3), can tolerate partial shade (45) | Moist, well drained (44) | | Will grow in coarse, medium, and fine textured soils, does not tolerate drought, preference to low nutrient conditions (45) |
| Ty spp | None (39, 40), but some populations have shown a better germination response to cold | 12 Weeks (39) | Requires light (3, 39) | Saturated, moist (not flooded)(2), best germination under 2 cm of water (39), T. angustifolia tolerates deeper water conditions than Ty la | Germinates best under 25-30° C (39) | Will germinate in 5.7-9.2 pH levels, unaffected at 4-12 pH soil conditions, will grow in soils composed of sand, silt, loam, and clay (39) |
| Vi am | Scarification increases germination time (1) | | Requires light (1) | Moist soil conditions (43) | | Sandy, clay, medium-textured, and high organic matter soils vary from acidic to moderately basic (42) |

Appendix A: Common PNW Tidal Wetland Plant Species and their Germination Requirements

Table A1: Part 3 - A collection of biological data on the growth and germination requirements of common tidal wetland plant species, all basic data collected from the USDA plants database- data from all other references are numbered and listed below the table.

| Code | Salinity Tolerance | Asexual Reproduction | Growth Season | Flowering | Establishment Phase | Mature Seeds | Seed Longevity | Seed dispersal | Other |
|--------|--|--|---|--|--------------------------|----------------------------------|--|---|--|
| Ag st | Found in Fresh, brackish, salt marsh conditions (10), germinates best <15 ppt salinity (11) | Rhizomes, stolons, moist, full light (13) | Spring - Fall (1) | | up to 90 days (1) | | >4 yrs (1) | Dispersed by wind, water, and animals | Found in high and low elevations (10) |
| Al ge | Medium, Found in freshwater marsh conditions (10) | Vegetatively from roots nodes (16) | Early spring (15) | | 2-4 Weeks (2,15) | June-Aug (16) | > 3 yrs (16), Short (15) | Dispersed by wind (16), water, and animals | Found in high elevations (10) |
| Al pl | Freshwater marsh conditions (10) | Stem divisions, saturated/flooded conditions (1, 3) | | June - Sept (1) | | | | Dispersed by wind, water, and animals | Found in high elevations (10) |
| Al pr | Moderate 12ppt (4), freshwater marsh conditions (10) | Rhizomes, moist, full light (4) | | | 2 Weeks (4) | | Short (4) | Dispersed by wind, water, and animals | Found in high elevations (10) |
| Ca ly | Can germinate in salinity conditions ≤ 20 ppt, best germination ≤10 ppt (6), Found in fresh, brackish, saline conditions (10) | Rhizomes, moist, full light (3) | May-August (8) | April-July (3) | | | | Dispersed by wind, water, and animals | Found in high and low elevations (10), less time inundated increases biomass (8) |
| El pa | Found in fresh and brackish marsh conditions (10, 17) | Rhizomes, stem divisions, saturated, full light (3) | | May-Aug (17) | 1-2 weeks (3) | Aug-Oct (3), Jul-Aug (17) | Seeds must be moist to maintain fertility (2) | Dispersed by wind, water, and animals | Found in high and low elevations (10), Nitrogen fixer (2) |
| Ga tr | Found in fresh and brackish marsh conditions (10) | Rhizomes, stolons, moist, damped light (19) | | June-August (19) | 17-28 days (19) | July-Sept (19) | | Dispersed by wind, water, and animals | Found in high elevations (10) |
| Ho spp | Potentially low tolerance to prolonged inundation or high salinity (20), Found in freshwater marsh conditions (10) | Tillering, not common (20) | | June-August (20, 21) | 1-2 Weeks (20) | June-Sept (21) | >12 yrs (20) | Can disperse by water, animals, birds, humans (20) | Found in high elevations (10), rapid growth rate - seedling growth rate greater than reed canarygrass (20), allelopathy may increase species ability to compete against other species (20, 21) |
| Ju ef | Tolerates salinities ≤ 14 ppt (1), Found in freshwater marsh conditions (10) | Rhizomes, moist (not saturated), full light (3) | Spring - Fall (1) | June through August, persist March October (2) | 7 days (1) - 30 days (2) | June - September (1) | >60 yrs (2) | Dispersed via wind, water, and animals (1) | Found in high elevations (10), Invasive tendencies (7) |
| Li oc | Found in brackish and saltmarsh conditions (10) | Rhizomes (22) | | | | | | Dispersed by water, remain buoyant in both fresh and saltwater for months, also dispersed by birds (22) | Found in low elevations (10) |
| Lo co | Tolerates some salinity (1,5), Found in freshwater marsh conditions (10) | Rhizomes, moist, full light (1, 5) | Spring - Fall (5) | June-September | Grows slowly (5) | | >11 yrs (4) | Disperse via ballochory, water, and animals (4) | Found in high elevations (10), Nitrogen fixer (1,4,5) |
| Oe sa | Not vary salt tolerant, found in freshwater marsh conditions (10) | Stem divisions (23) | | June-August (2) | 30 days (2) | August - Sept (2) | | Dispersed by wind, water, and animals | Found in high elevations (10) |
| Ph ar | May tolerate mildly saline conditions (25), found in freshwater and brackish marsh conditions (10) | Rhizomes, moist, full light (25) | Late winter/early spring, peaks mid-June and declines by mid- August (25) | June - July (25) | 8-10 days (25) | Late July - early August (25) | Can stay viable for 20 years in seed bank, submergence limits viability after 24 months (25) | Can disperse by water, wind, animals, birds, humans (25) | Found in high elevations (10) |
| Po an | Tolerates brackish wetland soil conditions (1), found in fresh, brackish, and saltmarsh conditions (10) | Rhizomes, stolons, moist, full light (1,27) | | | 14 days (27) | August - Sept (27) | 5-7 years (27) | Dispersed by wind, water, and animals | Found in high elevations (10) |
| Ra re | Semi-tolerant of saline conditions - grows in tidal wetlands (4, 32), found in freshwater marsh conditions (10) | Rhizomes, stolons, moist, full light (30) | Can germinate from early spring - fall, or in mild winter conditions (29) | March - August (30) | | Summer (30) | 20-80 yrs, acid or water-logged conditions increase duration of viability (32) | Seeds dispersed primarily through birds and mammals (30), also dispersed by wind and water (32) | |
| Sc la | It will grow best in alkaline, saline, and brackish soil (1), found in freshwater and brackish marsh conditions (10) | Rhizomes, saturated or flooded, full light (1, 36) | | June-August (2) | | August - Sept (1) | | | |
| | Tolerates a wide range of salinities, grows in brackish and freshwater conditions (34) | Rhizomes, saturated (not flooded), full light (2, 34) | | July-August (34) | 7-10 days (1) | July-Sept (34) | >20 yrs in the seed bank (34) | Dispersed by wind and water (36) | Found in high and low elevations (10) |
| Sc mi | Found in freshwater marsh conditions (10), may tolerate slightly brackish conditions "mean 0.534 mS/cm with a range of 0.305 to 0.922" (38) | Rhizomes, stem divisions, saturated, full light (3) | | April - Sept (37) | 30 days (3) | Late Summer - Early Fall (37) | < 5 yrs (38) | Dispersed by wind and water (1) | Found in high elevations (10) |
| Sy su | Tolerant of saline conditions (45), found in freshwater and brackish conditions (10) | Stem cuttings/divisions, well drained, full light (3) | Spring - Fall (44) | July - October (45) | | | | Dispersed by wind, water, and animals | Found in high elevations (10) |
| Ty spp | Germinates best under freshwater conditions (39), may tolerate salinities up to 8000ppm (2) and brackish conditions (39), Typha latifolia found in freshwater conditions (10), Typha angustifolia tolerates more saline conditions than Ty la (41) | Rhizomes/basal shoots, saturated (not flooded) (3,39,40) | May - Sept (40) | June-July (39) | Few weeks (2) | May-July (2), August - Sept (40) | >5yrs (2), Long-term (39), >70 yrs (40) | Dispersed by wind, water, and animals (39,40) | Typha latifolia found in high elevations (10), germination rate generally low, seedlings are fast growing (39) |
| Vi am | Can tolerate moderate salinity (42), found in freshwater marsh conditions (10) | Creeping rhizomes (42) | Early spring - summer (42) | July - August (43) | 3-14 days (1) | | | Dispersed by water and animals (no reference) | Found in high elevations (10), Nitrogen fixer (43) |

APPENDIX A: LITERATURE CITED IN GERMINATION TABLE

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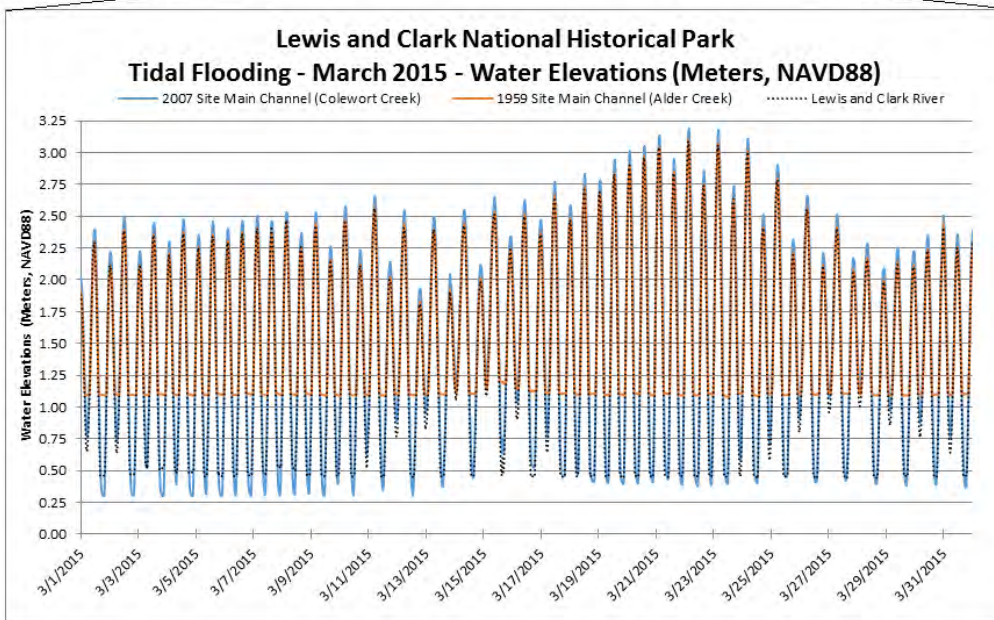
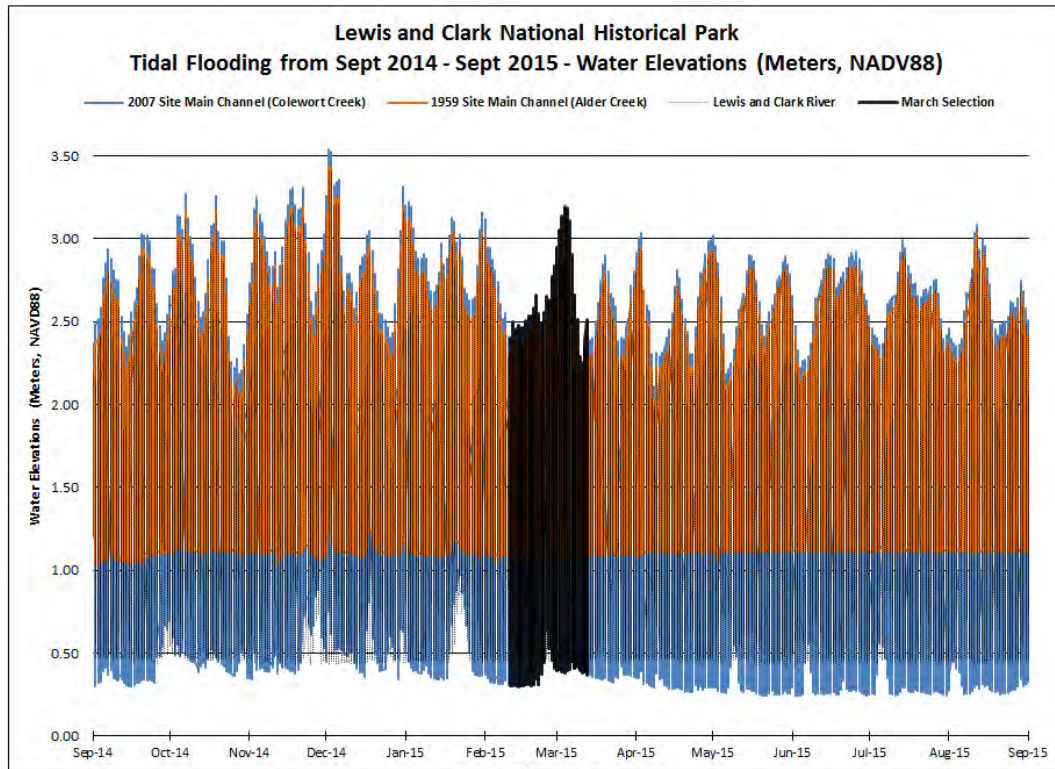
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41. Grace, J. B. and Harrison, J. S. 1986. The biology of Canadian weeds.73. *Typha latifolia* L., *Typha angustifolia* L. and *Typha* × *glauca* Godr. Canadian Journal of Plant Science. 66: 361-379.
42. Coladonato, M. 1993. *Vicia americana*. In: Fire Effects Information System, [Online]. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available: <http://www.fs.fed.us/database/feis/>
43. Plants for a Future (PFAF). 2012. *Vicia americana* Plant Profile. Plants For A Future, Dawlish, Devon, UK. Available: <http://www.pfaf.org/user/Plant.aspx?LatinName=Vicia+americana>
44. Laine, M. 2015. Douglas Aster Seed Germination. University of Minnesota Powerpoint Presentation. Available: <https://conservancy.umn.edu/bitstream/handle/11299/143435/Symphiotrichum%20subsp.licatum%20Mary%20Laine.pdf?sequence=1>
45. Silver Falls Seed Company. 2015. Aster Douglas, Oregon Native Seed Description. Available: <https://www.silverfallsseed.com/seed/Oregon-Native-Seed/Aster-Douglas.html>

APPENDIX B: TIDAL FLOODING AND SALINITY OBSERVATIONS AND DETERMINATION OF TREATMENT CONDITIONS

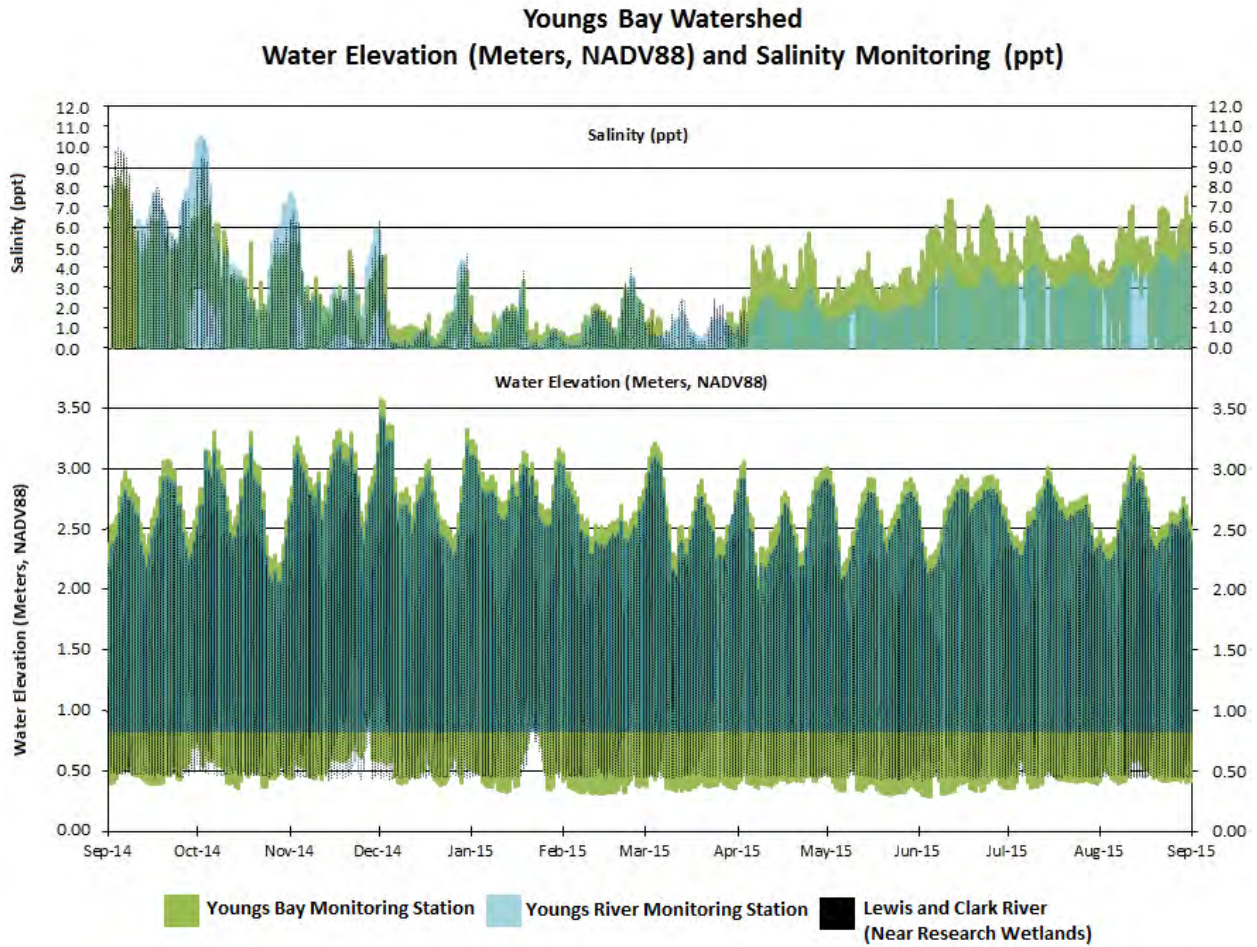
Observed tidal flooding water level elevations were similar among the two main tidal channels Alder Creek (1959 site) and Colewort Creek (2007 site) and the Lewis and Clark River which they feed into (Map 2, Figure 1). Overall, channel depth varied between the two sites (and at the location of water level monitoring), with Colewort Creek being much deeper (-0.25 m, NADV88) and with a constant freshwater upstream contribution, compared to the Alder Creek which was more shallow in depth (0.92 m, NADV88) and did not receive as much upstream input (Map 2). These differences in channel depth and fluvial input are likely explanatory factors for the differences in soil salinity observed among the sites and dominant plant communities. Colewort Creek is providing more freshwater to the 2007 site (compared to Alder Creek and the 1959 site), especially to the eastern portion of the site farthest away from the confluence of the Lewis and Clark River, where most of the *J. effusus* dominance occurred (Map 2). The *J. effusus* plant community soil had significantly lower salinity than the other plant communities surveyed (Appendix D Table 8).

Salinity measurements made in the Lewis and Clark River (river bank placement at 0.43 m, NADV88) between September 2014 and April 2015 indicate that tidal water salinity varied seasonally, with low salinities typically ranging from 0-3ppt persisting from the late fall through early spring and increasing during the summer, getting upwards of 10 ppt in September (Map 1 and 2, Figure 2). The water levels and salinity patterns of the Lewis and Clark River also tracked closely with those observed in Youngs Bay near the mouth of the Lewis and Clark River and those observed at the mouth of the Youngs River, both of which are located closer to the confluence of the Columbia River and the Pacific Ocean (Map 1, Figure 2). The salinity patterns observed track the seasonal changes in rain and flow, with more freshwater being present in the system locally and coming from increased flow of the Columbia during the rainier winter and spring months than in the drier summer and early fall months (Figure 3). The salinity ranges observed were used to inform the salinity treatments in the greenhouse experiment, with fresh (<1 ppt) and oligohaline (3 ppt) conditions representing those conditions typical of early and late spring and more brackish (10 ppt) conditions representing extreme salinity levels already experienced on the site during dry, low fluvial flow conditions. The brackish treatments are demonstrating possible future conditions on the site under extreme sea level rise and climate change scenarios (Glick et al. 2007).

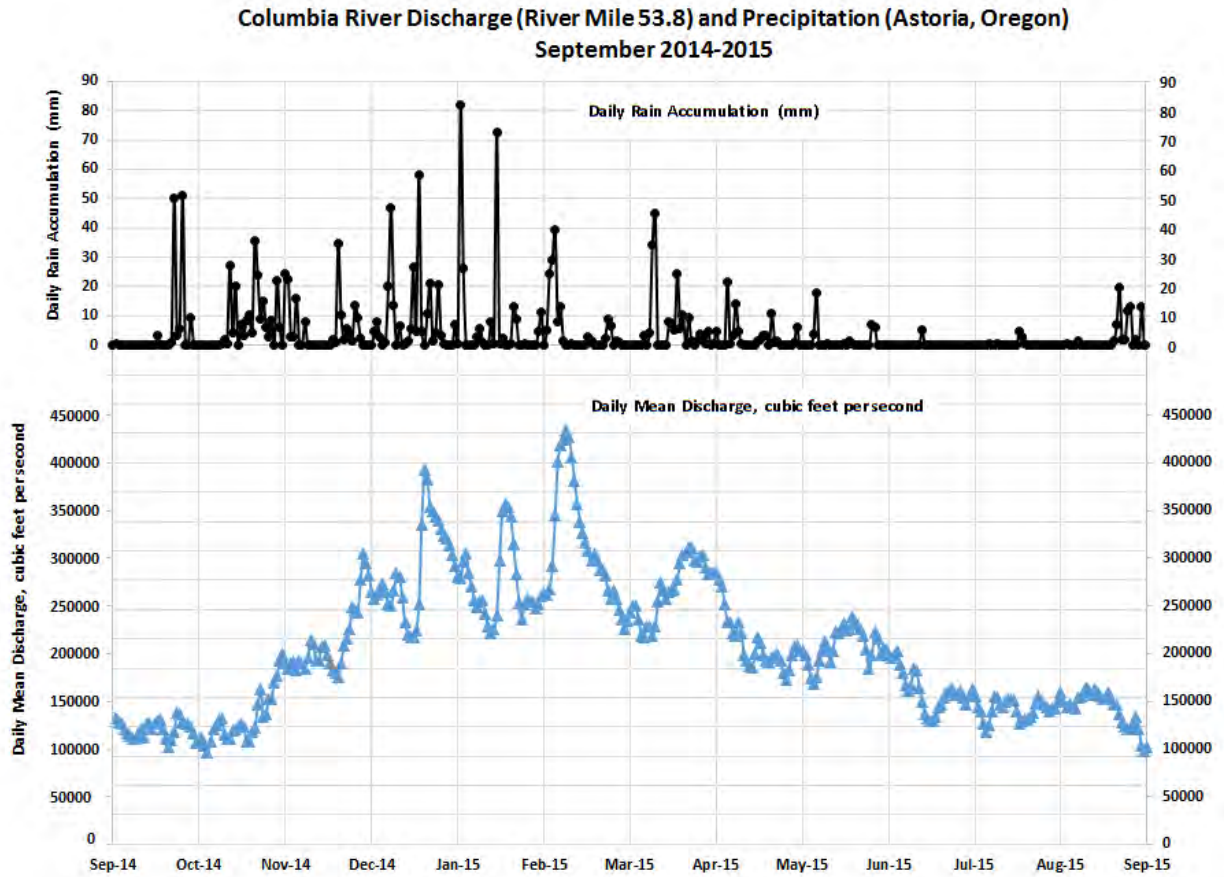
A clear gradient in elevation, flooding, soil ORP, and plant community distributions was observed between the high and low marsh wetland areas (Appendix D Table 8, Figure 4). The high marsh zone was found to be dominated by *P. arundinacea* and *J. effusus* plant communities with some overlap in elevation range, and with *P. arundinacea* tending to dominate slightly higher elevation areas than *J. effusus*. In the mid marsh zone, *C. lyngbyei* plant communities were generally found dominant at a significantly lower elevation range than *P. arundinacea* plant communities, but with some *J. effusus* plant community elevation overlap with both *C. lyngbyei* and *P. arundinacea*. *S. lacustris* dominant plant communities were found primarily in the low marsh zone, which was significantly lower in elevation than all of the other dominant plant communities (Appendix D Table 8, Figure 4). These significant differences were also observed in the corresponding flooding frequency, and duration characterizations and in the soil ORP levels observed within the plant communities (Appendix D Table 8, Figure 4 and 5). The differences in soil ORP levels provide further evidence of the biogeochemical conditions created by the tidal flooding (duration and frequency) observed along the elevation and plant community gradient (Kidd and Yeakley, in press). These flooding frequency and duration conditions, for the month of March, were used to inform the development of the tidal flooding treatments used in the greenhouse experiment (Figure 1, 4 and 5). It was estimated that > 60% of the time (March 2015) the high marsh zone plant communities, dominated by *P. arundinacea*, were only flooded once a day for ≤ 1.5 hr which was translated into a daily flooding treatment of 1 hr in the greenhouse (1 hr every 24 hr ~ 2.5 m) (Figure 5). The mid-marsh zone, where *C. lyngbyei* was found dominant, and *P. arundinacea* was mostly absent, was estimated to flood twice a day 60-95% of the time for approximately 1.5-3 hours, which was translated into two daily flooding treatments of 3 hours (a total of 6 hours over a 24 hr period ~ 2.2 m) (Figure 5). The low marsh zone, where *S. lacustris* was found dominant, was estimated to be flooded twice daily 95-100% of the time, for approximately 4.5-6.5 hr, which translated into two flooding treatments for 6 hours (a total of 12 hours over a 24 hr period ~ 1.5 m) (Figure 5). Overall, the flooding treatments were chosen to represent the average high marsh elevation and the lower (elevation) ends of the mid and low marsh gradient with the intention of reducing similarity and ambiguity among the treatment conditions tested.



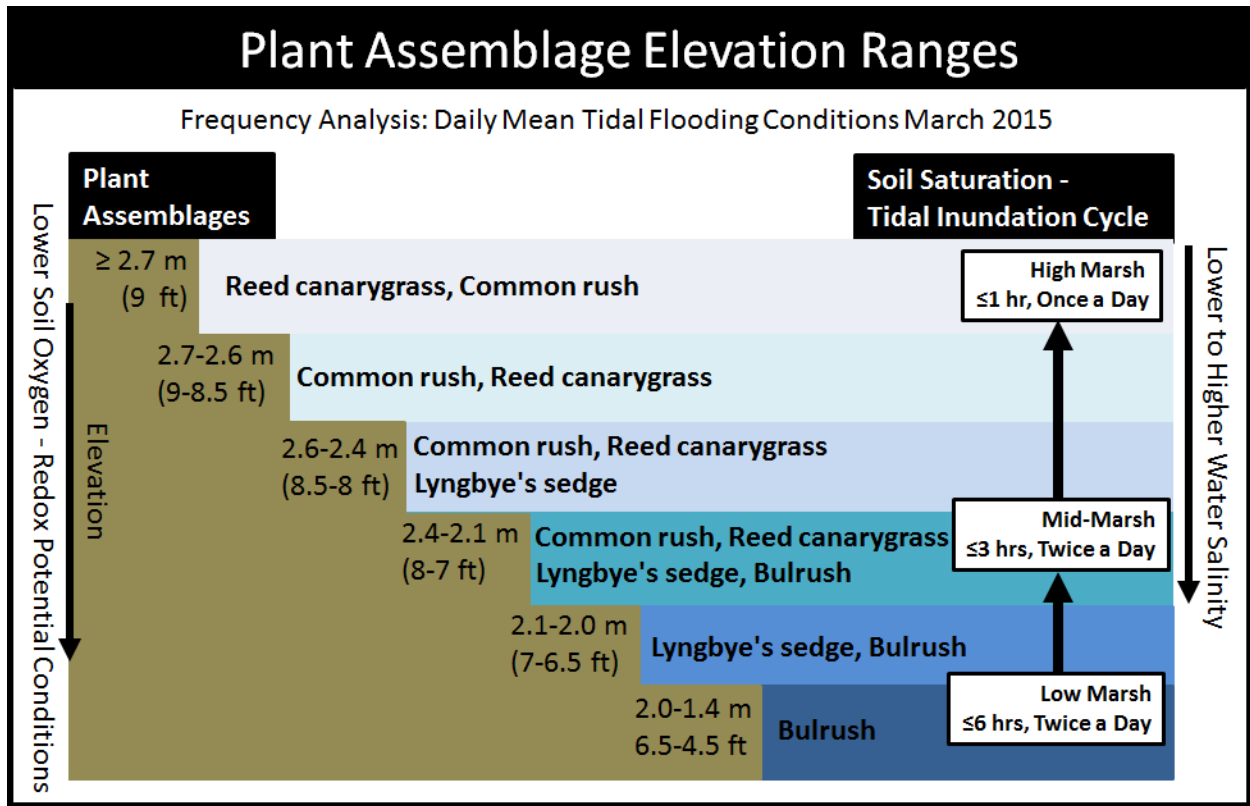
Appendix B Figure 1: Lewis and Clark River, Alder Creek, and Colewort Creek flooding from Sept 2014-2015 at the Lewis and Clark National Historical Park. See data logger location on Map 1. Within each tidal wetland channel the data logger was placed near the channel bottom, and in the Lewis and Clark river the data logger was placed at a non-vegetated low elevation point along the river bank. No water level elevation measurements were made below the elevation of the data loggers which is why there are different end points for water depth among the channels and river above. Wetland plants were generally not seen growing below 1.4 meters. The month of March is highlighted as these were the data used to calculate tidal flooding frequency and duration for the greenhouse study.



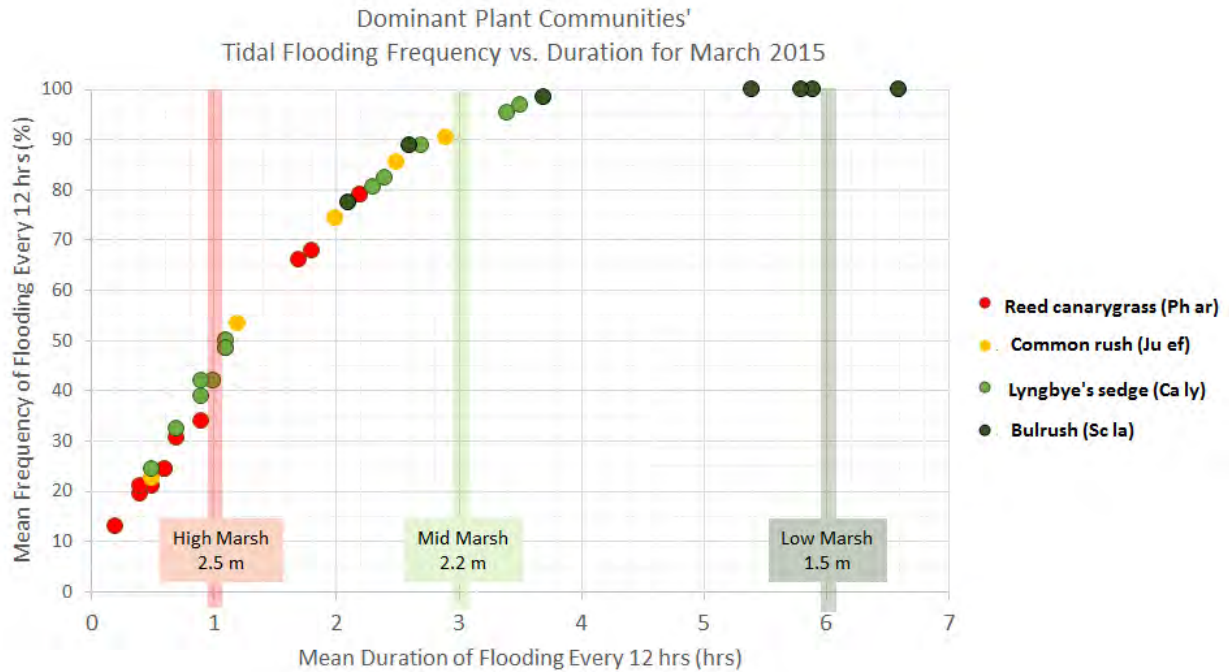
Appendix B Figure 2: Lewis and Clark River, Youngs Bay, and Youngs River flooding and salinity levels from Sept 2014-2015, Lewis and Clark River salinity data were only collected from September 2014 to April 2015 due to data logger malfunction, Youngs Bay salinity data were collected between Oct 2014 – Sept 2015. See data logger locations on Map 1 and 2.



Appendix B Figure 3: Daily mean Columbia River Discharge at river mile 53.8 (86.6 km) and daily precipitation collected at the Astoria Airport located near the Youngs Bay monitoring station on Map 1. These data were obtained from the USGS (2016). River discharge is not monitored on any of the tributaries within the Youngs Bay watershed or closer than river mile 53.8 in the Columbia River estuary which is approximately 45 miles (72 km) upstream from the mouth of Youngs Bay (Map 1).



Appendix B Figure 4: Plant assemblage elevation ranges and tidal flooding duration and frequency observed in the restoration wetlands. Salinity is assumed to increase with depth from the salt water (wedge) which normally is found below a layer of freshwater in estuary systems. Plant community data are taken from Appendix D.



Appendix B Figure 5: Graph showing the estimated average mean tidal flooding occurrence (% estimate likelihood plant community/elevation is flooded twice a day) vs. mean duration of flooding every 12 hours for the month of March 2015 for the elevations and plant communities sampled (these are highlighted with different colors and associated dominant species codes). The experimental flooding durations are highlighted – High marsh treatment, 2.5 m: 1 hr, once a day(24 hrs.) reflecting the low occurrence of flooding observed every 12 hours observed in this elevation zone (on average >50% of the time this area was only flooded once a day), mid-marsh treatment, 2.2 m: 3 hours of flooding every 12 hours, and low marsh treatment, 1.5 m: 6 hr of flooding every 12 hours. Both the mid and low marsh treatments were set to twice a day flooding (every 12 hours) reflecting the observed 90-100% occurrence of twice a day flooding observed in these elevation zones. These estimates are based on the dominant plant community elevations measured at the time of seed bank sampling (April 2015) and the main tidal channel water level elevations monitored using water level data loggers recording every 30 mins during this time period.

APPENDIX C: COMPLETE SEED BANK STUDY PLANT SPECIES INFORMATION AND OCCURRENCE

Appendix C Table 1: Seed bank study plant species occurrence and basic plant species information, plant species information collected from the USDA plants database-

| APPENDIX C TABLE 1: PLANT SPECIES INFORMATION AND OCCURRENCE: FIELD (STANDING PLANT COMMUNITY), SEED BANK (DIRECT SEED ID), AND IN THE GERMINATION EXPERIMENT (SEEDLING ID) | | | | | | | | | | | |
|--|-----------|-------------|--------------|------------------------------------|----------------------------|---------|-----------------|-----------|--------------|---------------|-------------------|
| FIELD | SEED BANK | GERMINATION | SPECIES CODE | LATIN | COMMON | GROUP | FAMILY | DURATION | GROWTH HABIT | NATIVE STATUS | WETLAND INDICATOR |
| X | X | X | Ag sp | <i>Agrostis sp.</i> | Bentgrass | Monocot | Poaceae | Perennial | Graminoid | N/A | FAC |
| | | X | Ag st | <i>Agrostis stolonifera</i> | Creeping bentgrass | Monocot | Poaceae | Perennial | Graminoid | Non-native | FAC |
| | | X | Al ge | <i>Alopecurus geniculatus</i> | Water foxtail | Monocot | Poaceae | Perennial | Graminoid | Non-native | OBL |
| X | X | X | Al pa a | <i>Alisma plantago-aquatica L.</i> | American water plantain | Monocot | Alismataceae | Perennial | Forb/herb | Native | OBL |
| | | X | Al pr | <i>Alopecurus pratensis L.</i> | Meadow foxtail | Monocot | Poaceae | Perennial | Graminoid | Non-native | FAC |
| | X | X | Al ru | <i>Alnus rubra</i> | Red alder | Dicot | Betulaceae | Perennial | Tree | Native | FAC |
| | X | X | Al sp | <i>Alopecurus</i> | Foxtail species | Monocot | Poaceae | Perennial | Graminoid | Non-native | FAC/OBL |
| X | | X | At fi | <i>Athyrium filix-femina</i> | Lady fern | Fern | Dryopteridaceae | Perennial | Forb/herb | Native | FAC |
| | X | | Br sp | <i>Brassicaceae family</i> | Yellow watercress (likely) | Dicot | Brassicaceae | Annual | Forb/herb | N/A | N/A |
| X | X | X | Ca ly | <i>Carex Lyngbyei</i> | Lyngbye's sedge | Monocot | Cyperaceae | Perennial | Graminoid | Native | OBL |
| | X | | Ca ob | <i>Carex obnupta</i> | Slough sedge | Monocot | Cyperaceae | Perennial | Graminoid | Native | OBL |
| X | | X | Ca st | <i>Callitriche stagnalis Scop.</i> | Water starwort | Dicot | Callitrichaceae | Perennial | Forb/herb | Non-native | OBL |

Appendix C: Complete Seed Bank Study Plant Species Information and Occurrence

| APPENDIX C TABLE 1: PLANT SPECIES INFORMATION AND OCCURRENCE: | | | | | | | | | | | |
|---|-----------|-------------|--------------|------------------------------|---------------------------|---------|------------|------------------|----------------|---------------|-------------------|
| FIELD (STANDING PLANT COMMUNITY), SEED BANK (DIRECT SEED ID), AND IN THE GERMINATION EXPERIMENT (SEEDLING ID) | | | | | | | | | | | |
| FIELD | SEED BANK | GERMINATION | SPECIES CODE | LATIN | COMMON | GROUP | FAMILY | DURATION | GROWTH HABIT | NATIVE STATUS | WETLAND INDICATOR |
| | X | | Ci sp | <i>Cirsium sp.</i> | Thistle | Dicot | Asteraceae | Perennial | Forb/herb | Non-native | FACU |
| | X | | Co ma | <i>Conium maculatum</i> | Poison hemlock | Dicot | Apiaceae | Biennial | Forb/herb | Non-native | FAC |
| | X | | El ob | <i>Eleocharis obtusa</i> | Blunt spike rush | Monocot | Cyperaceae | Annual/Perennial | Forb/herb | Native | OBL |
| X | X | X | El pa | <i>Eleocharis palustris</i> | Creeping spike rush | Monocot | Cyperaceae | Perennial | Graminoid | Native | OBL |
| | X | X | Ep ci | <i>Epilobium ciliatum</i> | purple leaved willow herb | Dicot | Onagraceae | Perennial | Forb/herb | Native | FACW |
| | X | | Er sp | <i>Eragrostis sp.</i> | Lovegrass | Monocot | Poaceae | Perennial | Graminoid | N/A | N/A |
| | X | | Fi sp | <i>Fimbristylis sp.</i> | Rush | Monocot | Cyperaceae | Annual | Graminoid | N/A | N/A |
| X | | X | Ga tr | <i>Galium triflorum</i> | Sweet smelling bedstraw | Dicot | Rubiaceae | Perennial | Forb/herb/vine | Native | FACU |
| | X | X | Gl sp | <i>Glyceria sp.</i> | Mannagrass | Monocot | Poaceae | Perennial | Graminoid | Native | OBL |
| | | X | Gn ul | <i>Gnaphalium uliginosum</i> | Cudweed | Dicot | Asteraceae | Annual | Forb/herb | Non-native | FAC |
| | | X | Ju ba | <i>Juncus Balticus</i> | Baltic rush | Monocot | Juncaceae | Perennial | Graminoid | Native | OBL |
| | X | X | Ju bu | <i>Juncus bufonius</i> | Toad rush | Monocot | Juncaceae | Perennial | Graminoid | Native | FACW |
| X | X | X | Ju ef | <i>Juncus effusus</i> | Soft rush | Monocot | Juncaceae | Perennial | Graminoid | Non-native | FACW |

| APPENDIX C TABLE 1: PLANT SPECIES INFORMATION AND OCCURRENCE: FIELD (STANDING PLANT COMMUNITY), SEED BANK (DIRECT SEED ID), AND IN THE GERMINATION EXPERIMENT (SEEDLING ID) | | | | | | | | | | | |
|--|-----------|-------------|--------------|--|-----------------------|---------|----------------|-----------|--------------|---------------|-------------------|
| FIELD | SEED BANK | GERMINATION | SPECIES CODE | LATIN | COMMON | GROUP | FAMILY | DURATION | GROWTH HABIT | NATIVE STATUS | WETLAND INDICATOR |
| | | X | Ju sp | <i>Juncus sp.</i> | Unknown juncus | Monocot | Juncaceae | Perennial | Graminoid | N/A | FACW |
| X | | | La ne | <i>Lathyrus nevadensis</i> | Purple peavine | Dicot | Fabaceae | Perennial | Forb/herb | Native | N/A |
| X | | X | Li oc | <i>Lilaeopsis occidentalis</i> | Western grasswort | Dicot | Apiaceae | Perennial | Forb/herb | Native | OBL |
| | | X | Li sc | <i>Lilaea scilloides</i> | Flowering quillwort | Monocot | Juncaginaceae | Annual | Graminoid | Native | OBL |
| X | X | X | Lo co | <i>Lotus corniculatus</i> | Birdsfoot trefoil | Dicot | Fabaceae | Perennial | Forb/herb | Non-native | FAC |
| | | X | Ly nu | <i>Lysimachia nummularia L.</i> | Moneywort | Dicot | Primulaceae | Perennial | Forb/herb | Non-native | FACW |
| | | X | My la | <i>Myosotis laxa Leh m</i> | Forget-me-not | Dicot | Boraginaceae | Perennial | Forb/herb | Native | OBL |
| | | X | My sp | <i>Myriophyllum spicatum L.</i> | Eurasian watermilfoil | Dicot | Haloragaceae | Perennial | Forb/herb | Non-native | OBL |
| X | X | X | Oe sa | <i>Oenanthe Sarmetosa</i> | water parsley | Dicot | Apiaceae | Perennial | Forb/herb | Native | OBL |
| X | X | X | Ph ar | <i>Phalaris arundinacea</i> | Reedcanary grass | Monocot | Poaceae | Perennial | Graminoid | Non-native | FACW |
| | X | | Pl sp | <i>Plantago sp.</i> | Plantain | Dicot | Plantaginaceae | Perennial | Forb/herb | Non-native | FAC |
| X | | X | Po an | <i>Potentilla anserine</i> | silverweed cinquefoil | Dicot | Rosaceae | Perennial | Forb/herb | Native | OBL |
| | | X | Po pe | <i>Polygonum persicaria (maculosa)</i> | Ladies thumb | Dicot | Polygonaceae | Annual | Forb/herb | Non-native | FACW |

| APPENDIX C TABLE 1: PLANT SPECIES INFORMATION AND OCCURRENCE: FIELD (STANDING PLANT COMMUNITY), SEED BANK (DIRECT SEED ID), AND IN THE GERMINATION EXPERIMENT (SEEDLING ID) | | | | | | | | | | | |
|--|-----------|-------------|--------------|--|----------------------|---------|-----------------|-----------|--------------|---------------|-------------------|
| FIELD | SEED BANK | GERMINATION | SPECIES CODE | LATIN | COMMON | GROUP | FAMILY | DURATION | GROWTH HABIT | NATIVE STATUS | WETLAND INDICATOR |
| | X | | Po pr | <i>Poa pratensis</i> L. | Kentucky bluegrass | Monocot | Poaceae | Perennial | Graminoid | Non-native | FAC |
| | X | | Po sp | <i>Polygonum Species (Cryptic)</i> | Polygonum (Cryptic) | Dicot | Polygonaceae | Annual | Forb/herb | Non-native | OBL |
| | X | | Po tr | <i>Poa trivialis</i> | Rough bluegrass | Monocot | Poaceae | Perennial | Graminoid | Non-native | FAC |
| | | X | Poa an | <i>Poa annua</i> | Annual bluegrass | Monocot | Poaceae | Perennial | Graminoid | Non-native | FAC |
| | X | | Poa sp | <i>Poa sp</i> | <i>Poa sp</i> | Monocot | Poaceae | Perennial | Graminoid | Non-native | FAC |
| | X | X | Ra re | <i>Ranunculus repens</i> | Creeping buttercup | Dicot | Ranunculaceae | Perennial | Forb/herb | Non-native | FAC |
| | X | | Ru ar | <i>Rubus armeniacus (fruticosus)</i> | Himalayan blackberry | Dicot | Rosaceae | Perennial | Subshrub | Non-native | FACU |
| | X | X | Ru sp | <i>Rumex sp.</i> | Dock | Dicot | Polygonaceae | Perennial | Forb/herb | N/A | N/A |
| X | X | X | Sc la | <i>Scirpus lacustris (tabernaemontani & validus)</i> | Bulrush | Monocot | Cyperaceae | Perennial | Graminoid | Native | OBL |
| X | X | X | Sc mi | <i>Scirpus microcarpus</i> | Paniced bulrush | Monocot | Cyperaceae | Perennial | Graminoid | Native | OBL |
| | | X | St hu | <i>Stellaria humifusa</i> | Salt marsh chickweed | Dicot | Caryophyllaceae | Perennial | Forb/herb | Native | OBL |
| X | | | Sy su | <i>Symphotrichum s ubspicatum</i> | Douglas aster | Dicot | Asteraceae | Perennial | Forb/herb | Native | FACW |
| | X | | Tr re | <i>Trifolium repens</i> | Clover sp. | Dicot | Fabaceae | Perennial | Forb/herb | Non-native | FAC |

| APPENDIX C TABLE 1: PLANT SPECIES INFORMATION AND OCCURRENCE: FIELD (STANDING PLANT COMMUNITY), SEED BANK (DIRECT SEED ID), AND IN THE GERMINATION EXPERIMENT (SEEDLING ID) | | | | | | | | | | | |
|--|-----------|-------------|--------------|--|--------------------------------|---------|------------------|-----------|--------------|---------------|-------------------|
| FIELD | SEED BANK | GERMINATION | SPECIES CODE | LATIN | COMMON | GROUP | FAMILY | DURATION | GROWTH HABIT | NATIVE STATUS | WETLAND INDICATOR |
| | X | | Tr sp | <i>Trifolium sp.</i> | Clover | Dicot | Fabaceae | Perennial | Forb/herb | Non-native | FAC |
| X | | X | Ty sp | <i>Typha sp (latifolia and angustifolia)</i> | Cattail | Monocot | Typhaceae | Perennial | Forb/herb | Unknown | OBL |
| | X | | UNK1 | Unknown seed | Unknown seed | Unknown | Unknown | Unknown | Unknown | Unknown | N/A |
| | | X | UNK2 | Unknown seedling | Unknown seedling | Unknown | Unknown | Unknown | Unknown | Unknown | N/A |
| | | X | UNK3 | Unknown | (possible Brassicaceae family) | Unknown | Unknown | Unknown | Unknown | Unknown | N/A |
| | | X | UNK4 | Unknown grass 1 | Unknown grass 1 | Unknown | Unknown | Unknown | Unknown | Unknown | N/A |
| | | X | UNK5 | Unknown grass 2 | Unknown grass 2 | Unknown | Unknown | Unknown | Unknown | Unknown | N/A |
| | | X | UNK6 | Unknown succulent | Unknown Succulent | Unknown | Unknown | Unknown | Unknown | Unknown | N/A |
| | | X | UNK7 | Unknown grass 3 | Unknown Succulent | Unknown | Unknown | Unknown | Unknown | Unknown | N/A |
| | X | | Va sp | <i>Vaccinium sp.</i> | Blueberry | Dicot | Ericaceae | Perennial | Shrub | Native | N/A |
| | | X | Ve am | <i>Veronica americana</i> | American Speedwell | Dicot | Scrophulariaceae | Perennial | Forb/herb | Native | OBL |
| X | | | Vi gi | <i>Vicia nigricans subsp. gigantea</i> | Giant vetch | Dicot | Fabaceae | Perennial | Vine | Native | FAC |

Appendix C: Complete Seed Bank Study Plant Species Information and Occurrence

Appendix C Table 2: Seed bank study overall mean plant species abundance across the field samples (standing plant community, % relative cover/m²), seed bank samples (seeds/m²), and the germination experiment (seedlings/m²).

| APPENDIX C TABLE 2: PLANT SPECIES AND MEAN OCCURRENCE: FIELD (STANDING PLANT COMMUNITY, % RELATIVE COVER/M ²), SEED BANK (SEEDS/M ²), AND THE GERMINATION EXPERIMENT (SEEDLINGS/M ²) | | | | | | | | | | | | | | |
|---|---------------|-------------------|------------------------------|--------------------------------|--|--------------------|-----------|-----------|---------------------|-----------|-----------|-------------------|-----------|-----------|
| SPECIES CODE | NATIVE STATUS | WETLAND INDICATOR | FIELD % COVER/M ² | SEED BANK SEEDS/M ² | OVERALL GERMINATION SEEDLINGS/M ² | FRESHWATER (0 PPT) | | | OLIGOHALINE (3 PPT) | | | BRACKISH (10 PPT) | | |
| | | | | | | HIGH MARSH | MID-MARSH | LOW MARSH | HIGH MARSH | MID-MARSH | LOW MARSH | HIGH MARSH | MID-MARSH | LOW MARSH |
| Ag sp | N/A | FAC | 0.3 | 789.6 | 42.5 | 0.0 | 127.4 | 0.0 | 0.0 | 0.0 | 152.8 | 0.0 | 101.9 | 0.0 |
| Ag st | Non-native | FAC | 0.0 | 0.0 | 17.0 | 0.0 | 0.0 | 0.0 | 152.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Al ge | Non-native | OBL | 0.0 | 0.0 | 693.4 | 789.6 | 1,554 | 1,325 | 1,248 | 229.2 | 815.1 | 127.4 | 0.0 | 152.8 |
| Al pl a | Non-native | OBL | 0.1 | 152.8 | 184.0 | 305.7 | 509.4 | 509.4 | 127.4 | 127.4 | 76.4 | 0.0 | 0.0 | 0.0 |
| Al pr | Non-native | FAC | 0.0 | 0.0 | 418.9 | 1,248 | 866.0 | 280.2 | 917.0 | 0.0 | 280.2 | 152.8 | 25.5 | 0.0 |
| Al ru | Native | FAC | 0.0 | 76.4 | 5.7 | 50.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Al sp | Non-native | FAC/OBL | 0.0 | 1,044 | 11.3 | 0.0 | 101.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| At fi | Native | FAC | 0.0 | 0.0 | 3,931 | 30,514 | 4,712 | 152.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Br sp | N/A | N/A | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ca ly | Native | OBL | 29.3 | 1,069 | 367.9 | 942.4 | 280.2 | 356.6 | 331.1 | 152.8 | 433.0 | 203.8 | 25.5 | 585.8 |
| Ca ob | Native | OBL | 0.0 | 50.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

| APPENDIX C TABLE 2: PLANT SPECIES AND MEAN OCCURRENCE: | | | | | | | | | | | | | | |
|---|---------------|-------------------|------------------------------|--------------------------------|--|--------------------|-----------|-----------|---------------------|-----------|-----------|-------------------|-----------|-----------|
| FIELD (STANDING PLANT COMMUNITY, % RELATIVE COVER/M ²), SEED BANK (SEEDS/M ²), AND THE GERMINATION EXPERIMENT (SEEDLINGS/M ²) | | | | | | | | | | | | | | |
| SPECIES CODE | NATIVE STATUS | WETLAND INDICATOR | FIELD % COVER/M ² | SEED BANK SEEDS/M ² | OVERALL GERMINATION SEEDLINGS/M ² | FRESHWATER (0 PPT) | | | OLIGOHALINE (3 PPT) | | | BRACKISH (10 PPT) | | |
| | | | | | | HIGH MARSH | MID-MARSH | LOW MARSH | HIGH MARSH | MID-MARSH | LOW MARSH | HIGH MARSH | MID-MARSH | LOW MARSH |
| Ca st | Non-native | OBL | 1.3 | 0.0 | 834.9 | 1,783 | 1,375 | 1,884 | 815.1 | 560.4 | 866.0 | 0.0 | 25.5 | 203.8 |
| Ci sp | Non-native | FACU | 0.0 | 229.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Co ma | Non-native | FAC | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| El ob | Native | OBL | 0.0 | 101.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| El pa | Native | OBL | 3.3 | 2,037 | 5.7 | 0.0 | 50.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ep ci | Native | FACW | 0.0 | 101.9 | 99.1 | 407.5 | 458.5 | 0.0 | 0.0 | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 |
| Er sp | N/A | N/A | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Fi sp | N/A | N/A | 0.0 | 280.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ga tr | Native | FACU | 0.2 | 0.0 | 11.3 | 101.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Gl sp | Native | OBL | 0.0 | 280.2 | 152.8 | 331.1 | 891.5 | 152.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Gn ul | Non-native | FAC | 0.0 | 0.0 | 135.8 | 178.3 | 636.8 | 382.1 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ju ba | Native | OBL | 0.0 | 0.0 | 348.1 | 229.2 | 636.8 | 967.9 | 738.7 | 407.5 | 50.9 | 0.0 | 76.4 | 25.5 |

| APPENDIX C TABLE 2: PLANT SPECIES AND MEAN OCCURRENCE: | | | | | | | | | | | | | | | |
|---|---------------|-------------------|------------------------------|--------------------------------|--|--------------------|-----------|-----------|---------------------|-----------|-----------|-------------------|-----------|-----------|--|
| FIELD (STANDING PLANT COMMUNITY, % RELATIVE COVER/M ²), SEED BANK (SEEDS/M ²), AND THE GERMINATION EXPERIMENT (SEEDLINGS/M ²) | | | | | | | | | | | | | | | |
| SPECIES CODE | NATIVE STATUS | WETLAND INDICATOR | FIELD % COVER/M ² | SEED BANK SEEDS/M ² | OVERALL GERMINATION SEEDLINGS/M ² | FRESHWATER (0 PPT) | | | OLIGOHALINE (3 PPT) | | | BRACKISH (10 PPT) | | | |
| | | | | | | HIGH MARSH | MID-MARSH | LOW MARSH | HIGH MARSH | MID-MARSH | LOW MARSH | HIGH MARSH | MID-MARSH | LOW MARSH | |
| Ju bu | Native | FACW | 0.0 | 687.7 | 25.5 | 50.9 | 0.0 | 0.0 | 178.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Ju ef | Non-native | FACW | 13.1 | 45,517 | 51,183 | 130,132 | 68,390 | 55,909 | 11,7116 | 19,332 | 34,921 | 21,956 | 8,380 | 4,508 | |
| Ju sp | N/A | FACW | 0.0 | 0.0 | 79.2 | 0.0 | 407.5 | 76.4 | 0.0 | 203.8 | 25.5 | 0.0 | 0.0 | 0.0 | |
| La ne | Native | N/A | 0.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Li oc | Native | OBL | 0.3 | 0.0 | 2.8 | 0.0 | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Li sc | Native | OBL | 0.0 | 0.0 | 8.5 | 0.0 | 50.9 | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Lo co | Non-native | FAC | 0.9 | 484.0 | 200.9 | 662.3 | 101.9 | 458.5 | 509.4 | 50.9 | 25.5 | 0.0 | 0.0 | 0.0 | |
| Ly nu | Non-native | FACW | 0.0 | 0.0 | 62.3 | 484.0 | 0.0 | 0.0 | 50.9 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | |
| My la | Native | OBL | 0.0 | 0.0 | 70.8 | 382.1 | 101.9 | 0.0 | 101.9 | 0.0 | 25.5 | 25.5 | 0.0 | 0.0 | |
| My sy | Non-native | OBL | 0.0 | 0.0 | 2.8 | 0.0 | 0.0 | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| Oe sa | Native | OBL | 5.8 | 1,477 | 232.1 | 560.4 | 509.4 | 229.2 | 560.4 | 101.9 | 101.9 | 0.0 | 25.5 | 0.0 | |
| Ph ar | Native | FACW | 30.1 | 6,724 | 3,000 | 7,361 | 4,789 | 3,006 | 5,094 | 2,140 | 2,191 | 1,223 | 611.3 | 585.8 | |

| APPENDIX C TABLE 2: PLANT SPECIES AND MEAN OCCURRENCE: | | | | | | | | | | | | | | |
|---|---------------|-------------------|------------------------------|--------------------------------|--|--------------------|-----------|-----------|---------------------|-----------|-----------|-------------------|-----------|-----------|
| FIELD (STANDING PLANT COMMUNITY, % RELATIVE COVER/M ²), SEED BANK (SEEDS/M ²), AND THE GERMINATION EXPERIMENT (SEEDLINGS/M ²) | | | | | | | | | | | | | | |
| SPECIES CODE | NATIVE STATUS | WETLAND INDICATOR | FIELD % COVER/M ² | SEED BANK SEEDS/M ² | OVERALL GERMINATION SEEDLINGS/M ² | FRESHWATER (0 PPT) | | | OLIGOHALINE (3 PPT) | | | BRACKISH (10 PPT) | | |
| | | | | | | HIGH MARSH | MID-MARSH | LOW MARSH | HIGH MARSH | MID-MARSH | LOW MARSH | HIGH MARSH | MID-MARSH | LOW MARSH |
| Pl sp | Non-native | FAC | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Po an | Native | OBL | 2.3 | 0.0 | 11.3 | 0.0 | 0.0 | 25.5 | 25.5 | 0.0 | 50.9 | 0.0 | 0.0 | 0.0 |
| Po pe | Non-native | FACW | 0.0 | 0.0 | 5.7 | 50.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Po pr | Non-native | FAC | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Po sp | Non-native | OBL | 0.0 | 50.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Po tr | Non-native | FAC | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Poa an | Non-native | FAC | 0.0 | 0.0 | 14.2 | 0.0 | 0.0 | 0.0 | 127.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Poa sp | Non-native | FAC | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ra re | Non-native | FAC | 0.0 | 178.3 | 84.9 | 152.8 | 229.2 | 229.2 | 101.9 | 0.0 | 50.9 | 0.0 | 0.0 | 0.0 |
| Ru ar | Non-native | FACU | 0.0 | 50.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ru sp | N/A | N/A | 0.0 | 50.9 | 19.8 | 25.5 | 25.5 | 101.9 | 0.0 | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 |
| Sc la | Native | OBL | 12.5 | 3,260 | 744.3 | 1,044 | 891.5 | 687.7 | 1,070 | 840.6 | 1,248 | 203.8 | 585.8 | 127.4 |

| APPENDIX C TABLE 2: PLANT SPECIES AND MEAN OCCURRENCE: | | | | | | | | | | | | | | |
|---|---------------|-------------------|------------------------------|--------------------------------|--|--------------------|-----------|-----------|---------------------|-----------|-----------|-------------------|-----------|-----------|
| FIELD (STANDING PLANT COMMUNITY, % RELATIVE COVER/M ²), SEED BANK (SEEDS/M ²), AND THE GERMINATION EXPERIMENT (SEEDLINGS/M ²) | | | | | | | | | | | | | | |
| SPECIES CODE | NATIVE STATUS | WETLAND INDICATOR | FIELD % COVER/M ² | SEED BANK SEEDS/M ² | OVERALL GERMINATION SEEDLINGS/M ² | FRESHWATER (0 PPT) | | | OLIGOHALINE (3 PPT) | | | BRACKISH (10 PPT) | | |
| | | | | | | HIGH MARSH | MID-MARSH | LOW MARSH | HIGH MARSH | MID-MARSH | LOW MARSH | HIGH MARSH | MID-MARSH | LOW MARSH |
| Sc mi | Native | OBL | 0.4 | 1,809 | 113.2 | 229.2 | 229.2 | 50.9 | 178.3 | 0.0 | 280.2 | 50.9 | 0.0 | 0.0 |
| St hu | Native | OBL | 0.0 | 0.0 | 25.5 | 101.9 | 127.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Sy su | Native | FACW | 0.02 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Tr re | Non-native | FAC | 0.0 | 178.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Tr sp | Non-native | FAC | 0.0 | 254.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ty sp | Native | OBL | 0.6 | 0.0 | 4,084 | 2,242 | 2,089 | 1,681 | 3,082 | 7,794 | 5,247 | 1,732 | 6,393 | 6,495 |
| UNK1 | Unknown | N/A | 0.0 | 178.3 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| UNK2 | Unknown | N/A | 0.0 | 0.0 | 268.9 | 331.1 | 484.0 | 101.9 | 458.5 | 152.8 | 127.4 | 280.2 | 331.1 | 152.8 |
| UNK3 | Unknown | N/A | 0.0 | 0.0 | 34.0 | 50.9 | 0.0 | 0.0 | 229.2 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 |
| UNK4 | Unknown | N/A | 0.0 | 0.0 | 59.4 | 0.0 | 25.5 | 50.9 | 254.7 | 203.8 | 0.0 | 0.0 | 0.0 | 0.0 |
| UNK5 | Unknown | N/A | 0.0 | 0.0 | 5.7 | 0.0 | 0.0 | 0.0 | 0.0 | 50.9 | 0.0 | 0.0 | 0.0 | 0.0 |
| UNK6 | Unknown | N/A | 0.0 | 0.0 | 34.0 | 0.0 | 25.5 | 0.0 | 76.4 | 0.0 | 25.5 | 0.0 | 50.9 | 127.4 |

**APPENDIX C TABLE 2: PLANT SPECIES AND MEAN OCCURRENCE:
FIELD (STANDING PLANT COMMUNITY, % RELATIVE COVER/M²), SEED BANK (SEEDS/M²), AND THE GERMINATION EXPERIMENT (SEEDLINGS/M²)**

| SPECIES CODE | NATIVE STATUS | WETLAND INDICATOR | FIELD % COVER/M ² | SEED BANK SEEDS/M ² | OVERALL GERMINATION SEEDLINGS/M ² | FRESHWATER (0 PPT) | | | OLIGOHALINE (3 PPT) | | | BRACKISH (10 PPT) | | |
|--------------|---------------|-------------------|---------------------------------|-----------------------------------|---|--------------------|-----------|-----------|---------------------|-----------|-----------|-------------------|-----------|-----------|
| | | | | | | HIGH MARSH | MID-MARSH | LOW MARSH | HIGH MARSH | MID-MARSH | LOW MARSH | HIGH MARSH | MID-MARSH | LOW MARSH |
| UNK7 | Unknown | N/A | 0.0 | 0.0 | 62.3 | 101.9 | 0.0 | 280.2 | 76.4 | 0.0 | 25.5 | 0.0 | 25.5 | 50.9 |
| Va sp | Native | N/A | 0.0 | 152.8 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Ve am | Native | OBL | 0.0 | 0.0 | 2.8 | 0.0 | 0.0 | 25.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Vi gi | Native | FAC | 0.9 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

APPENDIX D: SUPPLEMENTAL RESULTS - FIELD OBSERVATIONS STANDING PLANT COMMUNITY AND SEED BANK

Standing Plant Community and Seed Bank Data by Site - Overall 19 different plant species including 14 native, 4 non-native, and 1 of unknown status were identified during field surveys of the standing vegetation of the 1959 and 2007 restoration sites in April of 2015 (n=40 – 1 m² quadrats, Appendix D Table 10). All of the standing species surveyed were perennial in life duration, with a distribution of wetland indicator statuses of 10 OBL, 3 FACW, 4 FAC, 1 FACU, and 1 unknown. A total of 12 native and 2 non-native species were found on the 1959 restoration site (n=20), and a total of 9 native and 5 non-native species were identified in the 2007 restoration site (n=20). Overall, the 1959 site had the greatest abundance of *P. arundinacea* and *C. lyngbyei* plant communities, and the 2007 site had the greatest abundance of *J. effusus* and *S. lacustris* plant communities (Appendix D Table 10).

In comparison, the seed bank samples taken from these same field survey locations were composed of a total of 34 species including 13 native and 15 non-native species, in addition to 6 species of unknown origin (n=40 – 100 ml soil/1 m² quadrat, Appendix D Table 12). A majority of the seed bank species were perennial in life duration: 28 perennial, 5 annual, and 1 unknown, with a distribution of wetland indicator statuses of 9 OBL, 4 FACW, 11 FAC, 2 FACU, and 7 unknown. The seed bank of the 1959 site was composed of a total of 15 species, 8 native and 5 non-native, and the 2007 site seed bank consisted of a total of 34 species, 13 native and 15 non-native (n=20 each site, Appendix D Table 12). The most abundant species found in the 1959 site's seed bank were *P. arundinacea*, *S. microcarpus*, *S. lacustris*, *O. sarmetosa*, and *C. lyngbyei*, while the most abundant species found in the 2007 site's seed bank were *J. effusus*, *P. arundinacea*, *E. palustris*, *S. lacustris*, and *Alopecurus* species respectively (Appendix D Table 12). Only 10 of the total species identified in the seed bank were also found in the standing vegetation across the sites: 6 native, 3 non-native, 1 unknown (*Agrostis* sp) species (Table 1 and 2, Appendix A).

No significant difference was found in the standing plant community mean (n=20 - 1 m² quadrats per site) native and non-native cover (% relative abundance), total species richness, native species richness, or non-native species richness between the two sites (Table 3). In contrast, the seeds directly identified from the sites' seed banks did show significant differences in seed species composition among the sites (Table 3). The 1959 site had a mean (±SD) total seed bank species richness of 3.1 ± 1.2 which was significantly lower than the 2007 site which had a mean total species richness of 6.0 ± 2.2 (Table 3). This difference in seed bank species

richness among the sites was primarily from higher levels of non-native species identified out of the 2007 site, with that site having a significantly greater proportion of non-native species richness, 3.0 ± 1.1 , over the 1959 site, 1.3 ± 0.6 (Table 3). There was no significant difference found in native seed bank species richness between the sites (Table 3).

Appendix D Table 10: Field observations: standing plant species composition (% relative cover) by site, significant, Bonferroni corrected significance level $p < 0.01$, and marginally significant $p < 0.05$ differences highlighted.

| Field Observations: Standing Species Composition by Site - Mean Relative Cover (%) (n= 1 m ² , all species) | | | | | | | | | | | | | | |
|--|-------------------------|--------|------|---------|----------------|------|------|------------------|------|------|------------------|------|------|---------------|
| Latin | Common | Status | WIS | Code | Overall (n=40) | | | 1959 Site (n=20) | | | 2007 Site (n=20) | | | Man-Whitney U |
| | | | | | Mean | SD | SE | Mean | SD | SE | Mean | SD | SE | |
| <i>Agrostis sp.</i> | Bentgrass | NN | NI | Ag sp | 0.6 | 4 | 0.6 | | | | 1.3 | 5.6 | 0.9 | |
| <i>Alisma plantago-aquatica</i> | American water plantain | NAT | OBL | Al pa a | 0.2 | 0.8 | 0.1 | | | | 0.3 | 1.1 | 0.2 | |
| <i>Athyrium filix-femina</i> | Ladyfern | NAT | FAC | At fi | 0.03 | 0.2 | 0.03 | 0.1 | 0.2 | 0.04 | | | | N/A |
| <i>Carex Lyngbyei</i> | Lyngbye's sedge | NAT | OBL | Ca ly | 34.5 | 47 | 7.4 | 44.0 | 47.8 | 7.6 | 25.0 | 44.4 | 7.0 | 0.166 |
| <i>Callitriche stagnalis</i> | Starwort | NN | OBL | Ca st | 2.8 | 15.9 | 2.5 | 5.5 | 22.4 | 3.5 | 0.1 | 0.2 | 0.04 | |
| <i>Eleocharis palustris</i> | Spike rush | NAT | OBL | El pa | 5 | 14.1 | 2.2 | 0.5 | 2.2 | 0.4 | 9.5 | 19.0 | 3.0 | |
| <i>Galium triflorum</i> | Bedstraw | NAT | FACU | Ga tr | 0.2 | 0.8 | 0.1 | 0.5 | 1.1 | 0.2 | | | | |
| <i>Juncus effusus subsp. effusus</i> | Common rush | NN | FACW | Ju ef | 15.9 | 36.1 | 5.7 | | | | 31.8 | 46.3 | 7.3 | N/A |
| <i>Lathyrus nevadensis</i> | Purple peavine | NAT | NI | La ne | 0.2 | 0.8 | 0.1 | 0.3 | 1.1 | 0.2 | 0.1 | 0.2 | 0.04 | |
| <i>Lilaeopsis occidentalis</i> | Western grasswort | NAT | OBL | Li oc | 0.3 | 1.6 | 0.3 | 0.1 | 0.2 | 0.0 | 0.6 | 2.2 | 0.4 | |
| <i>Lotus corniculatus</i> | Birdsfoot trefoil | NN | FAC | Lo co | 1.7 | 8 | 1.3 | | | | 3.4 | 11.2 | 1.8 | |
| <i>Oenanthe sarmantosa</i> | Water parsley | NAT | OBL | Oe sa | 7.9 | 12.2 | 1.9 | 12.4 | 14.6 | 2.3 | 3.4 | 7.2 | 1.1 | |
| <i>Phalaris arundinacea</i> | Reed canarygrass | NN | FACW | Ph ar | 36.7 | 47.7 | 7.5 | 50.3 | 51.1 | 8.1 | 23.2 | 40.9 | 6.5 | 0.229 |
| <i>Potentilla anserina</i> | Silverweed cinquefoil | NAT | OBL | Po an | 3.8 | 9.9 | 1.6 | 3.4 | 7.3 | 1.1 | 4.2 | 12.1 | 1.9 | |
| <i>Scirpus lacustris</i> | Bulrush | NAT | OBL | Sc la | 17.5 | 38.5 | 6.1 | 10.0 | 30.8 | 4.9 | 25.0 | 44.4 | 7.0 | 0.184 |
| <i>Scirpus microcarpus</i> | Panicled bulrush | NAT | OBL | Sc mi | 0.5 | 2.5 | 0.4 | 0.8 | 3.4 | 0.5 | 0.3 | 1.1 | 0.2 | |
| <i>Symphotrichum subspicatum</i> | Douglas aster | NAT | FACW | Sy su | 0.03 | 0.2 | 0.03 | 0.1 | 0.2 | 0.0 | | | | |
| <i>Typha latifolia x angustifolia</i> | Cattail | Hybrid | OBL | Ty sp | 0.8 | 1.8 | 0.3 | 1.6 | 2.3 | 0.4 | 0.1 | 0.2 | 0.04 | 0.016 |
| <i>Vicia nigricans subsp. gigantea</i> | Giant vetch | NAT | FAC | Vi gi | 1.1 | 3.8 | 0.6 | 2.1 | 5.2 | 0.8 | | | | |

Appendix D: Supplemental Results - Field Observations Standing Plant Community and Seed Bank

Appendix D Table 11: Seed bank composition: frequency of occurrence of species identified through direct seed counts by site ($n=100\text{ ml/m}^2$ soil each), only species with a relative frequency $\geq 0.1\%$ shown. Significant, Bonferroni corrected significance level $p < 0.01$, and marginally significant $p < 0.05$ differences highlighted.

| Seed Bank Composition: Seed Direct Count by Site - Mean Relative Frequency (%) ($n=100\text{ ml soil each, only species with } \geq 0.1\%$) | | | | | | | | | | | | | | |
|---|-------------------------|--------|---------|---------|----------------|------|-----|------------------|------|-----|------------------|------|-----|---------------|
| Latin | Common | Status | WIS | Code | Overall (n=40) | | | 1959 Site (n=20) | | | 2007 Site (n=20) | | | Man-Whitney U |
| | | | | | Mean | SD | SE | Mean | SD | SE | Mean | SD | SE | |
| <i>Agrostis</i> | Bentgrass | N/A | FAC | Ag sp | 0.9 | 5.3 | 0.8 | | | | 1.8 | 7.5 | 1.7 | |
| <i>Alisma plantago-aquatica L.</i> | American water plantain | NN | OBL | Al pl a | 0.2 | 0.7 | 0.1 | | | | 0.3 | 1.0 | 0.2 | |
| <i>Alnus rubra</i> | Red alder | NAT | FAC | Al ru | 0.2 | 0.7 | 0.1 | 0.1 | 0.5 | 0.1 | 0.2 | 0.8 | 0.2 | |
| <i>Alopecurus</i> | Foxtail species | NN | FAC/OBL | Al sp | 1.1 | 4.1 | 0.7 | | | | 2.2 | 5.7 | 1.3 | |
| <i>Carex Lyngbyei</i> | Sedge | NAT | OBL | Ca ly | 6.4 | 15.7 | 2.5 | 11.7 | 20.8 | 4.7 | 1.1 | 3.4 | 0.8 | 0.0528 |
| <i>Carex obnupta</i> | Slough sedge | NAT | OBL | Ca ob | 1.5 | 6.5 | 1.0 | 2.9 | 9.1 | 2.0 | | | | |
| <i>Cirsium spp</i> | Thistle | NN | FACU | Ci spp | 0.8 | 3.8 | 0.6 | | | | 1.7 | 5.3 | 1.2 | |
| <i>Conium maculatum</i> | Poison hemlock | NN | FAC | Co ma | 0.1 | 0.7 | 0.1 | 0.2 | 1.0 | 0.2 | | | | |
| <i>Eleocharis obtusa</i> | Blunt spike rush | NAT | OBL | El ob | 0.1 | 0.7 | 0.1 | | | | 0.2 | 1.0 | 0.2 | |
| <i>Eleocharis palustris</i> | Spike rush | NAT | OBL | El pa | 2.2 | 8.7 | 1.4 | | | | 4.4 | 12.1 | 2.7 | |
| <i>Epilobium ciliatum</i> | Willow herb | NAT | FACW | Ep ci | 0.3 | 1.4 | 0.2 | | | | 0.5 | 1.9 | 0.4 | |
| <i>Eragrostis spp.</i> | Lovegrass | N/A | N/A | Er spp | 0.2 | 1.0 | 0.2 | 0.3 | 1.4 | 0.3 | | | | |
| <i>Fimbristylis spp.</i> | Rush | N/A | N/A | Fi spp | 0.7 | 4.3 | 0.7 | | | | 1.4 | 6.1 | 1.4 | |
| <i>Glyceria spp.</i> | Mannagrass | NAT | OBL | Gl spp | 0.3 | 0.9 | 0.1 | | | | 0.5 | 1.2 | 0.3 | |
| <i>Juncus bufonius</i> | Toad rush | NAT | FACW | Ju bu | 0.7 | 2.3 | 0.4 | | | | 1.4 | 3.2 | 0.7 | |
| <i>Juncus effusus</i> | Soft rush | NN | FACW | Ju ef | 34.8 | 38.3 | 6.0 | 1.2 | 3.1 | 0.7 | 68.4 | 24.8 | 5.6 | 0.000 |
| <i>Lotus corniculatus</i> | Birdsfoot trefoil | NN | FAC | Lo co | 0.5 | 1.3 | 0.2 | | | | 1.0 | 1.7 | 0.4 | |
| <i>Oenanthe Sarmetosa</i> | Water parsley | NAT | OBL | Oe sa | 7.4 | 17.7 | 2.8 | 13.7 | 23.6 | 5.3 | 1.2 | 2.6 | 0.6 | |
| <i>Phalaris arundinacea</i> | Reed canarygrass | NAT | FACW | Ph ar | 24.9 | 33.3 | 5.3 | 45.2 | 36.2 | 8.1 | 4.6 | 9.8 | 2.2 | 0.000 |
| <i>Poa pratensis L.</i> | Kentucky bluegrass | NN | FAC | Po pr | 0.1 | 0.4 | 0.1 | | | | 0.1 | 0.6 | 0.1 | |
| <i>Poa trivialis</i> | Rough bluegrass | NN | FAC | Po tr | 0.1 | 0.2 | 0.0 | | | | 0.1 | 0.3 | 0.1 | |
| <i>Poa sp unknown 1</i> | Poa sp unknown 1 | NN | FAC | Poa sp | 0.2 | 1.2 | 0.2 | 0.4 | 1.7 | 0.4 | | | | |
| <i>Ranunculus repens</i> | Creeping buttercup | NN | FAC | Ra re | 0.3 | 1.1 | 0.2 | | | | 0.6 | 1.5 | 0.3 | |
| <i>Rubus armeniacus (fruticosus)</i> | Himalayan blackberry | NN | FACU | Ru ar | 0.2 | 0.8 | 0.1 | 0.2 | 1.0 | 0.2 | 0.1 | 0.6 | 0.1 | |
| <i>Rumex sp.</i> | Dock | N/A | N/A | Ru sp | 0.1 | 0.2 | 0.0 | | | | 0.1 | 0.3 | 0.1 | |
| <i>Scirpus lacustris</i> | Bulrush | NAT | OBL | Sc la | 9.0 | 17.4 | 2.7 | 12.4 | 22.6 | 5.1 | 5.6 | 9.1 | 2.0 | 0.345 |
| <i>Scirpus microcarpus</i> | Panicle bulrush | NAT | OBL | Sc mi | 5.1 | 14.4 | 2.3 | 9.6 | 19.4 | 4.3 | 0.7 | 2.4 | 0.5 | |
| <i>Trifolium repens</i> | Clover | NN | FAC | Tr re | 0.2 | 0.6 | 0.1 | 0.1 | 0.5 | 0.1 | 0.3 | 0.7 | 0.2 | |
| <i>Trifolium sp</i> | Clover sp. | NN | FAC | Tr sp | 0.2 | 1.0 | 0.2 | | | | 0.3 | 1.4 | 0.3 | |
| <i>Unknown seed</i> | Unknown seed | N/A | N/A | UNK1 | 1.1 | 4.2 | 0.7 | 1.7 | 5.7 | 1.3 | 0.4 | 1.9 | 0.4 | |
| <i>Vaccinium sp.</i> | Blueberry | NAT | N/A | Va sp | 0.2 | 1.0 | 0.2 | 0.2 | 1.0 | 0.2 | 0.3 | 1.0 | 0.2 | |

Appendix D Table 12: Field and seed bank composition summary by site – standing vegetation and seeds identified out of the soil. Significant, Bonferroni corrected significance level $p < 0.004$, and marginally significant $p < 0.05$ differences highlighted.

| Field and Seed Bank Composition Summary by Site – Standing Vegetation and Seeds Identified out of the Soil | | | | | | | | | | | | | | |
|--|-----------------------------------|-------------------------|------|------|-----|-----------|------|------|-----|-----------|------|------|------|---------------|
| Parameters | | Overall | | | | 1959 Site | | | | 2007 Site | | | | Man-Whitney U |
| | | n | Mean | SD | SE | n | Mean | SD | SE | n | Mean | SD | SE | |
| Standing Vegetation | Native relative cover (%) | 40 | 53.7 | 44.8 | 7.1 | 20 | 53.3 | 43.4 | 9.7 | 20 | 54.0 | 47.3 | 10.6 | 0.466 |
| | Non-native relative cover (%) | 40 | 45.4 | 44.7 | 7.1 | 20 | 45.5 | 43.2 | 9.7 | 20 | 45.3 | 47.2 | 10.6 | 0.294 |
| | Species richness | 40 | 3.4 | 1.4 | 0.2 | 20 | 3.5 | 1.3 | 0.3 | 20 | 3.3 | 1.4 | 0.3 | 0.956 |
| | Native species richness | 40 | 2.3 | 1.4 | 0.2 | 20 | 2.6 | 1.3 | 0.3 | 20 | 2.0 | 1.4 | 0.3 | 0.256 |
| | Non-native species richness | 40 | 0.9 | 0.9 | 0.1 | 20 | 0.6 | 0.5 | 0.1 | 20 | 1.2 | 1.1 | 0.2 | 0.066 |
| | Shannon Diversity Index | 40 | 0.5 | 0.3 | 0.1 | 20 | 0.6 | 0.3 | 0.1 | 20 | 0.5 | 0.4 | 0.1 | 0.255 |
| Seed Composition | Native relative abundance (%) | 40 | 34.3 | 33.0 | 5.2 | 20 | 50.6 | 35.8 | 8.0 | 20 | 18.0 | 19.9 | 4.5 | 0.010 |
| | Non-native relative abundance (%) | 40 | 63.5 | 33.9 | 5.4 | 20 | 47.7 | 35.8 | 8.0 | 20 | 79.3 | 23.3 | 5.2 | 0.012 |
| | Total species richness | 40 | 4.6 | 2.3 | 0.4 | 20 | 3.1 | 1.2 | 0.3 | 20 | 6.0 | 2.2 | 0.5 | 0.000 |
| | Native species richness | 40 | 2.2 | 1.5 | 0.2 | 20 | 1.7 | 1.0 | 0.2 | 20 | 2.7 | 1.8 | 0.4 | 0.107 |
| | Non-native species richness | 40 | 2.1 | 1.2 | 0.2 | 20 | 1.3 | 0.6 | 0.1 | 20 | 3.0 | 1.1 | 0.2 | 0.000 |
| | | Shannon Diversity Index | 40 | 0.8 | 0.4 | 0.1 | 20 | 0.8 | 0.4 | 0.1 | 20 | 0.9 | 0.5 | 0.1 |

Environmental Conditions by Site - Environmental conditions across the sites and sample locations varied significantly. The elevation range of the 1959 site was significantly higher than the 2007 site, averaging about 0.2 meters (0.5 ft) higher, with a mean elevation of 2.4 ± 0.4 m (7.8 ± 1.3 ft) compared to the 2007 site which had a mean elevation of 2.2 ± 0.3 m (7.3 ± 0.9 ft) (Table 4). Given this difference in elevation the lower 2007 site experiences significantly greater tidal flooding (on average flooded 38% more frequently) and duration (on average flooded for 1.3 hours longer per high tide) than the higher 1959 site (Table 4, Map 2). This difference in tidal flooding frequency and duration was further highlighted by the difference in soil ORP conditions observed between the sites, with the 2007 site having significantly lower ORP conditions averaging at 149.5 ± 69.6 mV compared to the 1959 sites average of 210.4 ± 89.9 mV. Soil conductivity and salinity were significantly higher on the 1959 site likely due to the lack of freshwater fluvial input on the site compared to the 2007 site (Map 2). The 1959 site also had significantly greater soil salinity levels, 387.4 ± 145.8 ppm, compared to the 2007 site at 300.5 ± 119.1 ppm. Marginal differences (not significant with Bonferroni correction) in soil bulk density, organic matter, and available nitrogen (calculated based on organic matter content) were also observed, with the 1959 site having slightly more organic matter, estimated available nitrogen, and less bulk density than the 2007 site (Table 4). The 48-year difference in site age is a likely explanatory factor for these differences, soil organic matter accumulating and bulk density reducing slowly after tidal reconnection (Kidd and Yeakley, in press, Table 4). No significant differences were identified between the sites' soil moisture, pH, texture (% sand, silt, and clay), Phosphorus (Bray II) content, or Total Exchange Capacity (Table 4).

Appendix D Table 13: Field observations: environmental conditions summarized by site, significant, Bonferroni corrected significance level $p < 0.003$, and marginally significant $p < 0.05$ differences highlighted.

| Field Observations: Environmental Conditions by Site | | | | | | | | | | | | | |
|--|---------|-------|-------|------|-----------|-------|-------|-----|-----------|-------|-------|-----|---------------|
| Parameters | Overall | | | | 1959 Site | | | | 2007 Site | | | | Man-Whitney U |
| | n | Mean | SD | SE | n | Mean | SD | SE | n | Mean | SD | SE | |
| Elevation (ft) | 40 | 7.6 | 1.1 | 0.2 | 20 | 7.8 | 1.3 | 0.1 | 20 | 7.3 | 0.9 | 0.1 | 0.001 |
| Elevation (m) | 40 | 2.3 | 0.3 | 0.1 | 20 | 2.4 | 0.4 | 0.0 | 20 | 2.2 | 0.3 | 0.0 | |
| Abundance flooded twice a day (% , March 2015) | 40 | 59.5 | 28.8 | 4.6 | 20 | 40.2 | 23.4 | 5.2 | 20 | 78.8 | 19.5 | 4.4 | 0.000 |
| Duration of each flooding event (hr, March 2015) | 40 | 2.0 | 1.6 | 0.3 | 20 | 1.3 | 1.7 | 0.4 | 20 | 2.6 | 1.3 | 0.3 | 0.000 |
| Bulk density (g/cm ²) | 28 | 0.4 | 0.1 | 0.0 | 13 | 0.3 | 0.1 | 0.0 | 15 | 0.4 | 0.1 | 0.0 | 0.007 |
| Organic Matter (% OM) | 38 | 15.2 | 7.6 | 1.2 | 18 | 17.8 | 8.2 | 0.4 | 20 | 12.9 | 6.4 | 0.4 | 0.033 |
| Soil moisture (%) | 38 | 62.7 | 9.0 | 1.5 | 18 | 66.3 | 8.2 | 0.2 | 20 | 59.4 | 8.5 | 0.2 | 0.047 |
| Field Conductivity (µS/cm) | 40 | 801.1 | 290.6 | 46.0 | 20 | 973.8 | 227.3 | 2.2 | 20 | 628.4 | 242.8 | 2.2 | 0.000 |
| Field ORP (mV) | 40 | 180.0 | 85.2 | 13.5 | 20 | 210.4 | 89.9 | 1.3 | 20 | 149.5 | 69.6 | 1.3 | 0.004 |
| Field pH | 40 | 6.1 | 0.9 | 0.1 | 20 | 6.0 | 1.2 | 0.0 | 20 | 6.2 | 0.4 | 0.0 | 0.393 |
| Field Salinity (ppm) | 40 | 387.4 | 145.8 | 23.0 | 20 | 474.3 | 116.3 | 1.5 | 20 | 300.5 | 119.1 | 1.5 | 0.000 |
| Field Temp (°C) | 40 | 12.5 | 1.4 | 0.2 | 20 | 12.5 | 0.9 | 0.1 | 20 | 12.5 | 1.7 | 0.1 | 0.645 |
| Clay (%) | 32 | 3.7 | 2.6 | 0.5 | 15 | 3.5 | 1.3 | 0.4 | 17 | 3.9 | 3.4 | 0.4 | 0.433 |
| Sand (%) | 32 | 70.5 | 10.9 | 1.9 | 15 | 72.3 | 5.1 | 0.4 | 17 | 69.0 | 14.2 | 0.4 | 0.246 |
| Silt (%) | 32 | 25.8 | 9.2 | 1.6 | 15 | 24.2 | 5.1 | 0.5 | 17 | 27.1 | 11.6 | 0.5 | 0.261 |
| Bray II P (mg/kg) | 38 | 50.9 | 15.6 | 2.5 | 18 | 54.4 | 14.5 | 0.5 | 20 | 47.9 | 16.3 | 0.5 | 0.260 |
| Estimated Nitrogen Release (N/acre) *calculated based on % OM | 38 | 126.0 | 10.2 | 1.6 | 18 | 129.5 | 8.2 | 0.2 | 20 | 122.9 | 10.9 | 0.2 | 0.036 |
| Total Exchange Capacity (meq/100 g) | 38 | 28.2 | 8.8 | 1.4 | 18 | 26.0 | 6.8 | 0.4 | 20 | 30.1 | 10.0 | 0.4 | 0.303 |

Standing Plant Community and Seed Bank Data by Dominant Plant Species Seed

Bank Sample Locations - Among the dominant native and non-native standing plant communities sampled *P. arundinacea* had the greatest overall standing vegetation species richness with a total of 13 species, 9 native and 3 non-native identified, followed by *S. lacustris* with a total of 11 species, 7 native and 3 non-native, *C. lyngbyei* with a total of 10 species, 8 native and 1 non-native, and *J. effusus* with 7 total species, 4 native, and 3 non-native (Table 5). Only 4 species were found in common among all of the dominant plant communities including 1 non-native species, *P. arundinacea*, and 3 native species, *P. anserine*, *O. sarmetosa*, and *E. palustris* (Table 5). In comparison, the seed bank total species richness was also similar among the different plant communities with 19 total species identified in the *P. arundinacea* (8 native, 7 non-native, 4 unknown), *C. lyngbyei* (9 native, 7 non-native, 3 unknown), and *J. effusus* (7 native, 9 non-native, 3 unknown) seed banks and 20 total species in the *S. lacustris* (11 native, 6 non-native, 3 unknown) seed bank (Table 6). A total of 7 species were found in common among all of the different plant community seed banks including 4 native species, *S. lacustris*, *S. microcarpus*, *O. sarmetosa*, and *Glyceria sp.*, and 3 non-native species *J. effusus*, *Alopecurus sp.*, and *Trifolium repens* (Table 6).

On average native species richness was found to be significantly greater in the *S. lacustris* standing plant community, 3.4 ± 1.0 , compared to the *P. arundinacea*, 1.7 ± 1.2 , and *J. effusus*, 1.3 ± 1.0 , plant communities, but not significantly different than the *C. lyngbyei* plant community native species richness, 2.7 ± 1.3 (Table 7). Average non-native species richness was significantly greater in the *J. effusus* plant community, 2.3 ± 0.8 , and lowest in the *C. lyngbyei* plant community, 0.2 ± 0.4 (Table 7). Total standing species richness and Shannon Diversity Indices were not significantly different among the 4 different plant communities (Table 7).

In comparison, native seed bank species richness was found to be significantly greater in the *S. lacustris*, 3.6 ± 2.4 , and *C. lyngbyei*, 2.2 ± 0.8 , seed banks compared to the *P. arundinacea* seed bank, 1.4 ± 0.8 . The *J. effusus* seed bank's native species richness, 2.7 ± 1.8 , was not significantly different from the others (Table 7). On average the relative abundance of native seeds identified from the different seed banks was significantly greater in the *C. lyngbyei* seed bank, $55.1 \pm 33.8\%$, compared to the *P. arundinacea*, $26.2 \pm 33.5\%$, and *J. effusus*, $12.6 \pm 13.8\%$, seed banks. The *S. lacustris* seed bank's native seed abundance, $30.8 \pm 25.5\%$, was not found to be significantly different from the others (Table 7). There was no significant difference in relative non-native seed abundance, total species richness, non-native species richness, or Shannon Diversity Indices among the different seed banks.

Appendix D: Supplemental Results - Field Observations Standing Plant Community and Seed Bank

Appendix D Table 5: Dominant plant community field observations: standing species composition. Significant Kruskal-Wallis analysis, Bonferroni significance level $p < 0.004$, and marginally significant $p < 0.05$ differences highlighted. Pairwise, Man-Whitney U tests, significant differences within species among plant communities marked by differing letters, Bonferroni corrected significance level $p < 0.003$, marginally significant $p < 0.05$ differences also marked with an *.

| Dominant Plant Community Field Observations: Standing Species Composition – Mean Relative Cover (%; n= 1 m ²) | | | | | | | | | | | | | | | |
|---|--------|------|-----------------------------------|------|-----|-----------------------------|------|-----|---------------------------------|------|-----|-------------------------|------|------|----------------|
| Code | Status | WIS | Reed canarygrass (Ph ar, n=14) | | | Common Rush (Ju ef, n=6) | | | Lyngbyeï sedge (Ca ly, n=13) | | | Bulrush (Sc la, n=7) | | | Kruskal-Wallis |
| | | | Mean | SD | SE | Mean | SD | SE | Mean | SD | SE | Mean | SD | SE | |
| Ag sp | NN | NI | 1.8 | 6.7 | 1.8 | | | | | | | | | | NA |
| Al pa a | NAT | OBL | | | | | | | | | | 0.9 | 1.9 | 0.7 | NA |
| At fi | NAT | FAC | 0.1 | 0.3 | 0.1 | | | | | | | | | | NA |
| Ca ly | NAT | OBL | 3.6 ^a | 9.3 | 2.5 | | | | 100.0 ^b | 0.0 | 0.0 | 4.3 ^a | 11.3 | 4.3 | 0.000 |
| Ca st | NN | OBL | | | | | | | | | | 15.9 | 37.3 | 14.1 | NA |
| El pa | NAT | OBL | 1.8 ^a | 6.7 | 1.8 | 1.7 ^{ab} | 4.1 | 1.7 | 0.8 ^a | 2.8 | 0.8 | 22.1 ^{bc*} | 27.4 | 10.3 | 0.003 |
| Ga tr | NAT | FACU | 0.1 ^a | 0.4 | 0.1 | | | | 0.5 ^b | 1.4 | 0.4 | | | | 0.359 |
| Ju ef | NN | FACW | 2.5 ^a | 8.0 | 2.1 | 100.0 ^b | 0.0 | 0.0 | | | | | | | 0.000 |
| La ne | NAT | NI | 0.1 | 0.3 | 0.1 | | | | 0.5 | 1.4 | 0.4 | | | | 0.528 |
| Li oc | NAT | OBL | | | | 0.2 | 0.4 | 0.2 | | | | 1.7 | 3.7 | 1.4 | 0.009 |
| Lo co | NN | FAC | | | | 11.0 ^a | 19.5 | 8.0 | | | | 0.1 ^{b*} | 0.4 | 0.1 | 0.000 |
| Oe sa | NAT | OBL | 3.8 | 9.2 | 2.5 | 3.5 | 6.0 | 2.4 | 17.3 | 15.2 | 4.2 | 2.3 | 2.6 | 1.0 | 0.064 |
| Ph ar | NN | FACW | 100.0 ^a | 0 | 0 | 9.5 ^b | 19.9 | 8.1 | 0.5 ^c | 1.4 | 0.4 | 0.7 ^{c*} | 1.9 | 0.7 | 0.000 |
| Po an | NAT | OBL | 6.1 | 14.9 | 4.0 | 4.5 | 10.1 | 4.1 | 2.8 | 4.3 | 1.2 | 0.3 | 0.5 | 0.2 | 0.809 |
| Sc la | NAT | OBL | | | | | | | | | | 100.0 | 0.0 | 0.0 | NA |
| Sc mi | NAT | OBL | 0.4 | 1.3 | 0.4 | | | | 1.2 | 4.2 | 1.2 | | | | 0.801 |
| Sy su | NAT | FACW | | | | | | | 0.1 | 0.3 | 0.1 | | | | NA |
| Ty sp | Hybrid | OBL | 1.5 | 2.3 | 0.6 | | | | 0.8 | 1.9 | 0.5 | 0.1 | 0.4 | 0.1 | 0.261 |
| Vi gi | NAT | FAC | 3.0 | 6.1 | 1.6 | | | | | | | | | | NA |

Paired Man-Whitney U tests (sig level p-value <0.003, if denoted with a* then pairwise p-values ≤0.05 but >0.003) differing letters indicate significant differences

Appendix D: Supplemental Results - Field Observations Standing Plant Community and Seed Bank

Appendix D Table 6: Dominant plant community seed bank composition: frequency of occurrence of species identified through direct seed counts ($n=100 \text{ ml/m}^2$ soil each), only species with a relative frequency $\geq 1\%$ shown. Significant Kruskal-Wallis analysis, Bonferroni significance level $p < 0.01$, and marginally significant $p < 0.05$ differences highlighted. Pairwise, Mann-Whitney U tests, significant differences within summary metrics among plant communities marked by differing letters, Bonferroni corrected significance level $p < 0.002$, marginally significant $p < 0.05$ differences also marked with an*.

| Seed Bank Composition: Seed Direct Count Relative Frequency (%) by Dominant Plant Communities (n=100 ml soil each, only species with $\geq 0.1\%$) | | | | | | | | | | | | | | | |
|--|--------|---------|-----------------------------------|------|-----|-----------------------------|------|-----|---------------------------------|------|------|-------------------------|------|------|--------------------|
| Parameters | | | Reed canarygrass (Ph ar, n=14) | | | Common Rush (Ju ef, n=6) | | | Lyngbyei sedge (Ca ly, n=13) | | | Bulrush (Sc la, n=7) | | | Kruskal -Wallis |
| Code | Status | WIS | Mean | SD | | Mean | SD | SE | Mean | SD | SE | Mean | SD | SE | |
| Ag sp | N/A | FAC | 2.4 | 9.0 | 2.4 | | | | 0.2 | 0.6 | 0.2 | 0.1 | 0.3 | 0.1 | |
| Al pl a | NN | OBL | | | | 0.7 | 1.8 | 0.7 | | | | 0.2 | 0.6 | 0.2 | |
| Al ru | NAT | FAC | 0.2 | 0.6 | 0.2 | | | | | | | 0.5 | 1.4 | 0.5 | |
| Al sp | NN | FAC/OBL | 0.6 | 1.9 | 0.5 | 2.6 | 6.3 | 2.6 | 0.1 | 0.3 | 0.1 | 3.0 | 7.8 | 3.0 | |
| Ca ly | NAT | OBL | 0.5 ^a | 1.3 | 0.3 | | | | 19.1 ^b | 23.2 | 6.4 | 0.3 ^b | 0.8 | 0.3 | 0.003 |
| Ca ob | NAT | OBL | | | | | | | 4.5 | 11.1 | 3.1 | | | | |
| Ci spp | NN | FACU | 0.1 | 0.3 | 0.1 | | | | 0.7 | 2.1 | 0.6 | 3.3 | 8.7 | 3.3 | |
| Co ma | NN | FAC | 0.3 | 1.2 | 0.3 | | | | | | | | | | |
| El ob | NAT | OBL | | | | | | | | | | 0.6 | 1.6 | 0.6 | |
| El pa | NAT | OBL | | | | 0.2 | 0.4 | 0.2 | 2.9 | 10.4 | 2.9 | 7.2 | 15.3 | 5.8 | |
| Ep ci | NAT | FACW | | | | 0.4 | 1.0 | 0.4 | 0.6 | 2.3 | 0.6 | | | | |
| Er spp | N/A | N/A | | | | | | | | | | 0.9 | 2.4 | 0.9 | |
| Fi spp | N/A | N/A | | | | 4.6 | 11.2 | 4.6 | | | | | | | |
| Gl spp | NAT | OBL | 0.0 | 0.1 | 0.0 | 0.8 | 1.8 | 0.7 | 0.2 | 0.9 | 0.2 | 0.3 | 0.8 | 0.3 | |
| Ju bu | NAT | FACW | | | | 2.7 | 3.1 | 1.3 | | | | 1.7 | 4.5 | 1.7 | |
| Ju ef | NN | FACW | 18.6 ^a | 30.6 | 8.2 | 79.9 ^b | 18.9 | 7.7 | 28.3 ^a | 37.2 | 10.3 | 40.9 ^{ab} | 40.4 | 15.3 | 0.009 |
| Lo co | NN | FAC | | | | 2.2 | 2.3 | 0.9 | 0.3 | 0.7 | 0.2 | 0.5 | 1.2 | 0.5 | |
| Oe sa | NAT | OBL | 2.9 | 8.4 | 2.2 | 0.4 | 0.6 | 0.2 | 12.3 | 17.8 | 4.9 | 13.8 | 32.6 | 12.3 | |
| Ph ar | NAT | FACW | 59.9 ^a | 33.1 | 8.9 | | | | 12.1 ^b | 12.0 | 3.3 | 0.2 ^b | 0.4 | 0.2 | 0.000 |
| Pl sp | NN | FAC | | | | 0.2 | 0.4 | 0.2 | | | | | | | |
| Po pr | NN | FAC | | | | | | | 0.2 | 0.7 | 0.2 | | | | |
| Po tr | NN | FAC | | | | 0.2 | 0.4 | 0.2 | | | | | | | |
| Poa an | NN | FAC | | | | | | | | | | 1.1 | 2.9 | 1.1 | |
| Ra re | NN | FAC | 0.2 | 0.6 | 0.2 | 0.6 | 1.3 | 0.6 | 0.4 | 1.5 | 0.4 | | | | |
| Ru ar | NN | FACU | 0.5 | 1.4 | 0.4 | | | | | | | | | | |
| Ru sp | N/A | N/A | 0.1 | 0.2 | 0.1 | 0.2 | 0.4 | 0.2 | | | | | | | |
| Sc la | NAT | OBL | 2.4 ^a | 7.6 | 2.0 | 2.6 ^{ab} | 3.9 | 1.6 | 11.6 ^{ab} | 16.2 | 4.5 | 22.9 ^b | 30.3 | 11.4 | 0.023 |
| Sc mi | NAT | OBL | 10.3 | 19.7 | 5.3 | 0.1 | 0.3 | 0.1 | 3.8 | 13.9 | 3.8 | 1.5 | 2.8 | 1.1 | |
| Tr re | NN | FAC | 0.2 | 0.7 | 0.2 | 0.6 | 1.0 | 0.4 | 0.1 | 0.4 | 0.1 | | | | |
| Tr sp | NN | FAC | | | | 1.0 | 2.6 | 1.0 | | | | | | | |
| UNK1 | N/A | N/A | 0.6 | 1.7 | 0.5 | 0.0 | 0.1 | 0.0 | 2.6 | 7.1 | 2.0 | | | | |
| Va sp | NAT | N/A | 0.3 | 1.2 | 0.3 | | | | | | | 0.7 | 1.6 | 0.6 | |

Paired Man-Whitney U tests differing letters indicate significant differences (sig level p-value <0.002, if denoted with a* then pairwise p-values ≤ 0.05 but >0.002)

Appendix D Table 7: Field and seed bank composition summary dominant plant community status – standing vegetation and seeds identified from the soil. Significant Kruskal-Wallis analysis, Bonferroni significance level $p < 0.004$, and marginally significant $p < 0.05$ differences highlighted. Pairwise, Man-Whitney U tests, significant differences within summary metrics among plant communities marked by differing letters. Bonferroni corrected significance level $p < 0.001$, marginally significant $p < 0.05$ differences also marked with an *.

| Field and Seed Bank Composition Summary by Dominant Plant Community – Standing Vegetation and Seeds Identified out of the Soil | | | | | | | | | | | | | | | | | | |
|--|-----------------------------------|--------------------------|-------------------|------|-----|---------------------|-------------------|------|-----|------------------------|--------------------|------|-----|-----------------|--------------------|------|------|----------------|
| Parameters | | Reed canarygrass (Ph ar) | | | | Common rush (Ju ef) | | | | Lyngbyei sedge (Ca ly) | | | | Bulrush (Sc la) | | | | Kruskal-Wallis |
| | | n | Mean | SD | SE | n | Mean | SD | SE | n | Mean | SD | SE | n | Mean | SD | SE | |
| Standing Vegetation | Native relative cover (%) | 14 | 12.5 ^a | 13.8 | 3.7 | 6 | 6.6 ^a | 7.8 | 3.2 | 13 | 99.1 ^b | 1.6 | 0.5 | 7 | 91.9 ^{b*} | 15.9 | 0.6 | 0.000 |
| | Non-native relative cover (%) | 14 | 85.5 ^a | 16.3 | 4.3 | 6 | 93.4 ^a | 7.8 | 3.2 | 13 | 0.4 ^b | 1.1 | 0.3 | 7 | 7.9 ^{c*} | 15.9 | 6.0 | 0.000 |
| | Total species richness | 14 | 3.1 | 1.5 | 0.3 | 6 | 3.7 | 0.8 | 0.4 | 13 | 3.0 | 1.4 | 0.4 | 7 | 4.3 | 1.1 | 0.4 | 0.220 |
| | Native species richness | 14 | 1.7 ^a | 1.2 | 0.3 | 6 | 1.3 ^a | 1.0 | 0.4 | 13 | 2.7 ^{ab} | 1.3 | 0.4 | 7 | 3.4 ^{b*} | 1.0 | 0.4 | 0.009 |
| | Non-native species richness | 14 | 1.0 ^a | 0.6 | 0.1 | 6 | 2.3 ^{b*} | 0.8 | 0.3 | 13 | 0.2 ^c | 0.4 | 0.1 | 7 | 0.7 ^{ac*} | 0.8 | 0.3 | 0.000 |
| | Shannon Diversity Index | 14 | 0.5 | 0.4 | 0.1 | 6 | 0.5 | 0.4 | 0.1 | 13 | 0.5 | 0.3 | 0.1 | 7 | 0.7 | 0.2 | 0.0 | 0.335 |
| Seed Composition | Native relative abundance (%) | 14 | 16.6 ^a | 21.8 | 5.8 | 6 | 12.6 ^a | 13.8 | 5.6 | 13 | 55.1 ^{b*} | 33.8 | 9.4 | 7 | 50.0 ^{b*} | 34.5 | 13.1 | 0.003 |
| | Non-native relative abundance (%) | 14 | 82.3 ^a | 21.6 | 5.8 | 6 | 84.8 ^a | 17.1 | 7.0 | 13 | 42.3 ^{b*} | 33.3 | 9.2 | 7 | 47.1 ^{b*} | 37.6 | 14.2 | 0.006 |
| | Total species richness | 14 | 3.6 | 1.8 | 0.5 | 6 | 5.8 | 2.8 | 1.1 | 13 | 4.2 | 1.6 | 0.4 | 7 | 5.7 | 2.9 | 1.1 | 0.065 |
| | Native species richness | 14 | 1.4 ^{a*} | 0.8 | 0.2 | 6 | 2.7 ^{ab} | 1.8 | 0.7 | 13 | 2.2 ^b | 0.8 | 0.2 | 7 | 3.6 ^b | 2.2 | 0.8 | 0.020 |
| | Non-native species richness | 14 | 2.0 | 1.3 | 0.3 | 6 | 2.8 | 1.2 | 0.5 | 13 | 1.8 | 1.3 | 0.4 | 7 | 2.0 | 0.8 | 0.3 | 0.319 |
| | Shannon Diversity Index | 14 | 0.6 | 0.3 | 0.1 | 6 | 0.7 | 0.6 | 0.2 | 13 | 1.0 | 0.3 | 0.1 | 7 | 0.9 | 0.5 | 0.2 | 0.075 |

Paired Man-Whitney U tests differing letters indicate significant differences (sig level p-value < 0.001, if denoted with a* then pairwise p-values ≤ 0.05 but > 0.001)

Environmental Conditions by Dominant Plant Species Seed Bank Sample Locations -

Environmental conditions across the different standing plant communities (and seed bank sampling locations) varied significantly. The *P. arundinacea* plant community was on average significantly higher in elevation, 2.5 ± 0.2 m (8.2 ± 0.5 ft), than the *C. lyngbyei*, 2.3 ± 0.2 m (7.7 ± 0.5 ft), and *S. lacustris*, 1.8 ± 0.4 m (5.8 ± 1.4 ft), plant communities (Table 12). The *J. effusus* plant community, 2.4 ± 0.2 m (7.9 ± 0.6 ft), was not significantly different in elevation compared to *P. arundinacea* and *C. lyngbyei*, but was significantly higher than the *S. lacustris* plant community (Table 12). Overall, the *P. arundinacea* plant community experienced a mean flooding abundance/frequency of 41 ± 23 % which was significantly less than *C. lyngbyei*, 59 ± 26 %, *S. lacustris*, 95 ± 9 %, but not significantly different than *J. effusus*, 63 ± 26 %, plant communities. Additionally, the greatest duration of flooding during each high tide was identified in the *S. lacustris* plant community, 4.6 ± 1.8 hr, followed by the *C. lyngbyei* and *J. effusus*, both at 1.7 ± 1.0 hr and *P. arundinacea*, 1.0 ± 0.7 hr, plant communities (Table 12). This difference in tidal flooding abundance/frequency and duration was further highlighted by the significant difference in soil ORP conditions observed among the plant communities, with the *S. lacustris* plant community having the lowest ORP conditions averaging at 46.8 ± 60.0 mV, followed by progressively higher average ORP levels in the *C. lyngbyei*, 168.5 ± 56.3 mV, *J. effusus*, 210.9 ± 23.4 mV, and *P. arundinacea*, 243.9 ± 49.3 mV, plant communities.

Overall soil salinity was found to be significantly lower in the *J. effusus* plant community, 237.9 ± 115.9 ppm, which is about half as salty as all of the others which were not

found significantly different from one another (Table 8). Soil conductivity was also significantly lower in the *J. effusus* plant community, 499 ± 236 $\mu\text{S}/\text{cm}$, and highest in the *P. arundinacea*, 923 ± 302 $\mu\text{S}/\text{cm}$, followed by the *S. lacustris*, 825 ± 250 $\mu\text{S}/\text{cm}$, and *C. lyngbyei*, 797 ± 241 $\mu\text{S}/\text{cm}$, plant communities. Soil pH was found to vary among the plant communities with *P. arundinacea* and *S. lacustris* having the highest pH, with an average of 6.5, and *C. lyngbyei* having the lowest pH, with an average of 5.6, and *J. effusus* falling in the middle with an average pH of 6.0 (Table 8). Additionally, soil Phosphorous (P) was found to be significantly greater in the *C. lyngbyei* plant community with an average of 62.4 mg/kg, compared to the *P. arundinacea* and *S. lacustris* plant communities which both had an average of 48.4 mg/kg and *J. effusus* which had the lowest P levels with an average of 38.8 mg/kg. This elevated P is likely related to the slightly lower pH and slightly higher salinity levels also observed in the *C. lyngbyei* plant community, conditions which favor increases in P availability (Fox et al. 1986, House 1999, Sundareshwar and Morris 1999). No significant differences were identified among the plant communities' soil bulk density, organic matter, moisture, texture (% sand, silt, and clay), available nitrogen, or total exchange capacity (Table 8).

Appendix D: Supplemental Results - Field Observations Standing Plant Community and Seed Bank

Appendix D Table 8: Dominant plant community field observations: environmental conditions. Significant Kruskal-Wallis analysis, Bonferroni significance level $p < 0.003$, and marginally significant $p < 0.05$ differences highlighted. Pairwise, Man-Whitney U tests, significant differences within summary metrics among plant communities marked by differing letters; Bonferroni corrected significance level $p < 0.0005$, marginally significant $p < 0.05$ differences also marked with an*.

| Dominant Plant Community Field Observations: Environmental Conditions | | | | | | | | | | | | | | | | | |
|---|--------------------------|---------------------|-------|------|---------------------|---------------------|-------|------|------------------------|---------------------|-------|------|-----------------|---------------------|-------|------|----------------|
| Parameter | Reed canarygrass (Ph ar) | | | | Common Rush (Ju ef) | | | | Lyngbyei sedge (Ca ly) | | | | Bulrush (Sc la) | | | | Kruskal-Wallis |
| | n | Mean | SD | SE | n | Mean | SD | SE | n | Mean | SD | SE | n | Mean | SD | SE | |
| Elevation (ft) | 14 | 8.2 ^{a*} | 0.5 | 0.1 | 6 | 7.9 ^{ab} | 0.6 | 0.3 | 13 | 7.7 ^{b*} | 0.5 | 0.1 | 7 | 5.8 ^c | 1.4 | 0.5 | 0.001 |
| Elevation (m) | 14 | 2.5 ^{a*} | 0.2 | 0.0 | 6 | 2.4 ^{ab} | 0.2 | 0.1 | 13 | 2.3 ^{b*} | 0.2 | 0.0 | 7 | 1.8 ^c | 0.4 | 0.2 | |
| Abundance flooded twice a day (% , March 2015) | 14 | 41% ^a | 23% | 0.1 | 6 | 63% ^{ab*} | 26% | 0.1 | 13 | 59% ^{b*} | 26% | 0.1 | 7 | 95% ^{c*} | 9% | 0.0 | 0.000 |
| Duration of each flooding event (hr, March 2015) | 14 | 1.02 ^a | 0.68 | 0.2 | 6 | 1.66 ^{ab*} | 0.92 | 0.4 | 13 | 1.68 ^{b*} | 1.03 | 0.3 | 7 | 4.59 ^{c*} | 1.75 | 0.7 | 0.001 |
| Bulk density (g/cm ²) | 10 | 0.4 | 0.1 | 0.0 | 2 | 0.5 | 0.3 | 0.2 | 10 | 0.3 | 0.1 | 0.0 | 6 | 0.4 | 0.1 | 0.0 | 0.419 |
| Organic Matter (%) | 14 | 15.3 | 5.9 | 1.6 | 6 | 15.2 | 9.6 | 3.9 | 11 | 18.0 | 9.1 | 2.7 | 7 | 10.5 | 4.8 | 1.8 | 0.219 |
| Soil moisture (%) | 14 | 62.4 | 5.7 | 1.5 | 6 | 55.1 | 13.1 | 5.3 | 11 | 67.0 | 10.1 | 3.1 | 7 | 62.9 | 4.3 | 1.6 | 0.465 |
| Field Conductivity (µS/cm) | 14 | 922.8 ^{a*} | 301.9 | 80.7 | 6 | 498.8 ^{b*} | 236.0 | 96.3 | 13 | 796.9 ^{c*} | 240.7 | 66.8 | 7 | 824.6 ^{ac} | 250.1 | 94.5 | 0.025 |
| Field ORP (mV) | 14 | 243.9 ^a | 49.3 | 13.2 | 6 | 210.9 ^{ab} | 23.4 | 9.6 | 13 | 168.5 ^b | 56.3 | 15.6 | 7 | 46.8 ^c | 60.0 | 22.7 | 0.000 |
| Field pH | 14 | 6.5 ^{a*} | 0.5 | 0.1 | 6 | 6.0 ^b | 0.2 | 0.1 | 13 | 5.6 ^b | 1.3 | 0.4 | 7 | 6.5 ^{ab} | 0.6 | 0.2 | 0.034 |
| Field Salinity (ppm) | 14 | 455.1 ^{a*} | 151.9 | 40.6 | 6 | 237.9 ^{b*} | 115.9 | 47.3 | 13 | 379.0 ^a | 117.7 | 32.7 | 7 | 395.6 ^a | 126.2 | 47.7 | 0.023 |
| Field Temp (°C) | 14 | 12.5 | 1.4 | 0.4 | 6 | 12.1 | 1.7 | 0.7 | 13 | 12.8 | 1.4 | 0.4 | 7 | 12.3 | 1.1 | 0.4 | 0.415 |
| Clay (%) | 13 | 2.9 | 1.0 | 0.3 | 4 | 3.7 | 3.7 | 1.8 | 8 | 3.8 | 1.6 | 0.6 | 7 | 5.1 | 4.4 | 1.6 | 0.548 |
| Sand (%) | 13 | 72.7 | 4.7 | 1.3 | 4 | 75.3 | 16.5 | 8.3 | 8 | 70.4 | 5.5 | 1.9 | 7 | 64.1 | 18.0 | 6.8 | 0.551 |
| Silt (%) | 13 | 24.4 | 5.0 | 1.4 | 4 | 21.1 | 13.9 | 6.9 | 8 | 25.8 | 5.9 | 2.1 | 7 | 30.8 | 14.1 | 5.3 | 0.390 |
| Bray II P (mg/kg) | 14 | 48.4 ^a | 11.7 | 3.1 | 6 | 38.8 ^a | 13.1 | 5.4 | 11 | 62.4 ^{b*} | 13.0 | 3.9 | 7 | 48.4 ^{ab} | 19.3 | 7.3 | 0.033 |
| Estimated Nitrogen Release (N/acre) | 14 | 127.9 | 5.8 | 1.5 | 6 | 123.8 | 15.2 | 6.2 | 11 | 130.1 | 9.5 | 2.9 | 7 | 117.7 | 9.9 | 3.7 | 0.185 |
| Total Exchange Capacity (meq/100 g) | 14 | 25.7 | 6.3 | 1.7 | 6 | 29.1 | 10.8 | 4.4 | 11 | 29.5 | 10.9 | 3.3 | 7 | 30.1 | 8.4 | 3.2 | 0.764 |

Paired Man-Whitney U tests differing letters indicate significant differences (sig level p-value <0.0005, if denoted with a* then pairwise p-values ≤0.05 but >0.0005)