Native Wetland Plant Recovery Following Phragmites australis *Invasion*

by

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Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Statement of Contributions

This thesis work is my intellectual property and I take sole responsibility for it. However my thesis could not have been completed without help. To recognize this, I use “we” throughout the document. My intention is to publish Chapters 2 and 3 as co-authored manuscripts and my academic supervisor Dr. Rebecca Rooney will be a co-author on them both, with Dr. Courtney Robichaud as a co-author of the manuscript derived from Chapter 3.

Abstract

Invasive common reed (*Phragmites australis* ssp. *australis*) has negatively affected 25% of all Species at Risk in Ontario since its arrival in the twentieth century. This is of particular concern at Long Point and Rondeau, two wetland complexes located on the northern shore of Lake Erie. To combat the negative effects of *P. australis*, over 1500 ha of invaded marsh was treated with a glyphosate-based herbicide, beginning in 2016. We monitored the vegetation communities in these wetland complexes over five years to track changes in the wetland vegetation following herbicide application. In the two years following herbicide application, a secondary invasion by *Hydrocharis morsus-ranae* was observed but was short-lived. Three to five years following treatment, treated plots shifted towards native-dominated vegetation communities. However, with lower Lake Erie water levels predicted in the next five years, these communities are expected to change, as more seedlings will emerge from the wetland seedbank. To predict what may return to treated areas, and to determine the effects of herbicide treatment on viable *P. australis* seeds in the seedbank, we performed a greenhouse emergence experiment with seedbank samples collected from invaded, herbicide-treated, and native reference marsh. We determined that while a high abundance of viable *P. australis* remain in the seedbanks of invaded areas, treatment followed by flooding for a minimum of one year effectively reduced the number of viable *P. australis* seeds. The seedbanks of all three vegetation types contained many native seeds but contained many non-native seeds as well. Further monitoring of the vegetation communities that emerge as Lake Erie water levels decline is recommended to ensure that the vegetation communities at Long Point remain native-dominated, and that low water levels do not facilitate the reinvasion of *P. australis*.

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1.0 Literature review

Coastal marsh on the north shore of Lake Erie is plagued by biological invasions which compromise the ecological integrity of this important habitat and threaten its biodiversity. A particularly problematic invasive plant, *Phragmites australis* ssp. *australis*, has been actively managed in the Long Point region since 2015. Described as Canada's worst invasive plant (Catling 2005), *P. australis* has been demonstrated to harm turtles (Markle and Chow-Fraser 2018), alter the avian community (Robichaud and Rooney 2017), and transform the ecological function of the marsh (e.g., Yuckin 2018). In my thesis, I use a combination of field and greenhouse studies to better understand the recovery of native vegetation following active management of *P. australis* in the Long Point coastal marsh.

1.1 *Wetland ecology*

1.1.1 Wetland plant zonation and hydrology

Wetland plant communities are directly linked to water levels through a wetland continuum (Euliss et al. 2004). Wetland plant communities in systems with dynamic hydrology, such as coastal wetlands, are in a constant flux. Keddy and Reznicek (1986) recognize four wetland vegetation zones that occur in Great Lakes coastal marsh, stratified by water levels. These include, from highest to lowest water level, aquatic (a.k.a. open marsh (Weller and Spatcher 1965)), emergent marsh, meadow marsh, and shrub thickets (a.k.a. dry marsh (van der Valk and Davis (1978)). Each vegetation zone is characterized by its own hydrological conditions and hosts distinct vegetation communities (Keddy and Reznicek 1986).

Aquatic communities exist in deep water conditions that are persistently inundated. Aquatic marsh vegetation is characterized by its tolerance to flooding (Keddy and Campbell 2019) and comprises primarily submerged aquatic species such as pondweed species (*Potamogeton* spp. (L.)) and floating plants such as common duckweed (*Spirodela polyrhiza* (L.) Shchleid.) (Weller and Spatcher 1965; van der Valk and Davis 1978). Such species struggle to compete for light with plants having an emergent growth form, because emergent plant species can grow much taller than submersed or floating species and intercept incident light. However, plant species with emergent growth forms, such as cattail (*Typha* spp. (L.)) and bulrush (*Schoenoplectus* spp. (Rchb.) Palla) die off in response to sustained flooding and are therefore prevented from dominating in aquatic marsh (Harris and Marshall 1963).

Instead, emergent growth forms dominate in emergent marsh communities, which are characterized by lower water levels or fluctuating water levels through time (Weller and Spatcher 1965; van der Valk and Davis 1978). The water must be shallow enough for their photosynthetic tissues to protrude from the water while they remain rooted in the sediment and the physical disturbance of waves and wind must not be so severe as to rip out their root systems (Gathman et al. 2005). Emergent species are typically clonal perennials, able to reproduce vegetatively in deep water conditions via rhizomes or stolons. This is ideal for emergent marsh communities, as deep water does not provide sufficient light and oxygen for seeds to germinate (van der Valk and Davis 1978). Though emergent species like cattail produce abundant wind-dispersed seeds, they are not typically able to compete with sedges and forbs in shallower water where the seedbank is more commonly exposed to air and light, such as in meadow marsh.

Meadow marsh communities are highly diverse habitat (Keddy and Reznicek 1986; Keddy and Campbell 2019), aided by temporally variable ecological selection (*sensu* Vellend

2016). Because meadow marsh communities experience alternating periods of flooding and drought, they are often in the early stages of succession, allowing a high abundance of species to grow with relatively low competition (Keddy and Campbell 2019). Though clonal perennial sedges (e.g., *Carex aquatilis* Wahlenb.) and grasses (e.g., *Calamagrostis canadensis* (Michx.) P. Beauv.) can form dense monocultures in meadow marsh, the active seedbank also enables diverse flowering annuals to form important community members.

Finally, shrub thicket marsh exists in areas that are infrequently flooded or that have persistent moist but not fully saturated soils. Like meadow marsh, these areas are characterized by high seedling emergence from the seedbank (Keddy and Campbell 2019). Woody vegetation is held in check by periodic soil anoxia, so that it does not come to fully dominate the shrub thicket zone, lest it become a swamp, rather than a marsh or extend into the meadow marsh (van der Valk 2005).

1.1.2 Wet-dry cycling

Freshwater coastal marshes naturally undergo hydrological cycles on both short and long terms (Keough et al. 1999). Short-term events, such as seiche events (short periods of high and low-water levels caused by wind [Trebitz 2006]), affect plant and animal communities on a day-to-day basis. For example, perturbed sediment from seiche events causes increased nutrient availability for a short period of time (Keough et al. 1999). Wetlands also experience oscillations in water depths on an annual scale, with highest water levels present in summer, and low water levels present in winter (Fig 1-1; NOAA 2022). These oscillations provide stress to which many wetland plants have adapted (e.g., the development of thin stems to reduce stress caused by large-scale water level fluctuations; Keough et al. 1999).

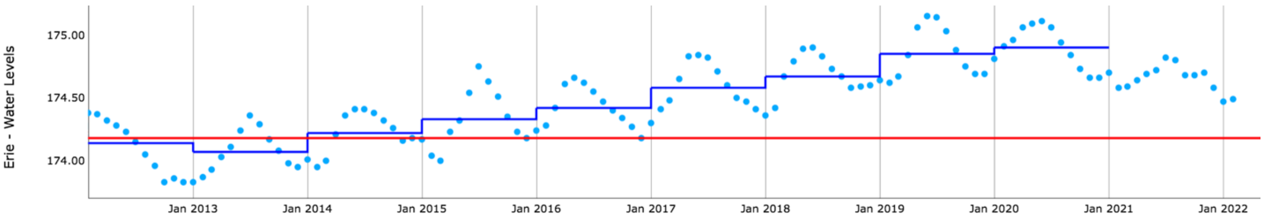


Figure 1-1. Lake Erie water levels from January 2012 to February 2022. The red line denotes average lake levels since 1918. Figure obtained from NOAA (2022). The solid blue line indicates annual average depths and the dotted blue line presents monthly average values.

In addition, wetlands naturally undergo longer-term cycles of low- and high-water levels, termed wet-dry cycling, which affect germination dynamics and community succession over decades (van der Valk 2005; Fig. 1-1). These cycles can vary in length, lasting anywhere from 4 to 35 years (Weller and Spatcher 1965). During these cycles, periods of high water promote the growth of submerged aquatic vegetation and floating plants (van der Valk and Davis 1978; Euliss et al. 2004; van der Valk 2005). However, sustained periods of high water can impede germination and growth of many emergent and meadow marsh species, decreasing overall native species diversity and richness (van der Valk 2005; Campbell et al. 2016). In contrast, periods of drawdown promote the germination of emergent annual species from the seedbank due to increased light and oxygen availability, encouraging the growth of meadow marsh communities (Delgado et al. 2018; Keddy and Campbell 2019). However, these low water levels can also promote the emergence and spread of invasive species such as European common reed (*Phragmites australis* ssp. *australis* (Cav.) Trin. ex Steud.)), which has historically expanded rapidly under periods of drawdown (Wilcox et al. 2003; Tulbure et al. 2007).

1.1.3 Wetland seedbanks

One major factor differentiating the aquatic and emergent marsh zones from the meadow and shrubby marsh zones is the influence of the seedbank. A wetland seedbank consists of the top 2-10 cm of sediment where viable seeds settle and collect, with the highest concentration of seeds in the top 2 cm (Leck and Graveline 1979). Once in the seedbank, seeds can either remain until they are no longer viable due to age or environmental damage, or until ideal environmental conditions promote germination and they sprout (van der Valk and Davis 1978). Identifying plant species present in a seedbank can predict species that may emerge under these environmental conditions.

The composition of a wetland seedbank can be highly variable. In some cases, seedbank composition closely reflects the present vegetation community (e.g., Rohal et al. 2021), whereas in others, the composition of the seedbank can differ substantially from the current vegetation community (e.g., Leck and Simpson 1995). In the case of substantial deviations, the seedbank may instead reflect vegetation communities from the past, as some seeds can remain viable for many years following community succession (Faist et al. 2013). However, longevity in the seedbank is highly variable. Some species may only survive for one growing season, while other species may remain viable for decades (Leck and Graveline 1979). Because of this, despite years of invasive species dominance, the seedbank may remain species-rich and diverse with long-lasting native seeds remaining dormant in the seedbank (Baldwin et al. 2010; Hazelton et al. 2014; Rohal et al. 2021), providing the sediment has not been extensively disturbed (e.g., dredged). The composition and longevity of a wetland seedbank is an important consideration in invasive species management projects. Seedbank composition will largely determine the pool of species able to naturally recolonize an area post-treatment, and thus it is important to be aware of the seed diversity and richness that is contained within the seedbank.

1.2 *Invasive* Phragmites australis *subsp.* australis

I use the definition of invasive species authored by Richardson et al. (2000), wherein invasive species are those introduced to an area across a geographical barrier by humans, that produce offspring and expand their range despite environmental and biotic barriers. Many of these species have negative impacts on the environment of the areas in which they invade, including decreases in the abundance and richness of native species (Pimentel et al. 2005; Vilà et al. 2011). The costs of invasive plant species treatment and the loss of native species are also consequences of invasion (Pimentel et al. 2005). Many invasive species possess traits that enhance their relative fitness and convey competitive superiority or the capacity to overcome barriers faced by native species, such as novel predator defenses, the capacity to access untapped resources, or ability to overcome dispersal limitations (van Kleunen et al. 2010). A common characteristic of invasive plants is that they are able to rapidly colonize and usurp available resources following disturbance.

This is a common outcome of invasions by European common reed, *Phragmites australis* ssp. *australis* (hereafter *P. australis*), which has been called North America's worst invasive plant (Catling 2005). *Phragmites australis* is a perennial plant that grows up to four metres tall in dense (>100 stems/m^2) stands (Haslam 1972; Packer et al. 2017; Robichaud 2021). It is highly tolerant of a variety of environmental conditions, and often grows in wetlands, areas of high wave action, and anthropogenically disturbed areas, such as the sides of roadways (Catling and

Carbyn 2006; Baldwin et al. 2010). *Phragmites australis* reproduces both sexually and asexually. Asexual reproduction is primarily via stolons or rhizomes (Haslam 1972; Packer et al. 2017), which allow established genets to spread locally and colonize habitats where seedlings could not survive. Vegetative reproduction is possible with rhizomes or stolons as small as 2 g (Bart and Hartman 2003), and long-distance dispersal of vegetative propagules along roadways is common (e.g., Jodoin et al. 2008). *Phragmites australis* also reproduces sexually with abundant small seeds that are dispersed primarily via wind (Haslam 1972), whose viability depends on the genetic diversity of the parent plants (e.g., Kettenring et al. 2011). The seeds of *P. australis* are highly tolerant of salinity and can germinate in a wide variety of environmental conditions (Galinato and van der Valk 1986). However, *P. australis* seeds and young seedlings are easily damaged by drought and frost (Haslam 1972). Additionally, *P. australis* seed germination is reduced in water depths greater than 3.5 cm (Baldwin et al. 2010) and in anoxic conditions (Wijte and Gallagher 1996). However, when environmental conditions are not ideal, *P. australis* seeds are able to remain in the seedbank for an unknown period of time until optimal conditions allow them to germinate (Kettenring and Whigham 2009; Wilcox 2012). Its ability to grow tall stems at high stem densities, paired with its ability to reproduce both sexually and asexually, makes *P. australis* a highly successful invader (Palma et al. 2021; Robichaud and Rooney 2022).

1.2.1 Effects of Phragmites australis *on native species*

Phragmites australis negatively affects approximately 25% of all Species at Risk in Ontario (Bickerton 2015). *Phragmites australis* alters critical turtle habitat, and acts as a barrier to movement, which results in turtles getting stranded in dense stands (Markle and Chow-Fraser 2018). These stands also alter fish habitat by outcompeting the native grasses that are essential spawning habitat (Weinstein and Balletto 1999), and many wetland birds of conservation concern do not use *P. australis* if there is native vegetation available (Robichaud and Rooney 2017). Importantly, *P. australis* stands drastically alter native plant communities. *Phragmites australis* outcompetes plant species via shading and overcrowding (Minchinton et al. 2006). Competition for light with *P. australis* resulted in native meadow marsh species, such as *Calamagrostis canadensis* ((Mich.) P. Beauv.) and *Carex aquatilis* (Wahlenb.)*,* assimilating less carbon (Robichaud and Rooney 2022). In addition to limiting resource acquisition, *P. australis* impedes the ability for native species to reproduce. A study by Minchinton et al. (2006) found that native plant seed dispersal was constrained to mere metres in dense *P. australis* stands. Because of this, vegetation communities in *P. australis*-dense areas tend to be primarily monotypic and less species-rich and diverse than areas free of *P. australis* (Wilcox 2012; Swarth et al. 2013; Bonello and Judd 2019). These dense *P. australis* stands can often replace native marsh communities, including meadow marsh and cattail marsh (Wilcox et al. 2003). Without proper methods of control and efforts to encourage wetland restoration, *P. australis* can easily dominate landscapes, negatively affecting many wetland plant and animal species.

1.3 Phragmites australis *treatment*

The goal of ecological restoration is to encourage the recovery of an ecosystem by reducing or eliminating the negative effects of a disturbance, and by restoring native plant and animal species (SER 2004). Successful restoration following invasive plant suppression activity ensures that ecosystem function and integrity are recovered (McDonald et al. 2016). However, restoration in these cases is not always straightforward, and can sometimes lead to unintended consequences (D'Antonio and Meyerson 2002). It is therefore critical to take an allencompassing view of the wetland before and after restoration has taken place to ensure that attempts at restoration do not cause harm to the ecosystem.

To control the spread of *P. australis* and re-establish native plant communities, many wetland managers choose to treat dense *P. australis* stands (Hazelton et al. 2014). However, *P. australis* treatment often requires a combination of treatment methods to effectively eliminate *P. australis* populations and restore native vegetation (Hazelton et al. 2014). These methods of treatment include both mechanical and chemical (herbicide) strategies.

1.3.1 Mechanical treatment

The mechanical treatment of *P. australis*, while less common than herbicide treatment, can be effective in reducing the density of *P. australis* stands. A review by Hazelton et al. (2014) determined that the most popular mechanical treatment methods in *P. australis* studies, in order of decreasing prevalence, included cutting or mowing (28%), burning (9%), grazing (5%), and flooding dense stands (4%), all of which work to varying levels of success (Hazelton et al. 2014). Unfortunately, mechanical methods of *P. australis* treatment are often work-intensive, as the extensive rhizome network must be controlled to ensure complete eradication (Smith 2005). A combination of mechanical treatment techniques is often needed to ensure *P. australis* removal and community restoration. For example, dense litter yielded by mowing *P. australis* stands may hinder native plant establishment following mowing (e.g., Rohal et al. 2019), and burning may be a valuable follow-up treatment to remove the thatch and encourage native plant regeneration. Due to a combination of these factors, *P. australis* often re-invades treated areas following mechanical treatment (Hazelton et al. 2014).

1.3.2 Herbicide treatment

The most commonly used method of *P. australis* removal is the application of broad-spectrum herbicides such as glyphosate and imazapyr (Cheshier et al. 2012; Hazelton et al. 2014) A study published by Martin and Blossey (2013) conducted of 285 land managers from US public and private conservation organizations determined that 94% reported using herbicides to manage *P. australis*. Additionally, in a survey of land managers across the Great Salt Lake watershed published by Rohal and colleagues in 2018, 97% reported using herbicide as their primary tool in *P. australis* management. Both glyphosate and imazapyr are post-emergence herbicides, which, when absorbed by plants, disrupt amino acid pathways, eventually causing plant death (Tu et al. 2001).

Though more effective than mechanical treatment alone (e.g., Rohal et al. 2019), the efficacy of herbicide-based *P. australis* suppression is also variable. While herbicide treatment has successfully enabled native vegetation re-establishment and increased floristic quality in some cases (e.g., Bonello and Judd 2019), it has failed to increase floristic quality in others (e.g., Judd and Francoeur 2019) and several studies have concluded that eradication is not an achievable goal (Martin and Blossey 2013; Quirion et al. 2018). Herbicide-treatment efficacy decreases with the size of the patch, as precise application is difficult in dense *P. australis* stands (Lombard et al. 2011). Multiple applications are therefore often needed to eradicate *P. australis* completely, meaning that treatment over multiple years is critical (Back and Holomuzki 2008; Bonello and Judd 2019).

Additionally, glyphosate- and imazapyr-based herbicides used in *P. australis* treatment may themselves have negative effects on the invaded wetland. Surfactants used in herbicides are widely understudied, and their broad effects on wetland species are yet to be determined (e.g., Breckels and Kilgour 2018). Additionally, few *P. australis* studies explore the effects of glyphosate and imazapyr on factors other than reducing *P. australis* populations, such as native species viability in the seedbank after treatment, and effects on invertebrate and bird species (Hazelton et al. 2014).

Finally, herbicide treatment can be extremely costly, making multi-year treatment projects difficult. For example, between 2010 and 2014, the Great Lakes Restoration Initiative spent $25 million towards *P. australis* herbicide treatment in the Great Lakes (GLRI 2015). In the United States, a survey of 285 land managers found that over $4.6 million was spent yearly between 2005 and 2009 on *P. australis* treatment (primarily herbicide treatment), with few of the projects resulting in complete eradication (Martin and Blossey 2013). Perversely, as glyphosate and imazapyr are broad-spectrum herbicides, they are effective not only on *P. australis* but on most plants with which they come into contact (Tu et al. 2001; Hazelton et al. 2014). Any accidental over-spray of these herbicides could therefore be potentially dangerous to surrounding plant species (Mozdzer et al. 2008) and may create open niche space (e.g., an increase in light and nutrient availability). This open niche space is ideal for native plant species to recolonize the area and re-establish a diverse community (e.g., Bonello and Judd 2019). However, this is also ideal other invasive species, such as European frog-bit (*Hydrocharis morsus-ranae* (L.)) and Eurasian milfoil (*Myriophyllum spicatum* (L.)), which may establish post-treatment (Zimmerman et al. 2018; Bonello and Judd 2019; Robichaud and Rooney 2021b). For example, a study by Bonello and Judd (2019) found that 11 of the 60 species that returned post-herbicide treatment were non-native species. These species, once established, may result in secondary invasions in treated areas. Unfortunately, as the effects of anthropogenic activities worsen and these disturbances become more frequent, invasions by non-native species may become more common (e.g., Mozdzer et al. 2016). The high cost of treatment, paired with the general inefficacy of herbicide treatment in the long term, results in *P. australis* often re-invading treated areas (Farnsworth and Meyerson 1999; Quirion et al. 2018; Zimmerman et al. 2018; Robichaud 2021).

1.4 *Great Lakes coastal wetlands and* Phragmites australis *invasion in Lake Erie*

1.4.1 Great Lakes coastal wetlands

The Great Lakes Coastal Wetlands provide numerous ecosystem services including nutrient sequestration and areas for recreation (Ball et al. 2003; Sierszen et al. 2012). Importantly, they provide critical habitat to threatened native plant and animal species and support high levels of biodiversity (Ball et al. 2003; Sierszen et al. 2012). Unfortunately, many of these wetlands have experienced degradation due to anthropogenic activities, including the introduction of invasive species and the contaminants (Ball et al. 2003).

Long Point Peninsula and Rondeau Provincial Park (hereafter Long Point and Rondeau, respectively) are both located on the northern shore of Lake Erie. Long Point, which is a sandspit peninsula approximately 6450 ha in area, extends 35 km into Lake Erie (Reznicek and Catling 1989). Long Point is designated as a Ramsar wetland, a World Biosphere Reserve, an Important Bird Area, and a provincially significant wetland, and is the largest remaining intact wetland on the north shore of Lake Erie (Ball et al. 2003; Environment Canada 2017). While separated into various management units, the majority of Long Point is currently owned by the Long Point Company, the Canadian Wildlife Service, and the Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry (Reznicek and Catling 1989; see Appendix 1.1). Rondeau, another sandspit peninsula, is a provincial park that is approximately one quarter of the size of Long Point (1560 ha in area) (Reznicek and Catling 1989). Rondeau, unlike Long Point, has a diverse upland deciduous forest community (Reznicek and Catling 1989). Rondeau hosts similar vegetation communities to Long Point, and supports many rare wetland plant and animal species (Reznicek and Catling 1989). Notably, both Long Point and Rondeau provide critical habitat for migrating birds, reptiles, and fish, as well as many rare and endangered plant species (Prince et al. 1992; Ball et al. 2003). Unfortunately, the effects of anthropogenic activities near Long Point and Rondeau have put many of these species at risk (e.g., Bickerton 2015).

1.4.2 Invasive plant species in the Great Lakes coastal wetlands

Within the past century, the Great Lakes coastal wetlands have experienced many biological invasions (Trebitz and Taylor 2007). A 2007 survey of the Great Lakes found nine invasive plant species present, with a higher prevalence of these species being found in Lake Erie and Lake Ontario in comparison to the other three Great Lakes, likely due to the large amount of anthropogenic activity surrounding these lakes (Trebitz and Taylor, 2007). Common reed (*Phragmites australis*), is one such invasive species currently found in Lake Erie coastal wetlands (Robichaud and Rooney 2021b).

1.4.3 Phragmites australis *invasion at Long Point and Rondeau Provincial Park*

Phragmites australis was first recorded at Long Point in 1945 in low densities (Wilcox et al. 2003), however it is possible it was present in the area before that time. During a period of low water levels in the mid-1990s, *P. australis* spread at an exponential rate throughout the Great Lakes area (Wilcox et al. 2003). It is believed that this drawdown period facilitated its spread, as *P. australis* favours low water levels, and is able to spread rapidly under these conditions (Wilcox et al. 2003). *Phragmites australis* subsequently established dense stands at both Long Point and Rondeau after this point, where it continues to expand and intensify (Jung et al. 2017).

Without treatment, *P. australis* has had negative effects on many native plant and animal species since its establishment.

A *P. australis* treatment program was launched in 2016 by the Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry (MNDMNRF). An Emergency Registration (no. 32356) was obtained, and in Fall 2016 and 2017, a glyphosate-based herbicide (Roundup® Custom for Aquatic & Terrestrial Use Liquid Herbicide, Bayer CropScience Inc., Canada) combined with a nonionic alcohol ethoxylate surfactant (Aquasurf®, registration no. 32152, Brandt Consolidated, Springfield, IL, USA) was used to treat over 1500 ha of invaded marsh. Additional details about the treatment method are provided in Chapter 2 and 3, but in brief, aerial treatment (via helicopter) took place in Fall 2016 at both Long Point and Rondeau, and a combination of both aerial (via helicopter) and ground (via Marsh Master^{TM}) treatment took place in 2017 at Long Point. There are plans to spot-treat remaining *P. australis* at Long Point in the coming years. The treated areas have undergone passive restoration, meaning that forms of active restoration (e.g., seeding treated areas with native seed mixes) have not taken place.

1.5 Research objectives

Re-invasion by *P. australis* (e.g., Quirion et al. 2018) and secondary invasions by other invasive species (e.g., Robichaud and Rooney 2021b) are important reasons as to why long-term timelines are essential to *P. australis* treatment studies. Unfortunately, long-term studies of the effects on vegetation communities post-treatment are uncommon in *P. australis* research (Hazelton et al. 2014). A review by Hazelton et al. (2014) found that 43% of *P. australis* treatment studies lasted for one year or less. Long-term wetland restoration studies (i.e., 3-5 years post-treatment) are crucial, as they can better capture the trajectory of vegetation communities. Studies that predict what phenomena may occur in the future are also important, as they allow managers to plan ahead and tailor future treatment to optimize their outcomes. My thesis incorporates both types of studies to determine how vegetation communities have changed since *P. australis* treatment took place, and how they may change moving forward.

In my first data chapter, I use an observational study design to determine the long-term vegetation community development following herbicide-treatment of *P. australis* for five years. I test how the vegetation differs between herbicide-treated and *P. australis*-dominated marsh via a spatially replicated before-after control-impact (BACI). In my second data chapter, I use a greenhouse study to examine emergence from the seedbanks of herbicide-treated, *P. australis*-dominated, and reference marsh under both a flooded and a moist watering regime. In my final thesis chapter, I summarize my findings, and explain how they can be applied to inform future *P. australis* management.

2.0 Determining the recovery of wetland vegetation communities following herbicide-based treatment of *Phragmites australis*

2.1 Introduction

Plant invasions by non-native species are a major threat to wetlands (Zedler and Kercher 2004), and invasive plant species management is a common challenge in wetland restoration (D'Antonio et al. 2016). The invasive plant *Phragmites australis* ssp. *australis* ([Cav.] Trin. ex Steud.) or European common reed (hereafter, *P. australis*) is one of the gravest threats to wetlands in North America, having even been designated as Canada's worst invasive plant (Catling 2005). *Phragmites australis* is a perennial plant that can grow over 4 m tall, often in dense (>100 stems/m^2) stands (Haslam 1972; Robichaud and Rooney 2021b). Invasion by *P. australis* alters the native wetland plant community via multiple mechanisms. *Phragmites australis* forms dense litter mats that are difficult for native plants to grow through, and dense stands hinder native seed dispersal (Minchinton et al. 2006). *Phragmites australis* also outcompetes native wetland plant species by intercepting photosynthetically active radiation (PAR) and reducing the carbon assimilation rates of native species (e.g., Robichaud and Rooney 2022). Due to these attributes, areas where *P. australis* grows tend to support less diverse vegetation communities than areas free of *P. australis* (Wilcox 2012; Swarth et al. 2013; Bonello and Judd 2019). *Phragmites australis* invasion also affects animal communities by altering critical habitat. Turtles (Markle and Chow-Fraser 2018), wetland birds (Robichaud and Rooney 2017), and amphibians (Greenberg and Green 2013) lose habitat due to *P. australis* invasion in marshes. So may fish (Weinstein and Balletto 1999), although some fish do use *P. australis* (e.g., Croft-White et al. 2021). In all, *P. australis* negatively affects 25% of all Species at Risk in

Ontario (Bickerton 2015). Due to the effects of *P. australis* on native species, managing its population in marsh ecosystems is important.

There are numerous approaches to suppressing *P. australis* populations including mechanical (e.g., rolling, cutting, or burning) and chemical (e.g., herbicide) approaches, or a combination thereof. Herbicide-based *P. australis* suppression is by far (>90%) the most dominant technique used by land managers in North America (Martin and Blossey 2013; Hazelton et al. 2014). To bolster the effects of herbicide, some managers also implement a secondary form of treatment, which may include rolling, cutting, or burning dead stands following the initial herbicide application. Secondary treatment may lead to improved floristic quality, though enhancements to plant richness and evenness may not be apparent (Bonello and Judd 2019). However, herbicide remains the predominant tool used in *P. australis* treatment across North America.

The herbicide can be applied aerially, typically by helicopter, or by ground-application using amphibious vehicles, boats, or backpack sprayers to penetrate the dense stands of *P. australis*. Aerial application has the advantage of being fast and cost-effective if larger areas of *P. australis* monoculture require treatment. It also minimizes risks to wetland wildlife, like turtles and snakes which might be harmed by traffic through the wetland (e.g., Angoh et al. 2021). The advantage of ground application is greater discretion in herbicide application rate, more precise application to irregularly shaped patches of *P. australis*, potential reductions in herbicide drift distances (Yates et al. 1978), and the ability to mitigate non-target effects, especially around sensitive plant communities. However, the relative performance of these application measures has never been assessed.

While herbicide treatment is typically effective at reducing the abundance and density of *P. australis* stands at least temporarily (e.g., Bonello and Judd 2019, Robichaud and Rooney 2021b), and is often more effective than mechanical methods alone (Rohal et al. 2019) complete eradication (i.e., the complete removal of *P. australis* from an area without reinvasion for a minimum of 3 years [Quirion et al. 2018]) is uncommon, and *P. australis* often re-invades in the years following treatment (Lombard et al. 2012; Quirion et al. 2018). In areas where *P. australis* fails to return in high densities, secondary invasions by other non-native plant species, such as European frog-bit (*Hydrocharis morsus-ranae* (L.)) and purple loosestrife (*Lythrum salicaria* (L.)), may occur (e.g., Bonello and Judd 2019; Robichaud and Rooney 2021b). Because of this, some studies have observed no significant changes to floristic quality post-treatment (e.g., Martin and Blossey 2013; Judd and Francoeur 2019). Due to a combination of these drawbacks, some researchers have concluded that *P. australis* suppression efforts may be futile (e.g., Lombard et al. 2012; Quirion et al. 2018).

One possible reason that many *P. australis* treatment programs report limited success in restoring native vegetation communities is that they almost universally adopt a “passive” or “natural” restoration approach to revegetation (see Atkinson and Bonser 2020). Simply removing invasive plant species may allow native vegetation community composition to recover unassisted. “Passive” wetland restoration involves ending the source of degradation, e.g., removing invasive species or plugging ditches, without intervening directly in the vegetation community assembly. This is in contrast with more costly and labour-intensive “active” or “reconstructive” restoration strategies wherein the vegetation community is manipulated by seeding or planting. “Passive” wetland restoration can lead to the recovery of a diverse wetland vegetation community (e.g., De Steven et al. 2010), though seeding or planting strategies may yield greater biodiversity than natural revegetation approaches in controlled empirical comparisons (e.g., Mitsch et al. 2012).

Prior research has concluded that the environmental conditions following invasive species removal will exert a strong influence on vegetation recovery by “passive” restoration (Frieswyk and Zedler 2006; Hazelton et al. 2018; Rohal et al. 2019). Consequently, the environmental conditions that predominate following invasive species removal will have a significant influence on which vegetation species that return. In particular, as wetland vegetation communities are directly linked to water levels (Euliss et al. 2004), water levels in coastal marsh will play a critical role in vegetation recovery. High water levels encourage the development of submerged aquatic vegetation and floating plant communities. Shallower water levels encourage the growth of dense emergent vegetation (Weller and Spatcher 1965; van der Valk and Davis 1978), whereas low water levels encourage the growth of marsh meadow communities, which tend to be more species-rich and diverse than the other communities (Keddy and Reznicek 1986; Keddy and Campbell 2019). Water level drawdowns, with increased light penetration and oxygen availability, encourage the emergence of seeds that may be native or non-native (Welling et al. 1988; Frieswyk and Zedler 2006; Wilcox 2012; Jung et al. 2017). Prevailing and projected water levels are therefore important to consider in wetland restoration projects, as they influence the composition of returning vegetation communities (Fig. 1-1).

The ultimate outcome of restoration actions for vegetation communities, be they passive or active, can take many years to manifest (Moreno-Mateos et al. 2012; Pezzati et al. 2018). Our understanding of the consequences of *P. australis* management actions is therefore hampered by the absence of long-term monitoring in restoration projects (Blossey 1999; Kettenring and Adams 2011; Hazelton et al. 2014). Despite repeated calls for longer-term studies of restoration involving invasive species management (e.g., Kettenring and Adams 2011; Hazelton et al. 2014; Quirion et al. 2018; Zimmerman et al. 2018), few have heeded these calls. Kettenring and Adams (2011) found 51% of invasive plant species treatment projects globally had monitoring programs that lasted for one growing season or less, which is not enough time to draw conclusions on the full effects of invasive species removal on wetland vegetation communities. Longer-term (i.e., 3-5 years following treatment) monitoring projects are needed to gain a full view of community succession after active restoration has taken place (Kettenring and Adams 2011).

To address this gap in longer-term monitoring data, we took advantage of a large-scale pilot project in Lake Erie coastal marshes the Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry (MNDMNRF), which began treating *P. australis* in 2016 (OMNRF 2017). To date, over 1500 ha of invaded marsh has been treated. We conducted yearly surveys of permanent plots originally established in 2016 and 2017. We addressed three research questions: 1) how effective is the aerial application of a glyphosate-based herbicide at suppressing *P. australis* and encouraging native species recovery over a five-year period?, 2) what is the successional trajectory of the vegetation communities in herbicide-treated areas over this time period?, and 3) what is the relative *P. australis* treatment efficacy of ground-based herbicide treatment compared to aerial-based herbicide treatment?

2.2 *Methods*

2.2.1 Field site

Long Point and Rondeau Provincial Park are both wetland complexes located on the north shore of Lake Erie. Both wetlands are biodiversity hotspots and host hundreds of threatened and endangered plant and animal species (Reznicek and Catling 1989; Bickerton 2015). Notably, Long Point is a designated Ramsar site (Wetland of International Importance) and a UNESCO World Biosphere Reserve, while both Rondeau and Long Point are designated as Important Bird Areas and provincially significant wetlands (Ball et al. 2003). Unfortunately, many native species at risk in Long Point and Rondeau, including herptiles, birds, and vascular plants, have been negatively affected due to *P. australis* invasion (e.g., Greenburg and Green 2013; Robichaud and Rooney 2017; Polowyk 2020).

2.2.2 Herbicide application

As part of a Nature Conservancy of Canada and MNDMNRF pilot program, an Emergency Use Registration (no. 32356) was obtained to treat dense *P. australis* at Long Point and Rondeau with a glyphosate-based herbicide (Roundup® Custom for Aquatic & Terrestrial Use Liquid Herbicide, Bayer CropScience, Whippany, New Jersey, USA), combined with a non-ionic alcohol ethoxylate surfactant (Aquasurf®, registration no. 32152 Brandt Consolidated Inc., Springfield, IL, USA). Herbicide (4210 g acid equivalent [a.e.] glyphosate/ha as an isopropylamine salt) was applied via helicopter ("aerial treatment") at a rate of 8.77 L ha^{-1} portions of the Long Point and Rondeau marshes in September and October of 2016. In September and October of 2017, additional areas of Long Point were treated with herbicide. Some sections of marsh were treated via aerial application, as in 2016, but other portions of the marsh were treated via ground application. Ground application used a range in active ingredient concentration, with of Roundup® Custom (1200-3600 g [a.e.] glyphosate/ha as an isopropylamine salt) and Aquasurf® surfactant applied via Marsh Master^{TM}. For both the 2016 and 2017 treatments in Long Point, secondary treatment involving rolling and/or cutting dead *P. australis* stems took place in the winter following herbicide application. Secondary treatment was not applied at Rondeau.

2.2.3 Study design

To assess how *P. australis* populations and vegetation communities respond to glyphosate-based treatments, we established a Before-After Control-Impact (BACI) study in Long Point and Rondeau in 2016. The spatially replicated BACI design is the gold standard in effects-based monitoring, as it allows us to compare control and treatment plots before and after treatment is applied to draw causal inferences about the effects of treatment, while controlling for things like regional changes in environmental conditions or weather patterns and pre-existing differences between control and treatment plots (Underwood 1991). This design is ideal for evaluating the effects of *P. australis* treatment in coastal marsh on Lake Erie, which is hydrologically dynamic, with high interannual variations in water levels that will influence *P. australis* and other wetland vegetation in a manner that could confound simple before-after studies.

Yearly in July and August since 2016, we surveyed permanent quadrats (1 m^2 in area) in high-density (>100 stems/m^2) *P. australis* (control sites) and herbicide-treated areas (treated sites). Forty sites were established at Rondeau in 2016 along a water-depth gradient (10 cm to 48 cm water depth; Fig. 2-1). These were divided into twenty control plots and twenty treatment plots (helicopter application of herbicide in September 2016). One Rondeau control plot (site ID RPC16; see Appendix 2.1) was accidentally treated in 2016 via helicopter overspray, resulting in 19 control plots and 21 treated plots in Rondeau Provincial Park.

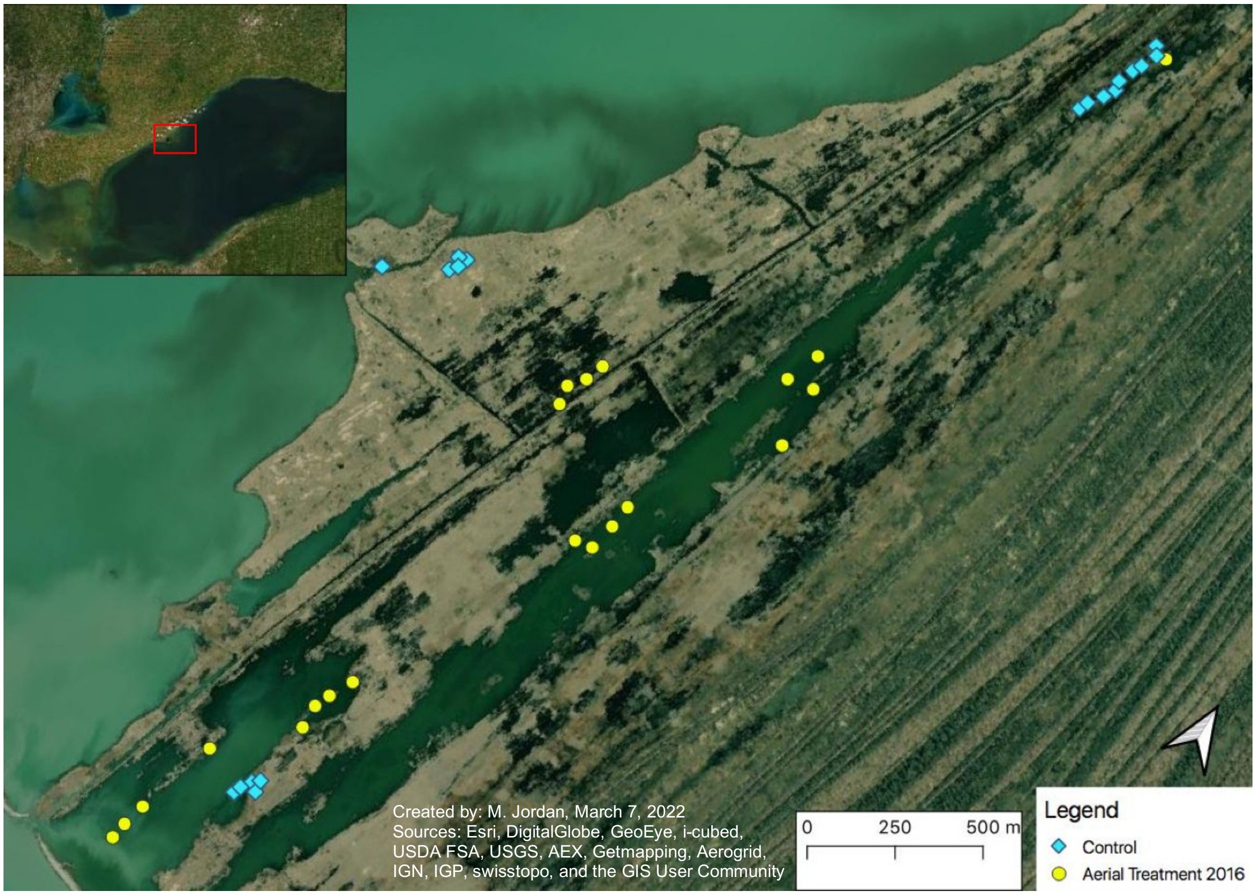


Figure 2-1. Locations of the experimental control plots (n = 19) and treated plots from aerial treatment in 2016 (n = 21) in Rondeau Provincial Park, Ontario.

In addition, forty plots were established in 2016 at Long Point along an equivalent water depth gradient (10-48 cm) to that used in Rondeau Provincial Park. This was similarly divided into twenty control plots and twenty treated plots that had herbicide applied aerially a month after we surveyed them in 2016 (Fig. 2-2). Following herbicide application to new areas of Long Point marsh in 2017, we established an additional forty plots: twenty treated plots that were aerially-treated and twenty that were ground-treated (Fig. 2-2). We first surveyed these plots in

August of 2018, after they were treated with herbicide. As such, no data from before herbicide application took place was obtained.

Created by: M. Jordan, March 7, 2022
Sources: Esri, DigitalGlobe, GeoEye, i-cubed, USDA FSA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community

Figure 2-2. Locations of the experimental control plots (n = 20) and treated plots from aerial treatment in 2016 (n = 20) and the ground-treated plots (n = 20) and aerial-treated plots from 2017 (n = 20) in Long Point, Ontario.

In 2016, we surveyed the Rondeau and Long Point control and aerial treatment plots prior to herbicide application and resurveyed them annually through 2020. In fall of 2020, the control plots in Long Point were treated via ground application of herbicide, and so in 2021 we only surveyed the treatment plots. One treatment site at Rondeau (site ID RPT41; see Appendix 2.1) was inaccessible in 2018 and 2019 but was accessed via airboat in September 2020. Four control sites at Rondeau (site ID RPC17-20; see Appendix 2.1) were inaccessible in 2020. The Long Point plots treated in 2017 were established after herbicide application, and first surveyed in 2018. All forty were surveyed annually until 2021.

2.2.3 Permanent plot surveys

Plot survey methods followed Robichaud and Rooney (2021b). In brief, we measured water depth (cm), water temperature (°C), canopy height (cm), live *P. australis* stem density (stems/m^2), and total *P. australis* stem density (stems/m^2) in each quadrat. To determine the amount of photosynthetically active radiation (PAR) available for plant species, we took simultaneous PAR measurements above the canopy and at the sediment or water's surface with a LI-COR LI-1500 light sensor with two LI-190 Quantum sensors that measure PAR in the 400 to 700 nm wavelength band in µmol m^{-2}s^{-1} to determine the percentage of incident PAR that penetrated the canopy. LI-COR measurements were taken on clear days with minimal cloud cover. At each plot, we measured the percent cover of each plant species as well as abiotic cover types, including plant litter, open water, or standing dead plants. These covers were relativized to sum to 100%. If species-level identification of plants was not possible (e.g., due to a lack of taxonomically-distinguishing seeds or flowers), we identified plants to the genus-level. Voucher samples of unknown plant species were taken for further examination if needed.

2.2.4 Statistical methods

Univariate analyses

To test for a significant interaction between year and treatment on a suite of response variables among the 2016 aerially-treated plots, we used a two-way ANOVA with year (2016 to 2020) and treatment (herbicide-treated vs. control) as fixed factors. These included variables reflecting the degree of *P. australis* suppression success (log transformed live *P. australis* stem density, log transformed total *P. australis* stem density, log transformed percent incident light reaching the water surface, and log transformed canopy height), and a suite of response variables indicating the diversity of recovering vegetation (species richness, Shannon-Weiner diversity, Simpson's diversity (1/D), Pielou's evenness (J), and mean coefficient of conservatism). Percent incident light data was log transformed. In a spatially replicated BACI design, a significant interaction between year and treatment supports the causal interpretation that herbicide application caused the observed change in the response variable. Data from the year 2021 was not included in the two-way ANOVAs as data was only collected from treated plots in this year. General linear models were analyzed using the *car* package (Fox and Weisberg 2019) in R v. 4.1.1 (R Core Team 2021).

To visualize the transition of vegetation through *P. australis*-dominated states to either states dominated by native species or by other "secondary" non-native plant species, we categorized all species as native or non-native according to the USDA Plants Database (USDA 2022; Appendix 2.2). This analysis combined the 2016 and 2017 fall treatments into a single analysis. Next, we categorized plots based on the total cover of native vs. non-native species (Table 2-1).

Table 2-1. Description of vegetation types used to categorize permanent plots from 2016-2021.

Category name	**Description**
Native-dominated	>50% of native plant species cover
Non-native dominated	>50% of non-native plant species cover, not including *P. australis* (e.g., *H. morsus-ranae*, *M. spicatum*, *Typha* spp. (see Appendix 2.2))
P. australis-dominated	>50% of *P. australis* cover
No dominant vegetation type	Equal cover of native and non-native species
No vegetation	No vegetation present in the plot

The coefficient of conservatism value for each species was extracted from the searchable Michigan database (https://www.michiganflora.net/). These values range from 0 to 10 and are assigned by expert botanists based on the relative sensitivity or tolerance of each wetland plant species to disturbance. Exotic species receive a value of 0, weeding species a value of 1, and common species a value of 3. In contrast, species considered extremely sensitive and of high conservation value are assigned a score of 10. Values for the species encountered in our study are reported in Appendix 2.2. We then used these values to calculate the mean CC_s value for each plot (i.e., the average conservatism coefficient of the vegetation in each plot), following the mean CC_s equation from Kutcher and Forrester (2018), where S represents species richness:

$$\text{Mean CCs} = \underset{,/o}{\overset{-}{\boldsymbol{)}}}(++_{,})\,/S$$

Multivariate analyses

To rank plots on the basis of similarity in their plant community composition, we performed an agglomerative, polythetic hierarchical cluster analysis with a Bray-Curtis dissimilarity matrix on arcsine square root-transformed percent cover data, as recommended by

McCune and Grace (2002). To reduce the sparsity in the community composition data, rare species (fewer than 4 occurrences out of 393 observations) were removed for this analysis, which removed a total of 41 species (Appendix 2.3). We performed an indicator species analysis (ISA; Dufrene and Legendre 1997) to determine the ideal place to prune the resulting dendrogram.

An ISA (Dufrene and Legendre 1997) was performed on the same arcsine square root-transformed plant community composition data to determine which species or abiotic cover types were indicative of the dendrogram clusters. Multivariate statistics were carried out in PC-ORD v 7.08 (McCune and Mefford 2018).

2.3 *Results*

2.3.1 Analysis of 2016 aerially-treated and control plots

Univariate analyses

All four response variables pertaining to *P. australis* suppression efficacy were significantly altered by the herbicide application, as evidenced by the dramatic change in plot values (Fig. 2-3) in treated plots immediately after aerial application of herbicide in fall 2016, compared to baseline measures in the summer of 2016. For example, live *P. australis* stem density in treated plots was >95% lower than in contemporary control plots for each year from 2017 to 2020, when control plots were finally treated with herbicide. Similarly, there was an increase of 1.3 times the light penetration in treatment plots relative to contemporary controls, and a reduction of 1.4 times the canopy height. This is supported by statistically significant interaction terms between year and treatment for all two-factor ANOVAs (Table 2-2). Note the effects on live *P. australis* stem density, light penetration and canopy height were immediate and persisted for the monitoring period of five years post-treatment (Fig. 2-3). The response of total *P. australis* stem density was more gradual, taking until 2019 to transition to a new state (Fig. 2-3).

Table 2-2. Two-way ANOVA test results for live *P. australis* stem density, total *P. australis* stem density, light penetration, and canopy height for the 2016 permanent efficacy monitoring plots, from 2016 to 2020.

	Treatment (control vs. herbicide-treated)			**Year** (2016-2020)			**Treatment x Year**		
	df	F	p	df	F	p	df	F	p
Log live stem density	1, 383	0.084	0.772	4, 383	5.978	<0.001	4, 383	122.074	<0.001
Log total stem density	1, 383	0.087	0.768	4, 383	0.415	0.798	4, 383	54.045	<0.001
Log percent incident light	1, 383	0.818	0.366	4, 383	14.429	<0.001	4, 383	27.596	<0.001
Log Canopy height	1, 383	0.002	0.963	4, 383	2.280	0.060	4, 383	31.776	<0.001

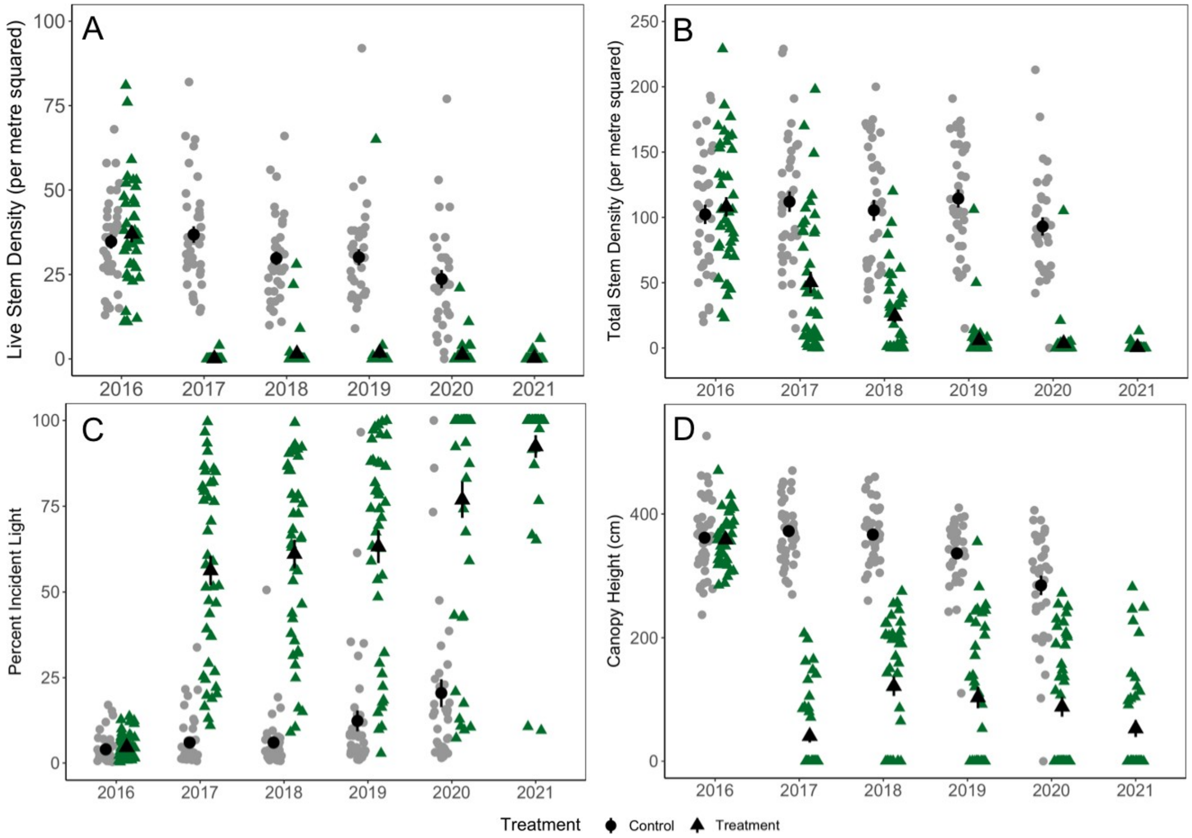


Figure 2-3. Jitter plot comparison of live *P. australis* stem density (A), total *P. australis* stem density (B), percent incident light passing through the canopy (C), and canopy height (D) between control and herbicide-treated plots established in 2016, both pre-treatment (2016), and post-treatment (2017-2021). Control plots were not surveyed in 2021 as they were treated in Fall 2020. Grey circles represent control sites, and green triangles represent treated sites. Black symbols represent the mean, and error bars represent standard deviation.

Response variables indicative of plant community diversity were also significantly altered by herbicide application (Fig. 2-4; Table 2-3). Species richness, Shannon-Weiner diversity, Simpson's diversity (1/*D*), Pielou's evenness (*J*), and the mean coefficient of conservatism (CC_s) were suppressed in treated plots in the first 1-2 years following herbicide application, but rebounded 3-5 years after treatment took place. Once these variables rebounded, they remained consistently higher in treated plots in comparison to control plots. For example, species richness dropped 50% from the baseline average of four species per m^2 to only two species per m^2 in 2017 and 2018, but then increased to 1.3 times the richness in contemporary control plots in 2020 (Figure 2.4). Additionally, mean coefficient of conservatism values remained similar to baseline values in 2017 and 2018, but then increased to 1.6 times the mean coefficient of conservatism in contemporary control plots in 2020 (Figure 2.4).

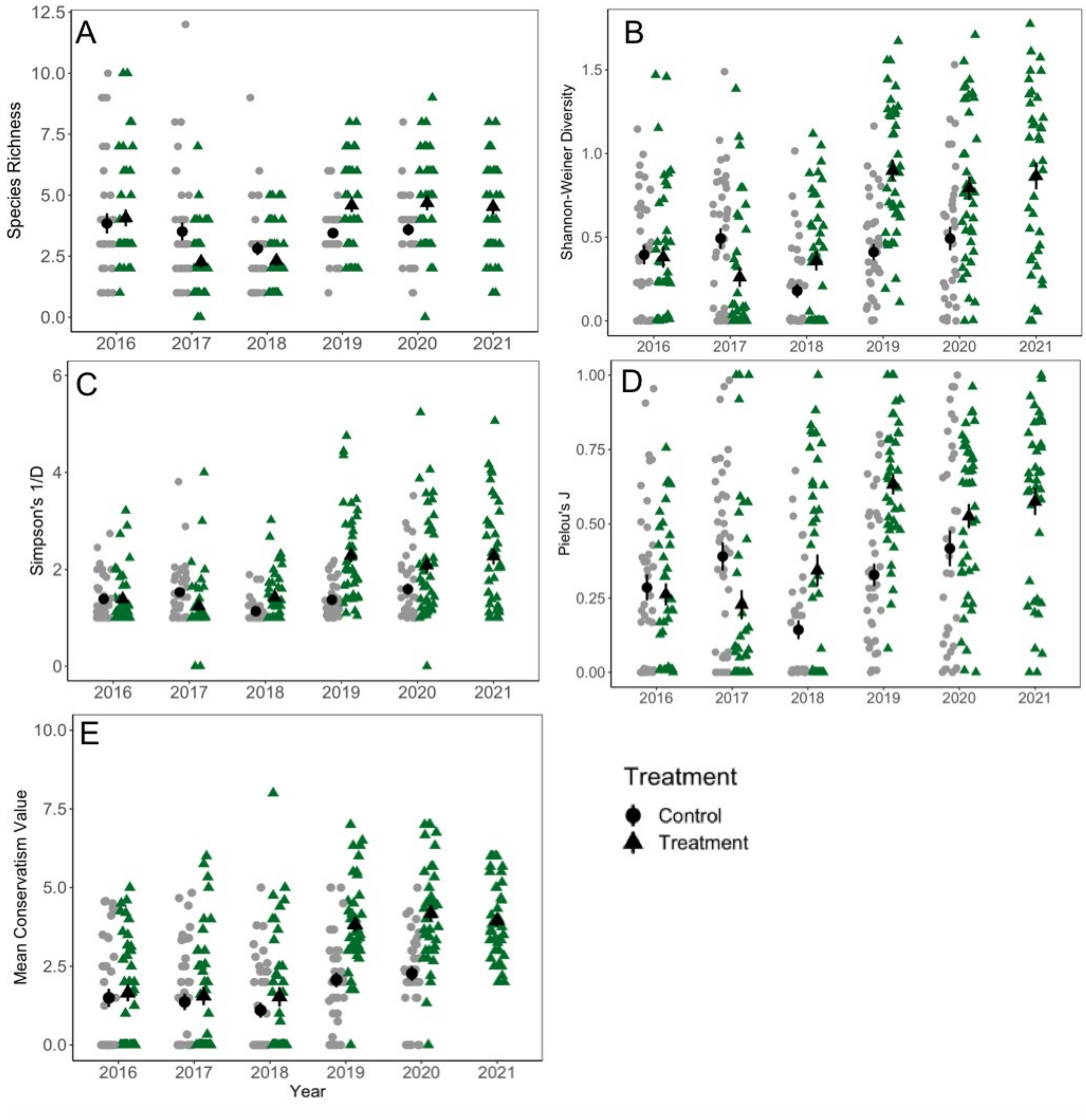


Figure 2-4. Comparison of species richness (A), Shannon-Weiner diversity (*H'*) (B), Simpson's diversity (1/*D*) (C), Pielou's evenness (*J*) (D), and mean coefficient of conservatism value (CC_s) (E) between control and herbicide-treated plots established in 2016, both pre-treatment (2016), and post-treatment (2017-2021). Control plots were not surveyed in 2021 as they were treated in

fall 2020. Grey circles represent control sites, and green triangles represent treated sites. Black symbols represent the mean, and error bars represent standard deviation.

Table 2-3. Two-way ANOVA test results for species richness, Shannon-Weiner diversity (*H'*), Simpson's diversity (*1/D*), Pielou's evenness (*J*), and mean coefficient of conservatism value (CC_s) for the 2016 aerially-treated permanent efficacy monitoring plots.

	Treatment			**Year**			**Treatment x Year**		
	df	F	p	df	F	p	df	F	p
Species richness	1, 383	0.302	0.583	4, 383	1.624	0.168	4, 383	6.079	<0.001
Shannon-Weiner diversity	1, 383	0.030	0.863	4, 383	4.401	0.002	4, 383	11.000	<0.001
Simpson's 1/D	1, 383	0.0002	0.988	4, 383	2.711	0.030	4, 383	9.959	<0.001
Pielou's J	1, 383	0.141	0.708	4, 383	5.683	<0.001	4, 383	8.763	<0.001
Mean conservatism value	1, 383	0.168	0.68	4, 383	3.17	0.014	4, 383	5.012	<0.001

Multivariate analyses

The cluster analysis yielded a dendrogram that orders the sites based on their similarity in community composition, merging groups of progressively greater dissimilarity (Fig. 2-5). Using the results of the indicator species analysis (Table 2-4) we determined there were 2 main groupings of sites. Nested within Group 1 was two subgroups. Group 1 included pre-treatment treated plots and control plots from the entire monitoring period. Group 2 included post-treatment treated plots. Nested within this second group were six subgroups. These plant communities tended to have higher beta diversity compared to Group 1, judging by the percent of information remaining at which they merged in the dendrogram (Fig. 2-5). Each group was characterized by at least one statistically significant indicator (Table 2-5).

Table 2-4. Results of indicator species analysis that determined the ideal pruning location for the resulting dendrogram. Asterisks indicate number of groups (2) or sub-groups (8) where pruning was optimal.

Number of groups	Mean p-value
2*	0.1599
3	0.1848
4	0.2158
5	0.1984
6	0.2268
7	0.2063
8*	0.1793
9	0.2146
10	0.2413
11	0.2405
12	0.2514
13	0.2163

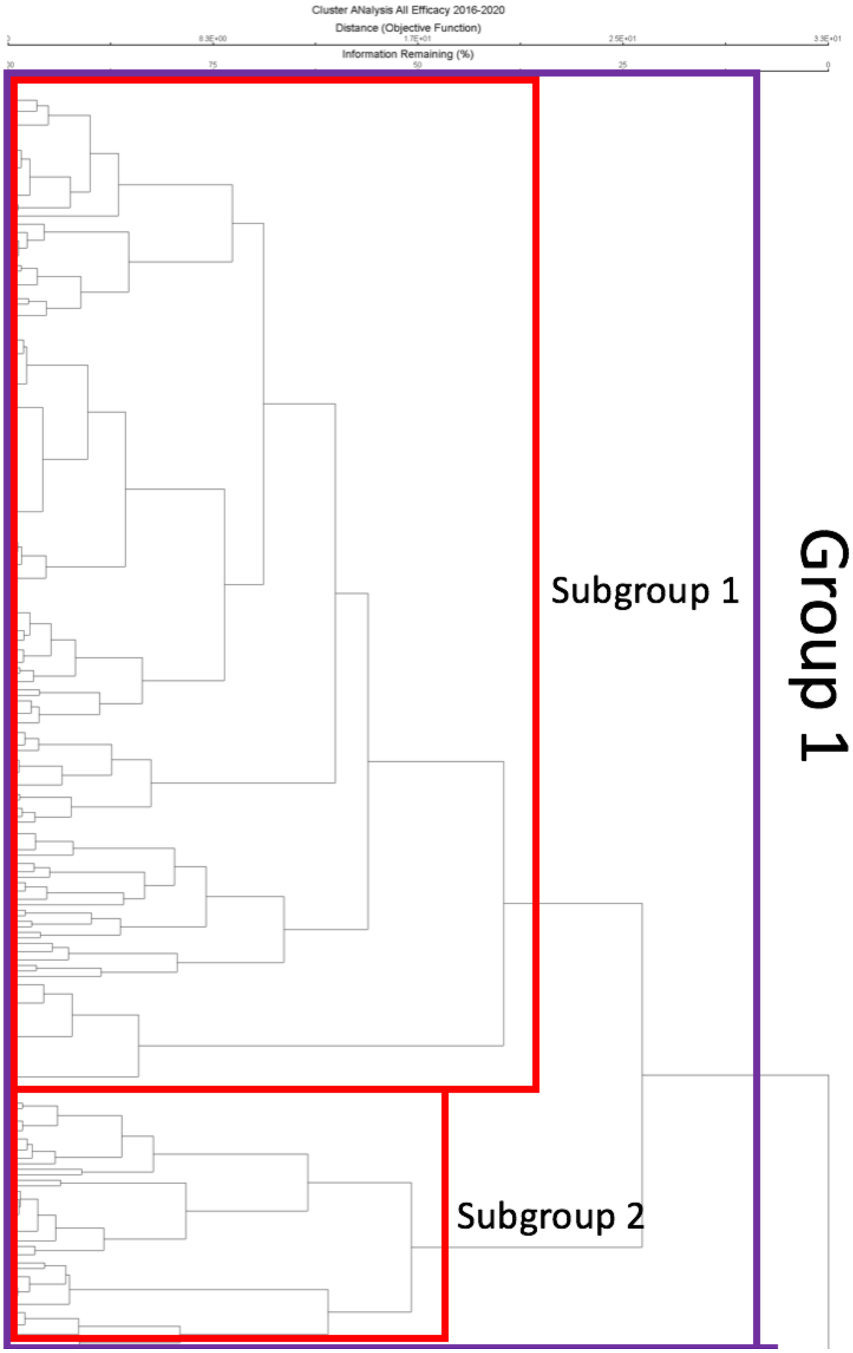


Figure 2-5. Cluster analysis for all 2016 aerially-treated and control permanent monitoring plots at Long Point and Rondeau (n = 80), both pre-treatment (2016), and post-treatment (2017-2020). Control site data was not collected in 2021. The full dendrogram at a coarser resolution can be viewed in Appendix 2.4.

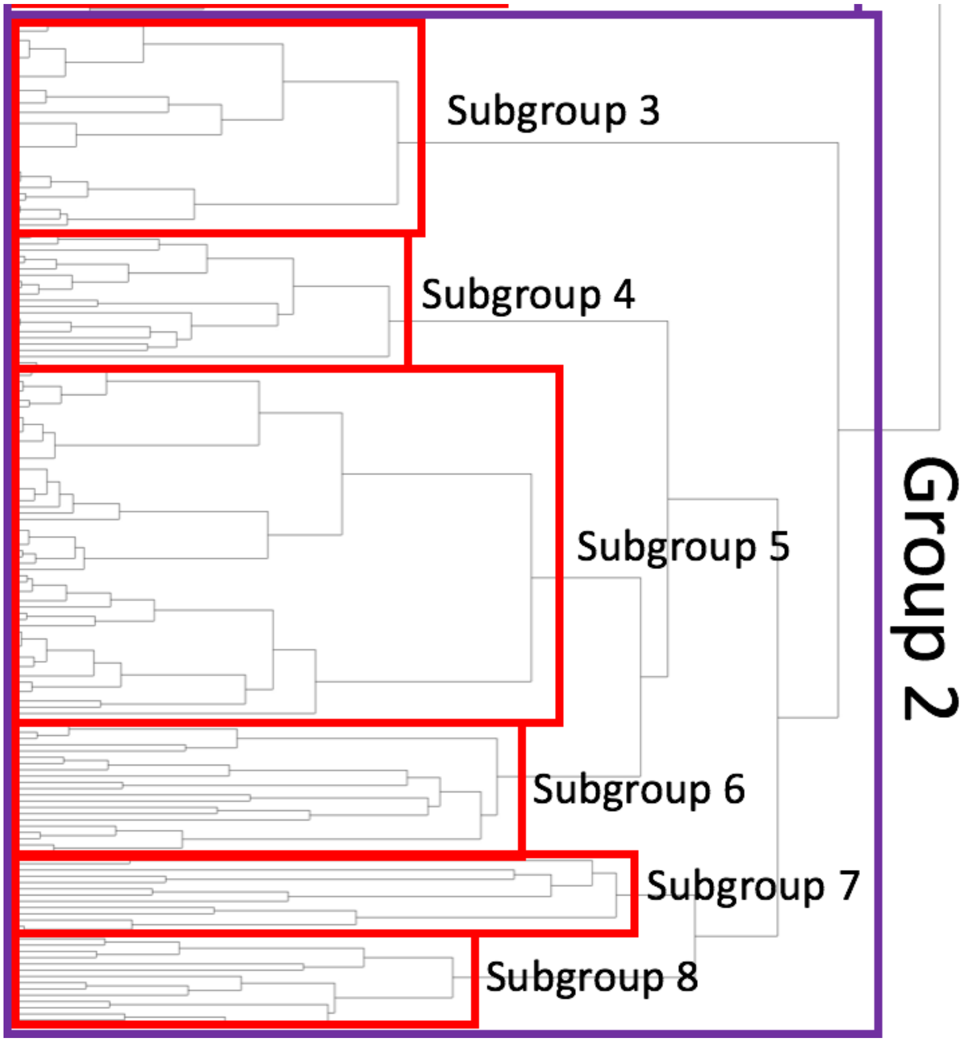


Figure 2-5. (continued) Cluster analysis for all 2016 aerially-treated and control permanent monitoring plots at Long Point and Rondeau (n = 80), both pre-treatment (2016), and post-treatment (2017-2020). Control site data was not collected in 2021. The full dendrogram at a coarser resolution can be viewed in Appendix 2.4.

Table 2-5. Results of the indicator species analysis using the eight subgroups defined from the cluster analysis for vegetation monitoring plots. For an alpha value of 0.05, only indicators with p-values below 0.05 are considered significantly faithful and exclusive in their group membership.

Indicator	**Group**	**Observed Indicator Value (IV)**	**p value**
Phragmites australis	**1**	**59.1**	**0.0002**
Calamagrostis canadensis	**1**	**10.7**	**0.0380**
Dulichium arundinaceum	1	8.6	0.0534
Eleocharis palustris	1	4.8	0.1398
Carex comosa	1	3.6	0.3103
Carex lasiocarpa	1	3.2	0.4311
Campanula aparinoides	1	2.7	0.4881
Polygonum spp.	1	2.2	0.5247
Standing dead	**2**	**37.7**	**0.0002**
Hydrocharis morsus-ranae	**3**	**45.9**	**0.0002**
Lemna minor	3	1.3	0.8512
***Typha* spp.**	**4**	**48.8**	**0.0002**
Unknown seedling	**4**	**8.7**	**0.0170**
Spirodela polyrhiza	4	8.9	0.0886
Nuphar lutea	4	5.5	0.0888
Equisetum fluvitale	4	5.2	0.3155
Sagittaria spp.	4	3.0	0.4719
Water	**5**	**30.7**	**0.0002**
Litter	**5**	**21.2**	**0.0018**
Utricularia intermedia	5	3.8	0.2611
Lythrum salicaria	5	2.7	0.4791
Decodon verticillatus	5	1.3	0.8542
Sagittaria latifolia	5	2.3	0.9110
Myriophyllum sibiricum	**6**	**34.2**	**0.0002**
Potamogeton richardsonii	**6**	**16.2**	**0.0016**
***Nitella* spp.**	**6**	**12.5**	**0.0058**
***Chara* spp.**	**6**	**11.3**	**0.0140**
Brasenia schreberi	**6**	**10.9**	**0.0150**
Nymphaea odorata	6	7.0	0.0732
Scirpus acutus	6	2.8	0.6317
Zizania palustris	**7**	**21.5**	**0.0010**
Carex aquatilis	**7**	**18.2**	**0.0028**
Pontederia cordata	**7**	**13.5**	**0.0028**
Najas flexilis	**7**	**13.0**	**0.0054**
Sparganium eurycarpum	7	7.9	0.0504

Indicator	**Group**	**Observed Indicator Value (IV)**	**p value**
Potamogeton praelongus	7	3.8	0.2567
Persicaria amphibia	7	5.1	0.3533
Elodea canadensis	**8**	**75.4**	**0.0002**
***Myriophyllum* spp.**	**8**	**70.3**	**0.0002**
Potamogeton foliosus	**8**	**27.2**	**0.0002**
Potamogeton zosteriformis	**8**	**35.2**	**0.0002**
Utricularia vulgaris	**8**	**18.6**	**0.0036**
***Potamogeton* spp.**	**8**	**15.6**	**0.0046**
Sparganium spp.	8	7.7	0.0562
Myriophyllum spicatum	8	4.1	0.2633

2.3.2 Community transitions among vegetation categories

The ratio of native and non-native-dominated plots changed through time (Fig. 2-6). Most plots in the two years immediately after herbicide application were non-native-dominated (>50% cover), but there was a shift towards native-dominated plots three years post-treatment. Moreover, no spatial aggregations in the trends of native- and non-native-dominated plots were observed through time (Fig. 2-7 & 2-8).

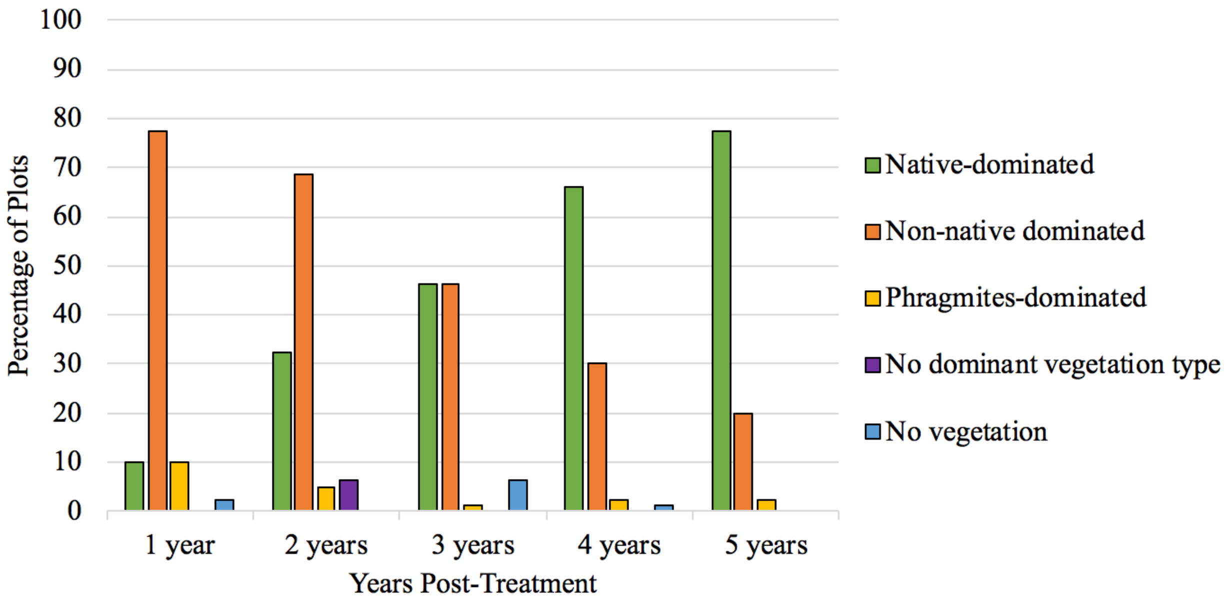


Figure 2-6. Percentage of 2016-herbicide-treated permanent monitoring plots that are native-, non-native- or *Phragmites australis*-dominated, have no dominant vegetation type, or have no vegetation present, one to five years post-treatment. Note that *P. australis* was counted separately from secondary non-native species, and so non-native-dominated plots were characterized by species such as European frog-bit (*Hydrocharis morsus-ranae*) and Eurasian milfoil (*Myriophyllum spicatum*) (see Appendix 2.2).

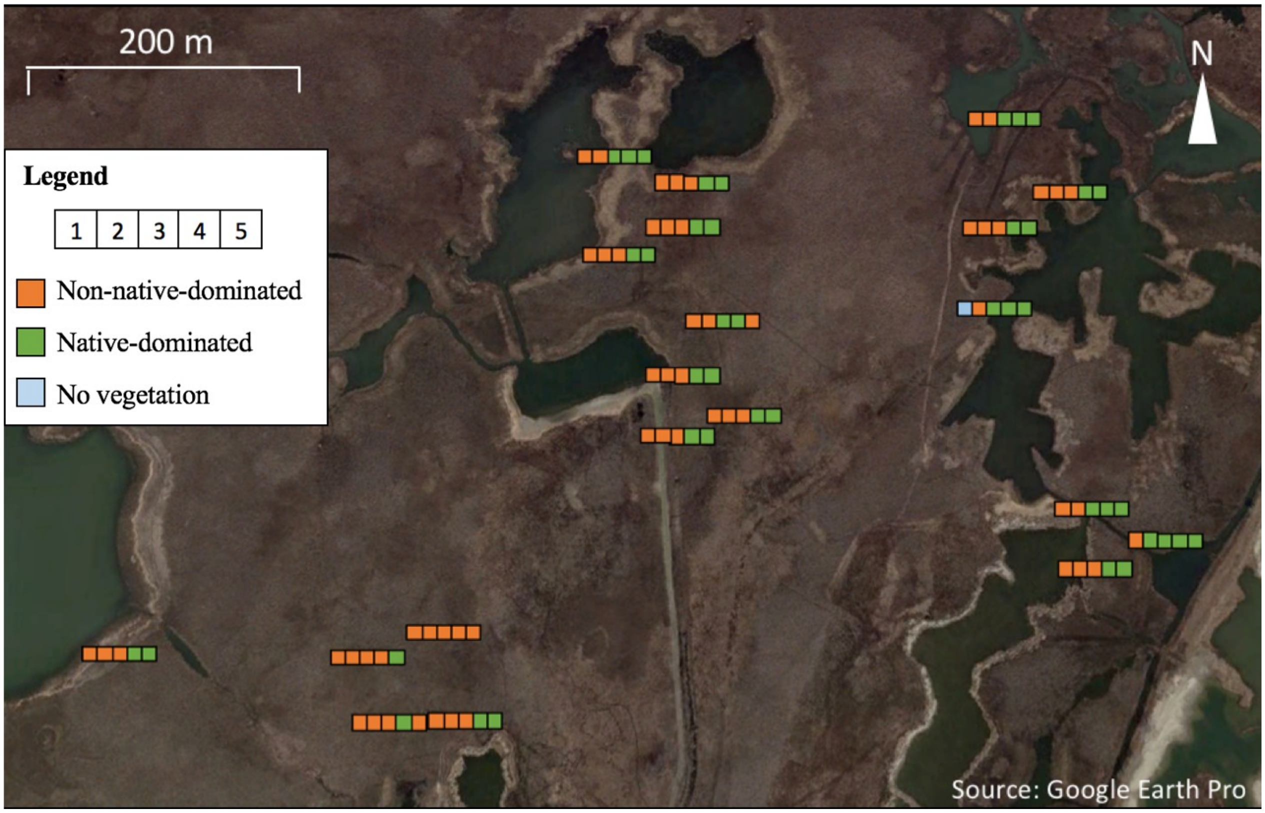
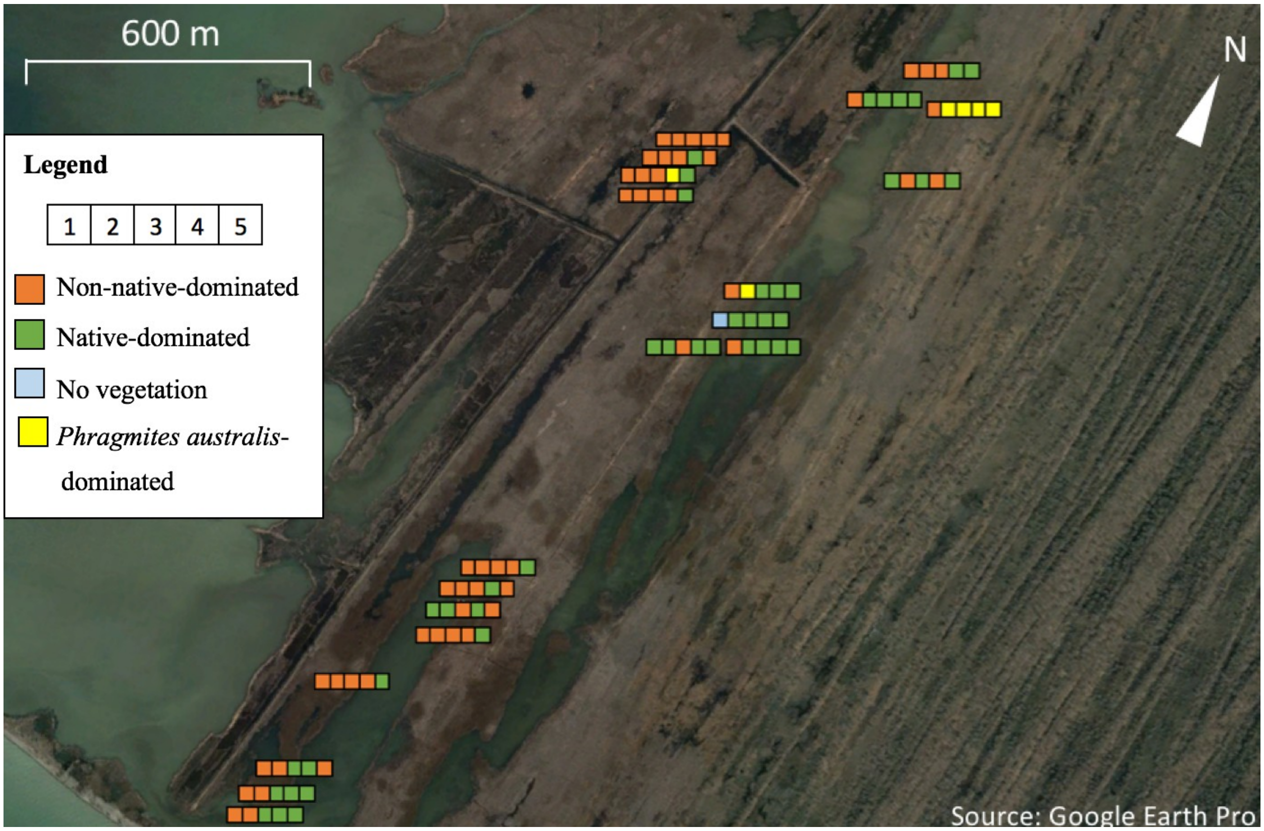


Figure 2-7. Vegetation trajectory of the 2016 aerially-treated plots at Long Point Peninsula (n = 20). Sites are classified as non-native- or native-dominated based on the dominant vegetation type (i.e., >50% of the vegetation present). Note that *P. australis* was counted separately from secondary non-native species, and so non-native-dominated plots were characterized by species such as European frog-bit (*Hydrocharis morsus-ranae*) and Eurasian milfoil (*Myriophyllum spicatum*) (see Appendix 2.2). The number signifies years post-treatment.



Source: Google Earth Pro

Figure 2-8. Vegetation trajectory of the 2016-treated plots at Rondeau Provincial Park (n = 20). Sites are classified as non-native-, native-, or *Phragmites australis*-dominated based on the dominant vegetation type (i.e., >50% of the vegetation present). Note that *P. australis* was counted separately from secondary non-native species (see Appendix 2.2). The number signifies years post-treatment.

2.3.3. Comparison between ground- and aerially-treated plots

Both ground- and aerially-treated plots followed similar trajectories, with primarily invasive-dominated plots in the first year post-treatment (Fig. 2-9 & 2-10). However, ground-treated plots overall experienced a greater delay in transitioning to native-dominated sites in comparison to aerially-treated plots. Namely, 40% of the ground treated sites were *P. australis*-

dominant one year following herbicide application (Fig. 2-9). While 15% (n = 3) of aerially treated plots contained *P. australis* in the year following herbicide application, 0% of these sites were *P. australis*-dominant (Fig. 2-10). However, there was a shift towards more native-dominated plots two to three years post-treatment in both the ground- and aerially-treated plots. Four years post-treatment, there has been a shift towards more invasive-dominated plots, as *M. spicatum* abundance has increased in both the ground- and aerially-treated plots. There was no spatial aggregation in vegetation type throughout the experiment (Fig. 2-11).

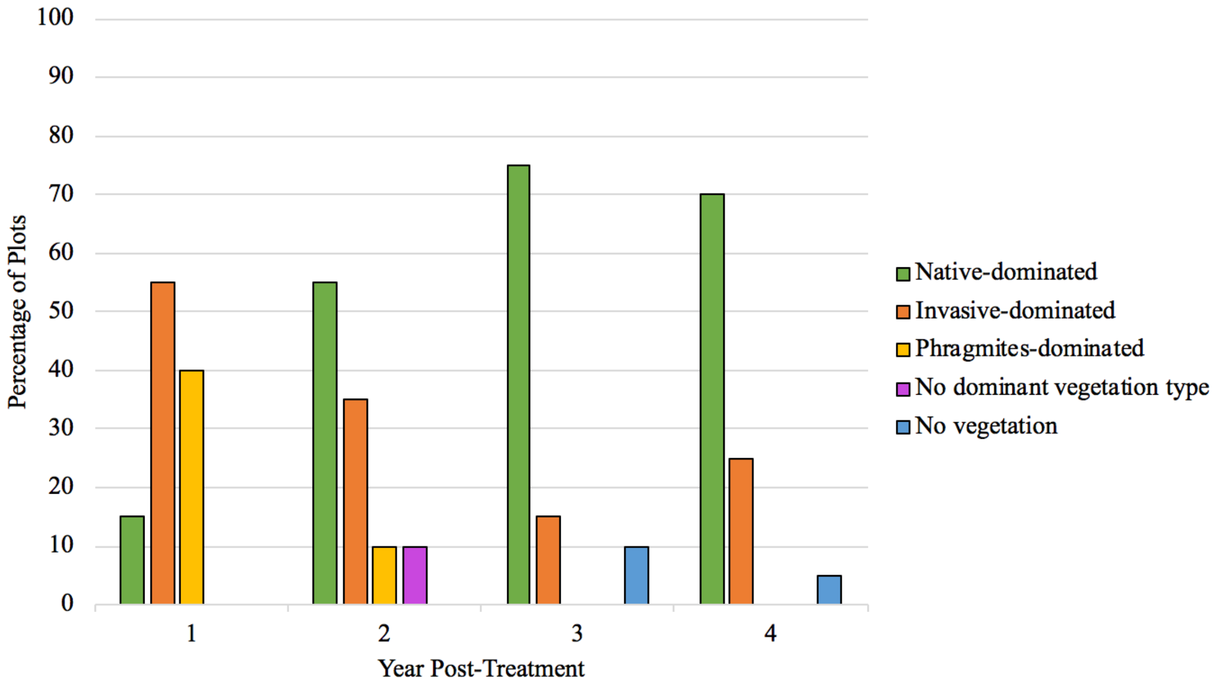


Figure 2-9 Vegetation types in 2017 ground-treated permanent plots at Long Point (n = 20), 1 to 4 years following herbicide application. Note that *P. australis* was counted separately from secondary non-native species, and so non-native-dominated plots were characterized by species such as European frog-bit (*Hydrocharis morsus-ranae*) and Eurasian milfoil (*Myriophyllum spicatum*) (see Appendix 2.2).

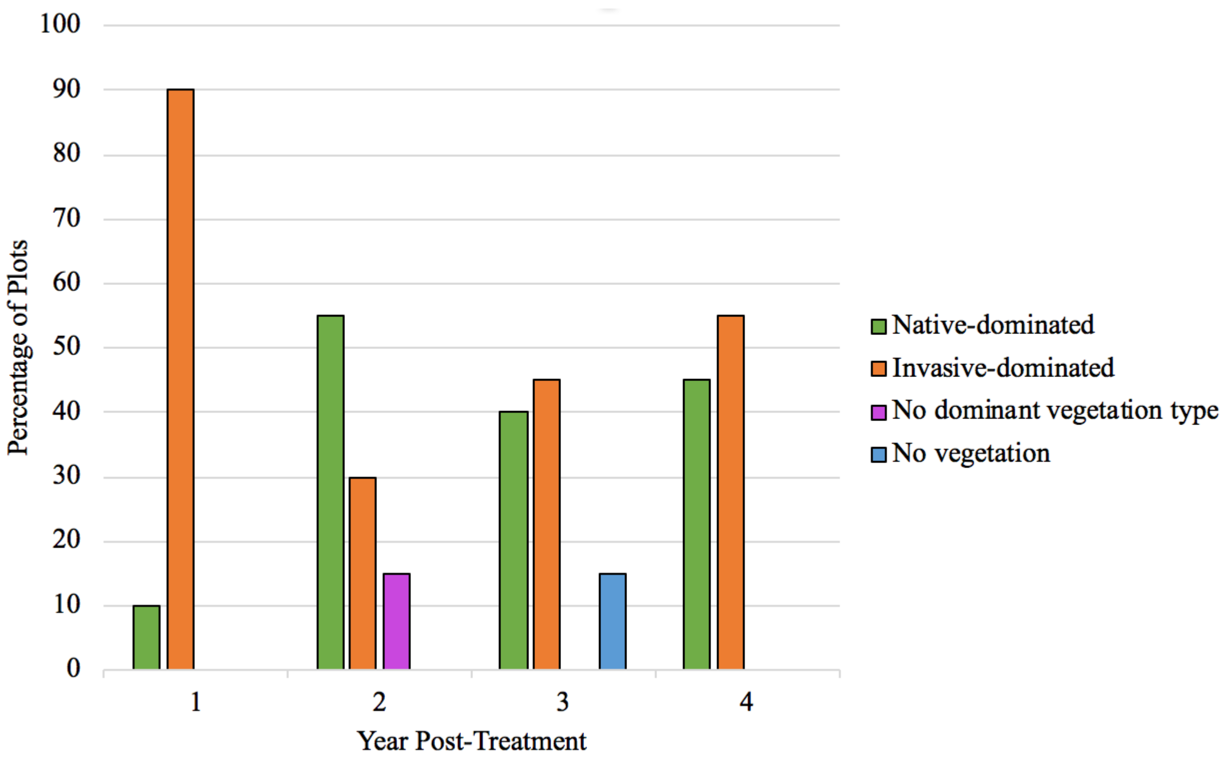
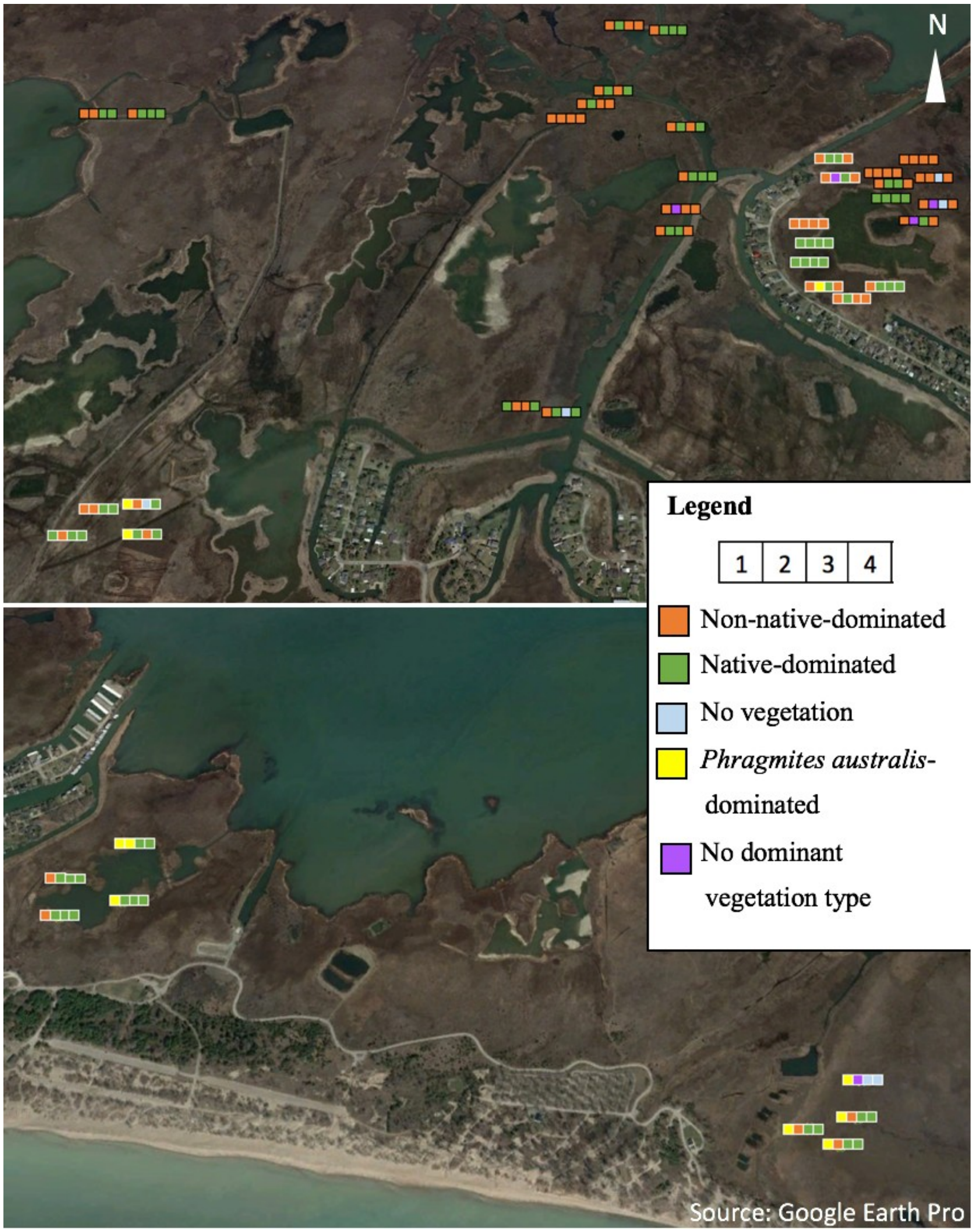


Figure 2-10. Vegetation types in 2017 aerially-treated permanent plots at Long Point (n = 20), 1 to 4 years following herbicide treatment. Note that *P. australis* was counted separately from secondary non-native species, and so non-native-dominated plots were characterized by species such as European frog-bit (*Hydrocharis morsus-ranae*) and Eurasian milfoil (*Myriophyllum spicatum*) (see Appendix 2.2).



Source: Google Earth Pro

Figure 2-11. Vegetation trajectory of the 2017-treated plots at Long Point Peninsula. Sites outlined in black were aerially-treated (n = 20), and sites outlined in white were ground-treated (n = 20). Sites are classified as non-native-, native-, or *Phragmites australis*-dominated based on the dominant vegetation type (i.e., more than 50% of the vegetation present). Sites with no vegetation were classified as No Vegetation, and sites with equal percent cover of non-native and native species were classified as No Dominant Vegetation Type. Note that *P. australis* was counted separately from secondary non-native species (see Appendix 2.2) The number signifies years post-treatment.

2.4 Discussion

The management of *P. australis* populations to encourage wetland restoration in Long Point and Rondeau Provincial Park was highly successful over a five-year period. *Phragmites australis* density was significantly reduced in herbicide-treated areas, with stem densities reduced by over 99% compared to contemporary control plots in the first year post-treatment, and remained more than 95% lower than baseline levels for the duration of monitoring. This is contrary to results obtained by Lombard et al. (2012) and Quirion et al. (2018), who found that *P. australis* persisted following herbicide application. While the reasons for this difference is unknown, they may be partially explained by Lake Erie water levels and the diverse coastal wetland seedbank. Despite this, there was a short-term reduction in species richness, evenness and diversity was observed in the first 2 y post treatment, and secondary invasion by European frog-bit (*Hydrocharis morsus-ranae*) occurred in many of the herbicide-treated areas. Yet this was followed by a transition of treated plots to a native vegetation community beginning 3 y after herbicide application by either aerial- or ground-application methods. This transition was accompanied by a 47% increase in richness (n = 20) and a 307% increase in mean coefficient of conservatism (n = 20) from 2016 (before herbicide application) to 2021 (5 y post-herbicide application), revealing improvements in floristic quality. The longer duration of monitoring was critical to determining the success of treatment, as changes that occurred in the first 2 y post-treatment were short-lived. This emphasizes the need for more long-term monitoring of wetland restoration outcomes.

2.4.1 Diversity of vegetation communities following herbicide treatment

The cluster analysis indicates that there was low beta diversity between the treated plots before herbicide application (i.e., plots were homogenous in terms of their community composition), and control plots remained this way throughout the monitoring period. Baseline and control plots were dominated by dense *P. australis* and Canada bluejoint grass (*Calamagrostis canadensis* (Michx.) P. Beauv). They were characterized by having a high canopy which intercepted light, hindering the emergence and growth of other species (Haslam 1972; Robichaud and Rooney 2019). The species richness and diversity indices were consequently low in the baseline and control plots in comparison to the treated plots after they were treated with herbicide (Robichaud and Rooney 2019).

In contrast, the treated plots developed higher beta diversity following herbicide application, yielding six distinct groups in the hierarchal cluster analysis. Two of these groups were non-native-dominated communities, dominated mainly by invasive or hybrid cattail (*Typha* spp.), and European frog-bit (*Hydrocharis morsus-ranae*), while three of the groups were native-dominated. The remaining group was dominated by open water, and was more common in the 2 y immediately following herbicide application. The higher level of beta diversity observed among the treated plots after herbicide application contrasts with the relative homogeneity of the control plots and baseline measures from the treatment plots.

With *P. australis* removed, more light penetrating the canopy, and dense *P. australis* stands not hindering native seed dispersal or forming obstructive litter mats (Minchinton 2006), the vegetation communities diversified, resulting in more species-rich communities with greater evenness. This shift towards more diverse communities is similar to results observed by Bonello and Judd (2019), who found that vegetation diversity was elevated in treated sites 6 to 10 years post-treatment in comparison to untreated sites. As Lake Erie water levels continue to drop in the near future (Fig. 1-1), emergence from the seedbank in treated areas may continue to increase community diversity as marsh meadow communities become more prevalent (Keddy and Reznicek 1986; Keddy and Campbell 2019).

2.4.2 Community succession

The trajectory of the vegetation communities following herbicide application also emphasizes the need for long-term studies, as the community succession appears to be biphasic. The non-native-dominated plant community groups comprised treated site × year combinations primarily in the first 1-3 y post-treatment, whereas the native-dominated groups comprised treated site × year combinations primarily 3-5 y post-treatment, revealing a pattern in community succession from *P. australis* domination before treatment, to an early phase of dominance by secondary invasives, followed by eventual dominance by native plant species.

In the 2 y post-treatment, many of the vegetation communities were dominated by invasive species, likely due to the open niche space afforded by the removal of dense *P. australis* stands (Robichaud 2021). The persistence of standing dead *P. australis* culms likely exacerbated this, combining with high water levels to hinder the emergence of native seedlings from the seedbank. High water levels and shade are not ideal for germination (Welling et al. 1988), and prior research found that the litter from invasive *P. australis* inhibited the recovery of native vegetation (Bonello and Judd 2019). In many plots, the open niche-space was therefore filled by Eurasian milfoil (*Myryiophyllum spicatum* (L.)) and European frog-bit (*Hydrocharis morsus-ranae*), invasive plants that were present at low abundances in the monitoring plots prior to herbicide treatment and could therefore rapidly colonize open water areas once *P. australis* was removed. Invasion of herbicide-treated areas by secondary invasive plants or reinvasion by *P.*

australis following herbicide application is common in the literature (e.g., Lombard et al. 2012; Quirion et al. 2018; Bonello and Judd 2019; Robichaud and Rooney 2021b).

Hydrocharis morsus-ranae was the invasive species most common in the invasive-dominated plots post-treatment. *Hydrocharis morsus-ranae* is a free-floating plant that reproduces primarily vegetatively (Catling et al. 2003). Once mature, it forms dense mats, obscuring light from submersed aquatic vegetation growing beneath it (Catling et al. 2003). Dense mats tend to grow in sheltered areas, predominantly in areas with emergent vegetation, as *H. morsus-ranae* is unable to grow in areas with high wave action (Monks et al. 2019). While many native species require large amounts of incident light to germinate (Welling et al. 1988), *H. morsus-ranae* is shade tolerant, giving it a competitive advantage under the canopy of emergent wetland plants (Zhu et al. 2014). While not present in Lake Erie during surveys conducted in 2007 (Trebitz and Taylor 2007), *H. morsus-ranae* was present in many of our monitoring plots at baseline, where it co-occurred with *P. australis* and *Typha* spp. (Robichaud 2021).

While *H. morsus-ranae* was dominant in treated plots between 2017 and 2019 and was present in many plots thereafter at lower relative abundance, its dominance in the treated plots decreased after 2019. The precise drivers of this community transition are unclear, but the transition coincides with the final collapse of standing dead *P. australis* stems, which contributed to light interception, as well as a dramatic increase in water depth between 2018 and 2019 across Lake Erie. Due to the inability of *H. morsus-ranae* to withstand wave action, likely deeper water and increased hydrodynamic driven damage to plants played an important role.

As standing dead *P. australis* stems decreased in density and water levels increased in the lake, richness and diversity rebounded, leading to a more rich and diverse community than was present before herbicide application. Notably, many native submerged aquatic species, primarily Canadian waterweed (*Elodea canadensis* (Michx.)), nodding waternymph (*Najas flexilis* (Willd.) Rostk. & Schmidt), and waterweed species (*Potamogeton* spp. (L.)), and floating species such as water-shield (*Brasenia schreberi* J.F. Gmel.) became dominant in many of the plots. The transition to vegetation communities dominated by native submersed aquatic and floating species was no doubt facilitated by the increase in Lake Erie water levels (Fig. 1-1). Submersed and floating species that commonly propagate vegetatively via plant fragments likely have a dispersal advantage over rooted emergent plants in flooded marsh conditions, as rooted emergent species that propagate vegetatively do so more typically by rhizome or stolon. Being connected to the parent plant, the expansion by rhizomes and stolons will be delayed compared to the colonization ability of plants that reproduce by fragments transported in the water. Even longer may be required for native emergent species to colonize flooded areas after *P. australis* removal, however drawdown and exposure of the sediment opens another pathway for dispersal via sexual reproduction and emergence from the seedbank.

Though the general trend in vegetation succession in our herbicide-treated monitoring plots is a shift towards dominance by native species, some plots that were native-dominated in 2020 transitioned back to a non-native-dominated state in 2021. Interestingly, in 2021 the non-native-dominated plots were primarily dominated by the submersed aquatic plant Eurasian milfoil (*Myriophyllum spicatum*) rather than the floating species *H. morsus-ranae*. *Myriophyllum spicatum* was present in Lake Erie before treatment began (Trebitz and Taylor 2007). While *M. spicatum* was not present in the 2016 permanent monitoring plots before treatment took place, it was present in 6 of the treated plots in 2017, one year following herbicide application, afterwhich *Myriophyllum spicatum* can grow in water up to 6 m deep and, unlike *H. morsus-ranae*, is able to withstand wave action (Eiswerth et al. 2000). Thus, we suspect the continued high water levels are responsible for the replacement of *H. morsus-ranae* with *M. spicatum* as the most common secondary invasive plant species in our monitoring plots.

2.4.3 Comparison of aerial- and ground-treated plots

Both the ground- and aerially-treated plots that were treated in 2017 followed similar trajectories over time. As we observed in the treatment plots that had aerial application in 2016, the vegetation trajectory of the plots treated by ground and aerial application methods in 2017 was biphasic. We saw the same initial period of dominance by secondary invasive plants, transitioning into more native-dominated plots three to four years post-treatment. While the ground and aerial plots followed similar trajectories over time, there was a slight delay in the efficacy of ground treatment in comparison to aerial treatment, with *P. australis* re-invading a greater number of plots in the year post-treatment, compared to the aerially-treated plots. Other studies of ground application methods to treat *P. australis* reported slow progress in *P. australis* suppression and that efficacy decreases with the size of the patch (Lombard et al. 2012; Quirion et al. 2018). As such, re-invasion in the years following ground-treatment is common (Lombard et al. 2012; Quirion et al. 2018). One clear difference between helicopter and MarshMaster™ application methods that might explain their differing suppression efficacy is that the two methods had different prescribed application rates for the active ingredient in the herbicide formulation. Whereas the helicopter applied glyphosate at a consistent rate of 4210 g a.e. glyphosate/ha, the MarshMaster™ applied it at a range of 1200-3600 g a.e. glyphosate/ha, depending on the stem density of *P. australis*. This reflects the level of operator discretion possible with ground application that is not achievable from a moving helicopter. However, we suspect that the relatively lower suppression efficacy achieved with ground-application was not simply due to the lower prescribed herbicide application rate. In our case, the relatively poor performance of ground-application was likely a water quality issue, as the MarshMaster™ took water from the wetland to blend with a concentrated solution of herbicide to achieve the appropriate a tank-mix (pers. comm. June 2017, Eric Giles, Giles Restoration Services, St. Williams, ON). In contrast, the helicopter applying herbicide from the air used potable water to achieve the target herbicide formulation (per. comm. June 2017, Eric Cleland, Nature Conservancy of Canada). Glyphosate, the active ingredient in the herbicide, is known to bind to suspended sediment and anions dissolved in the water, and this can lead to reduced herbicide activity levels (Buhler and Burnside 1983; Whitford et al. 2009). Thus, we anticipate that the actual activity of glyphosate in the ground-treated areas was below the target, resulting in more *P. australis* surviving the initial treatment. Subsequently, the licensed herbicide applicator conducting this treatment (Giles Restoration Services) incorporated a 100 µm mesh filter to improve the quality of water being used to generate the herbicide mix and we recommend that all ground-based treatment operations ensure adequate water quality if drawing from a local water body to both ensure the wise use of herbicide, and to maximize *P. australis* suppression (Cleland 2020). Despite the initial differences in *P. australis* suppression, both the ground- and aerially-treated plots resulted in successful *P. australis* removal within 4 y of treatment.

2.5 *Conclusions*

The aerial and ground herbicide application at Long Point and Rondeau successfully reduced the density of, or eradicated *P. australis* in treated sites. Changes that occurred in the first 2 y post-treatment, such as the presence of *H. morsus-ranae*, and low diversity and species richness in several of the plots, were short-lived, and the trajectory has now shifted towards more native-dominated communities that are both species-rich and diverse. With these results, we conclude that *P. australis* suppression can be successful, though vegetation community succession may take three or more years before native vegetation recovers. We caution that the presence of standing dead culms of *P. australis* persist past herbicide application and continue to influence vegetation succession. Further, water depth appears to play a crucial role in determining the direction of vegetation community development. Importantly, our study took place during a period of high water on Lake Erie, and consequently vegetative propagation of plants played a greater role in revegetation than seedlings. With Lake Erie water levels now entering a period of decline (NOAA 2022), continued monitoring is needed to determine how germination from the seedbank influence the direction of vegetation development and to determine if further invasions by non-native species, such as *M. spicatum* and invasive *Typha* spp., or re-invasions by *P. australis* may reverse the encouraging changes in species diversity, evenness, and floristic quality we observed in our study. Finally, we conclude that both ground and aerial herbicide application methods are effective over a four-year period, but proper filtration of the water used in ground treatment is crucial to ensure the herbicide is highly effective in suppressing *P. australis*.

3.0 Examining the seedbank of an invaded coastal wetland

3.1 Introduction

Wetland restoration following non-native species invasions is complex and frequently leads to unintended consequences (D'Antonio and Meyerson 2002). Even decades after restoration, the plant community may not return to reference conditions (Moreno-Mateos et al. 2012). Usually within 5-11 y, the trajectory of plant communities in restored wetlands will begin to converge with the species composition of degraded wetlands, typically due to non-native species (Matthews and Spyreas 2010). To encourage successful long-term restoration, multiple variables need to be considered (Zedler 2000), chief among them the seedbank. Many restoration activities do not involve active re-planting of native species, instead, practitioners expect the native species that are present in the seedbank and in neighboring wetland habitats to re-colonize a restored wetland: a process called "passive" or "natural" restoration (see Atkinson and Bonser 2020). Knowledge of the seedbank, where seeds settle and collect (Leck and Graveline 1979; Hazelton 2018), can help predict which species will return following wetland restoration.

Seedbanks can be highly variable, both in terms of their species composition and seed longevity. Some seedbanks closely resemble the vegetation community present (e.g., Rohal et al. 2021), while others differ substantially from the mature plants growing in a location (e.g., Leck and Simpson 1995; Frieswyck and Zedler 2007). Additionally, some seeds may remain viable for a single year in the seedbank, while others may remain viable for decades (Leck and Graveline 1979; Telewski and Zeevaart 2002). Due to this variability, the seedbank may instead reflect historic plant assemblages, including those no longer present or not capable of growing under current conditions (Rohal et al. 2021). The seedbank of a wetland where non-native plants have been eradicated may therefore still contain viable propagules ready to recolonize once conditions allow. The seedbank can therefore be considered an indicator of wetland resilience (Frieswyck and Zedler 2007).

This is of high concern in areas invaded by European common reed, or *Phragmites australis* subsp. *australis* (Cav.) Trin. ex Steud. (hereafter *P. australis*). *Phragmites australis* is an aggressively invasive perennial grass and has been termed North America's worst invasive species (Catling 2005). *Phragmites australis* grows in dense stands (>100 stems/m^2) up to 4 m tall (Haslam 1972; Chapter 2). While it often reproduces via vegetative methods (i.e., stolons and rhizomes) (Haslam 1972), *P. australis* also produces lightweight seeds, with each culm capable of producing 200-5000 seeds (Wijte and Gallagher 1996). Though once considered unimportant in invasion, the role of *P. australis* seeds in spreading the invasion is now well recognized (e.g., Kettenring et al. 2009; Kettenring et al. 2011; McCormick et al., 2020).

Phragmites australis invasion affects many animal species by altering critical habitat (e.g., Markle and Chow-Fraser 2018; Robichaud and Rooney 2021b). In total, *P. australis* negatively affects 25% of all Species at Risk in Ontario (Bickerton 2015). Most of its influence on wildlife is indirect, whereby *P. australis* outcompetes and replaces native plant species that provide the habitat structure required by nesting birds, basking turtles, or juvenile fish (Weinstein and Baletto 1999; Robichaud and Rooney 2017; Markle and Chow-Fraser 2018). As *P. australis* grows in dense monotypic stands, it also hinders native plant seed dispersal (Minchinton et al. 2006), and its dense canopy intercepts light and shades out native plant species (Haslam 1972; Robichaud and Rooney 2021b). Few plant species can grow alongside *P.*

australis, which results in low richness and diversity in invaded areas (Wilcox 2012; Bonello and Judd 2019; Chapter 2).

To treat *P. australis* and to encourage the re-establishment of native vegetation communities, managers often choose to perform *P. australis* treatment via mechanical (e.g., mowing or burning) or chemical (e.g., herbicide) methods (Hazelton et al. 2014; Chapter 2). The most popular method of *P. australis* treatment in North America involves herbicide; for example, a literature review by Hazelton et al. (2014) found that 27 of 34 papers published on invasive *P. australis* treatment focused on herbicide use, either alone or in combination with secondary treatments. This agreed with a results of a survey that Martin and Blossey (2013) conducted of 285 land managers from US public and private conservation organizations, of whom 94% reported using herbicides to manage *P. australis*. It also aligns with a more recent survey by Rohal et al. (2018) of land managers from 42 wetland management units across the Great Salt Lake watershed, which found that 97% use herbicide as their primary *P. australis* management tool. Both glyphosate and imazapyr are commonly used to manage invasive *P. australis* on dry land and in standing water in the US. In Canada, glyphosate is commonly used to treat *P. australis,* but only in areas without standing water (Pest Management Regulatory Agency's Re-evaluation Decision RVD2017-01). However, in March 2021 imazapyr was approved for use over standing water (Pest Management Regulatory Agency's Registration Decision RD2021-03). Both are post-emergence herbicides, and therefore act solely on the adult plant, not directly on seeds (Tu et al. 2001; Hazelton et al. 2014). Viable *P. australis* seeds contained in the seedbank therefore hold the capacity to re-establish *P. australis*, leading to rapid re-invasions of treated areas (e.g., Elsey-Quirk and Leck 2021).

While these herbicides are typically more effective than mechanical methods at suppressing *P. australis* (Rohal et al. 2019), they do not necessarily achieve complete eradication (Lombard et al. 2012; Quirion et al. 2018; Chapter 2). Eradication in the context of *P. australis* has been defined by Quirion et al. (2018) as the failure of *P. australis* to return for a minimum of 3 years post-treatment, and based on our literature review it is uncommon. *Phragmites australis* often returns shortly following treatment (e.g., Lombard et al. 2012; Martin and Blossey 2013; Hazelton et al. 2014; Quirion et al. 2018). This is potentially due to recolonization from remnant patches, neighboring properties, or the seedbank. The relative importance of these three sources is unclear.

Even in areas where *P. australis* does not re-establish in high densities, open niche space afforded by treatment can result in secondary invasion by other invasive species (Pearson et al. 2016). For example, Robichaud and Rooney (2021b) reported that after herbicide was used to suppress *P. australis* in Long Point and Rondeau, Ontario the treated areas became dominated by European frog-bit (*Hydrocharis morsus-ranae* (L.)), non-native hybrid cattail (*Typha* x *glauca* (Godr. (pro sp.)), and Eurasian milfoil (*Myriophyllum spicatum* (L.)). Although the continued monitoring through a period of extremely high-water reported in Chapter 2 revealed that native species eventually displaced these secondary invaders, particularly *H. morsus-ranae*, it remains unclear whether this desirable pattern of succession would be widespread. Work from Chesapeake Bay (Hazelton et al. 2018) indicates that active seeding may be necessary to revegetate wetlands following *P. australis* removal, especially in areas subject to hydrologic disturbance (Rohal et al. 2019).

Studies exploring the effects of herbicide treatment on the seedbank of treated areas are relatively uncommon in *P. australis* research (Hazelton et al. 2014; Galatowitch et al. 2016;

Elsey-Quirk and Leck 2021; Rohal et al. 2021; Robinson 2022). However, it has been suggested that herbicide application and subsequent removal of *P. australis* results in a marked reduction to the number and viability of *P. australis* seeds present in the seedbank (Rohal et al. 2021). This is perhaps due to the relatively short period of seed viability in *P. australis*, such that if plants are prevented from setting seed in fall, very little viable seed remains in the seedbank to germinate the following spring (Howell 2017). It has also been suggested that despite years of *P. australis*-dominance, the seedbank may remain diverse with native seeds (Hazelton et al. 2018; Rohal et al. 2021). This suggests that herbicide treatment can successfully control *P. australis* at the seedbank level, and that native vegetation recovery is possible without forms of "active" restoration (i.e., seeding treated areas with native seed mixes). However, if *P. australis* persists in the seedbank, it should be expected to spread at an exponential rate under low water level conditions (e.g., Wilcox 2012; Jung et al. 2017). Additionally, if the seedbank does not remain diverse, vegetation recovery will likely be dispersal limited (Kettenring and Galatowitch 2011) or seed limited (Robinson 2022). It is therefore important to understand the contents of the seedbank in places where restoration is taking place (Kettenring and Tarsa 2020) as it can indicate a wetland's resilience (Frieswyk and Zedler 2006).

One type of experiment that may be used to determine the abundance and diversity of seeds in the seedbank is an emergence experiment. These experiments involve collecting seedbank samples and growing them in a greenhouse under controlled conditions. Once a seedling is identifiable, it is typically removed (*sensu* van der Valk and Davis 1978), meaning that competition and priority effects are not a factor in seedling emergence. The diversity of wetland plant species detected using this method can be maximized by using two watering regimes: a flooded regime, where the water level is maintained above the soil surface, and a moist regime, where the soil is kept moist but never flooded (*sensu* van der Valk and Davis 1978). However, the comparison of emergence between the moist and flooded conditions can also be of direct scientific interest where water levels are known to fluctuate periodically (Mushet et al. 2018). While the emergence method is often inaccurate in determining the precise number of viable seeds in the seedbank, it does give an indication of the composition of viable seeds contained in the sample (Poiani and Johnson 1988; Brown 1998).

We implemented an emergence experiment using seedbank samples collected from dense patches of *P. australis* (controls), regions where *P. australis* had recently been treated with herbicide (herbicide-treated), and reference sites still dominated by native marsh species (reference). The seedbank samples were exposed to both a flooded regime and a moist regime to test whether *P. australis* herbicide treatment influenced the wetland seedbank composition. Our research had two objectives: 1) to determine how seedling emergence, including *P. australis* emergence, differed between vegetation types and watering regimes, and 2) to determine how community composition varied between these factors.

3.2 *Methods*

3.2.1 Field site

Long Point Peninsula is a 35 km long sandspit, including a diverse wetland complex located on the northern shore of Lake Erie (Reznicek and Catling 1989). Long Point supports critical habitat for many threatened and endangered plant and animal species (Reznicek and Catling 1989). However, this habitat was significantly altered by a large-scale *P. australis* invasion, which occurred in the mid-1990s. The invasion is believed to have been facilitated by a period of low water levels (Wilcox 2012, Jung et al. 2017). Dense *P. australis* stands have since negatively affected numerous Species at Risk at Long Point (e.g., Green and Greenburg, 2013; Markle and Chow-Fraser 2018; Polowyk 2020).

To treat *P. australis* at Long Point and to encourage the growth of native species, a pilot *P. australis* suppression project was launched in 2016 by the Ontario Ministry of Northern Development, Mines, Natural Resources and Forestry (MNDMNRF). Environment and Climate Change Canada and Canadian Wildlife Service joined the project in 2019, when they first engaged in *P. australis* suppression in the adjoining Big Creek unit of the Big Creek National Wildlife Area (see Appendix 1.1). An Emergency Use Registration (no. 32356) was obtained for this pilot project, enabling partners to use a glyphosate-based herbicide (Roundup® Custom for Aquatic & Terrestrial Use Liquid Herbicide, Bayer CropScience, Whippany, New Jersey, USA), combined with a non-ionic alcohol ethoxylate surfactant (Aquasurf®, registration no. 32152 Brandt Consolidated Inc., Springfield, IL, USA). Herbicide (4210 g acid equivalent [a.e.] glyphosate/ha as an isopropylamine salt) was applied via helicopter in 2016 at the Long Point sites, and the same blend of Roundup® Custom (1200-3600 g [a.e.] glyphosate/ha as an isopropylamine salt) was applied via MarshMaster^{TM} in 2019 at Big Creek sites.

To date, over 1500 ha of invaded marsh has been treated under the Emergency Use Registration. While this treatment has largely been effective in reducing the density of *P. australis* (Robichaud and Rooney 2021b: Chapter 2), high water levels that have been present since treatment occurred have hindered germination from the seedbank (Fig. 1-1; NOAA 2022). However, Lake Erie water levels undergo natural cycling in water depths, with about 1 m difference between low and high periods on decadal scales (Figure 1-1). Lake Erie is now entering a period of drawdown (NOAA 2022), and water levels are expected to remain low until about 2025. As such, we expect the seedbank will begin to play a crucial role in the direction of revegetation and vegetation succession following *P. australis* suppression.

3.2.2 Site selection

In July 2020, we selected a total of 60 sites, located in areas of dense untreated *P. australis* (>100 stems/m^2) (control sites) (n = 20), herbicide-treated *P. australis* (treated sites) (n = 20), and uninvaded reference marsh (reference sites) (n = 20) (Fig. 3-1). Treated and reference marsh sites were at minimum 50 m away from living *P. australis* stands.

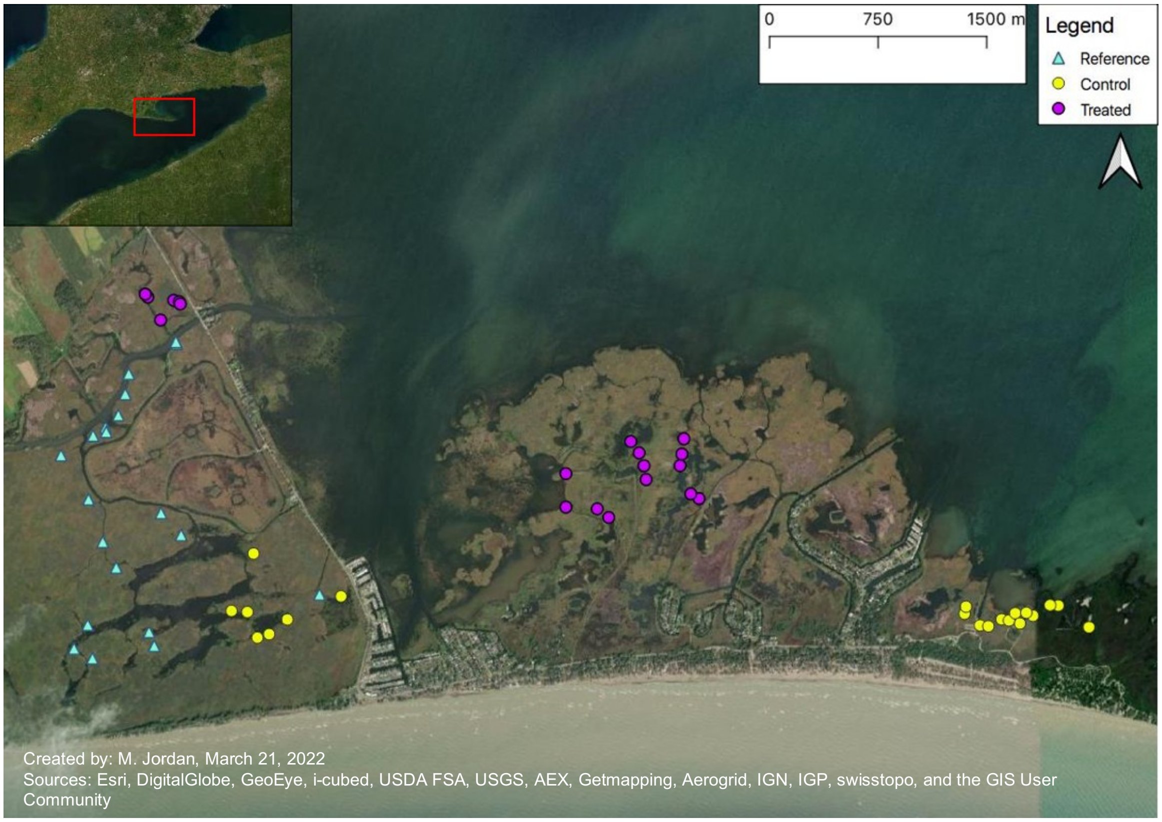


Figure 3-1. Location of reference (n = 20), control (n = 20), and treated (n = 20) sites from which seedbank samples were collected in 2020.

3.2.3 Plant surveys and seedbank collection

To determine the community composition in the wetland, we completed a timed survey of the plant species at each site in July 2020, during peak aboveground biomass. We began a 15-minute timer at the centre of the site, then travelled away from the centre in a spiral formation. When we encountered a new plant species, we stopped the timer and recorded the species, after which we restarted the timer. Voucher samples of unknown plant species were taken for further examination if needed. We identified a total of 53 species via the timed surveys (Appendix 3.1).

At each site, we also collected a seedbank sample, comprising the top 2 cm of sediment, within which the highest density of seeds in a wetland seedbank occurs (Leck and Graveline 1979). We collected the seedbank samples with a soil corer, cutting off the top 2 cm of soil from each core. We collected 400 cm^3 of seedbank samples from each site, the equivalent amount of soil for two 10 cm^2 x 2 cm deep samples. We kept the samples refrigerated at 4 °C in sealed plastic bags until we processed them. We filtered each seedbank sample through three sets of sieves (4000 µm, 212 µm, and 120 µm) to remove large debris, turions, and *P. australis* rhizomes and stolons, to ensure that any *P. australis* that emerged from the samples emerged from seed. We reserved the material remaining in the two finest sieves, resulting in sixty seedbank slurries. We rinsed equipment thoroughly between samples to prevent cross-contamination among samples. Timed surveys and seedbank collection was completed in July 2020.

3.2.4 Greenhouse methods

We used two watering regimes to maximize seedling emergence (*sensu* van der Valk and Davis 1987). The first was a flooded regime, where we continuously maintained the water level approximately 2-4 cm above the sediment, and the second was a moist regime, where we maintained soil moisture, but the soil was never flooded. In the flooded regime, we filled translucent plastic cups with approximately 4 cm of moistened peat-based soil, topped with 1 mm of sand to prevent downward seed migration. In the moist regime, we used 72-cell trays filled with the same thicknesses of soil and sand. We divided half of the seedbank slurry, by weight, into each regime. We spread the slurry approximately 0.5 cm thick over three plastic cups in the flooded regime and over 7-8 cells in the 72-cell trays for the moist regime. We also used six blanks per regime, where we added no seedbank slurry to ensure that no seedlings that emerged originated from the soil, sand, or the other plants in the greenhouse bay. No seedlings emerged from these blanks throughout the experiment.

The seedlings were exposed to a 12-hour day/night cycle under high-pressure sodium lamps and ambient temperatures in the University of Waterloo greenhouse (as described in Howell 2017). We watered the seedlings every 1-2 days to maintain the water level in the flooded regime, and to maintain soil moisture in the moist regime. For the moist regime, we added sufficient water to reach maximum soil moisture content each time these samples were watered. We observed the seedlings every 1-2 days and surveyed them once per week to record new emergence. We identified seedlings to the species level, or if species-level identification was not possible (e.g., due to a lack of seeds or flowers), we made a genus-level identification. One dicot (Moist Regime Unknown Dicot 15) senesced before identification was possible.

Once we identified the seedlings to the species- or genus-level, we removed them from the cup or cell in which they grew. When no further seedlings emerged from the sample, we stirred the top layer of soil and repeated the observation and survey process to ensure all viable seeds emerged. When no further seedlings emerged for one week after mixing, we concluded the experiment. The greenhouse experiment ran for approximately seven months, beginning on September 28, 2020, and ending on April 30, 2021.

3.2.5 Statistical methods

Univariate methods

To address our first objective, we calculated 1) total seedling abundance, 2) species richness, 3) Shannon-Weiner diversity (*H'*), 4) Simpson's diversity (*1/D*), 5) Pielou's evenness

(*J*), 6) *P. australis* seed density, and 7) native seed density of each sample. To determine if there was a difference in any of these seven response variables among vegetation types (i.e., control, herbicide-treated, reference) and between watering regimes (i.e., moist soil or flooded), we performed seven two-way ANOVAs with interaction, with vegetation type and watering regime as fixed factors. Species richness, total seedling abundance, native seedling abundance, and *P. australis* seedling abundance were log-transformed prior to analysis to improve model residuals. Where an interaction was determined to be significant, we reported Type III sums of squares. Else, we reported Type II sums of squares. Two-way ANOVAs were computed using the *car* package (Fox and Weisberg 2019) using R v. 4.1.1 (R Core Team 2021).

Multivariate methods

To address our second objective and determine if there were differences in community composition among the vegetation types and watering regimes, we performed an indicator species analysis (ISA; Dufrene and Legendre 1997) on seedling emergence, with vegetation type as a fixed factor. Due to the differences in community composition between moist and flooded regimes, each watering regime was analyzed separately. Multivariate statistics were carried out using PC-ORD v. 7.08 (McCune and Mefford 2018).

***3.3** Results*

Univariate analyses

We identified a total of 23 species in the greenhouse experiment. Fifteen species emerged under the moist regime, and 12 species emerged under the flooded regime (Appendix 3.2). Of the species identified in the greenhouse experiment, 15 (65%) were observed in the field and 8 (35%) were not (see Appendix 3.2).

While no significant interaction between vegetation type and watering regime predicted total seedling abundance (Table 3-1, Fig. 3-2), total seedling abundance differed significantly between vegetation types, with a higher abundance of seedlings emerging from the treated (average = 373 seedlings per m^2 ± 54.5 std. error) and control (average = 251 seedlings per m^2 ± 31.5 std. error) conditions than the reference condition (average = 128 seedlings per m^2 ± 12.9 std. error).

Log transformed species richness was not predicted by an interaction between vegetation type and watering regime (Table 3-1, Fig. 3-3A), nor did it differ among vegetation types (Table 3-1). However, species richness did differ between watering regimes (Table 3-1), with, on average, 20% higher species richness in the moist regime (average = 2.82 species per sample ± 0.171 std. error) compared to the flooded regime (average = 2.27 species per sample ± 0.148 std. error).

Log transformed Shannon-Weiner diversity (*H')* differed significantly between watering regimes (Table 3-1; Fig. 3-3B), with 56% higher diversity in the moist regime (average = 0.646 ± 0.053 std. error) compared to the flooded regime (average = 0.414 ± 0.045 std. error).

Square root transformed Simpson's diversity (*1/D*) also differed significantly between watering regimes (Table 3-1; Fig. 3-3C), with 28% higher diversity in the moist regime (average = 1.81 ± 0.789 std. error) compared to the flooded regime (average = 1.41 ± 0.505 std. error).

While a significant interaction between watering regime and vegetation type did not predict Pielou's evenness (*J*), it differed significantly between watering regime (Table 3-1; Fig. 3-3D), with 38% greater evenness in the moist regime (average = 0.598 ± 0.040 std. error) compared to the flooded regime (average = 0.432 ± 0.049 std. error), and among vegetation types, with greater evenness in control sites (average = 0.570 ± 0.049 std. error) and reference sites (average = 0.561 ± 0.050 std. error) compared to treated sites (average = 0.414 ± 0.050 std. error).

A significant interaction between vegetation type and watering regime predicted log *P. australis* abundance (Table 3-1; Fig. 3-4), with greatest *P. australis* seedling abundance emerging from control sites in the moist watering (185 seedlings/m^2 ± 43.8 std. error), and a similar abundance of *P. australis* seedlings emerging from the control flooded condition (average = 3 seedlings/m^2 ± 1.3 std. error), the moist and flooded treated (average = 9.5 seedlings/m^2 ± 4.5 std. error, and average = 1 seedlings/m^2 ± 0.7 std. error, respectively) and moist and flooded reference sites (average = 3.5 seedlings/m^2 ± 1.1 std. error, and average = 0.5 seedlings/m^2 ± 0.5 std. error, respectively).

Log transformed native seedling abundance did not differ significantly between vegetation type (Table 3-1; Fig. 3-5), or between watering regimes (Table 3-1; Fig. 3-5) (average = 39.9 seedlings/m^2 ± 5.15 std. error) (n = 120).

Table 3-1. Two-way ANOVA test results for seedling abundance, species richness and diversity measures, and total *P. australis* and native seedling abundance. Treatment consisted of herbicide-treated, invaded, or native vegetation types and regime consists of flooded or moist regimes.

	Treatment			**Regime**			**Treatment x Regime**		
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
Log total seedling abundance	2, 114	3.470	0.034	1, 114	0.835	0.363	2, 114	0.842	0.434
Log species richness	2, 114	0.180	0.835	1, 114	5.260	0.024	2, 114	0.314	0.731
Log Shannon-Weiner diversity (*H'*)	2, 114	0.841	0.434	1, 114	10.514	0.002	2, 114	0.900	0.409
Square root Simpson's diversity (*1/D*)	2, 114	0.898	0.410	1, 114	8.223	0.005	2, 114	0.584	0.560
Pielou's evenness (*J*)	2, 114	3.436	0.036	1, 114	9.180	0.003	2, 114	2.276	0.107
Log *P. australis* abundance	2, 114	0.425	0.655	1, 114	157.865	<0.001	2, 114	41.392	<0.001
Log native seedling abundance	2, 114	0.875	0.420	1, 114	0.054	0.816	2, 114	0.738	0.481

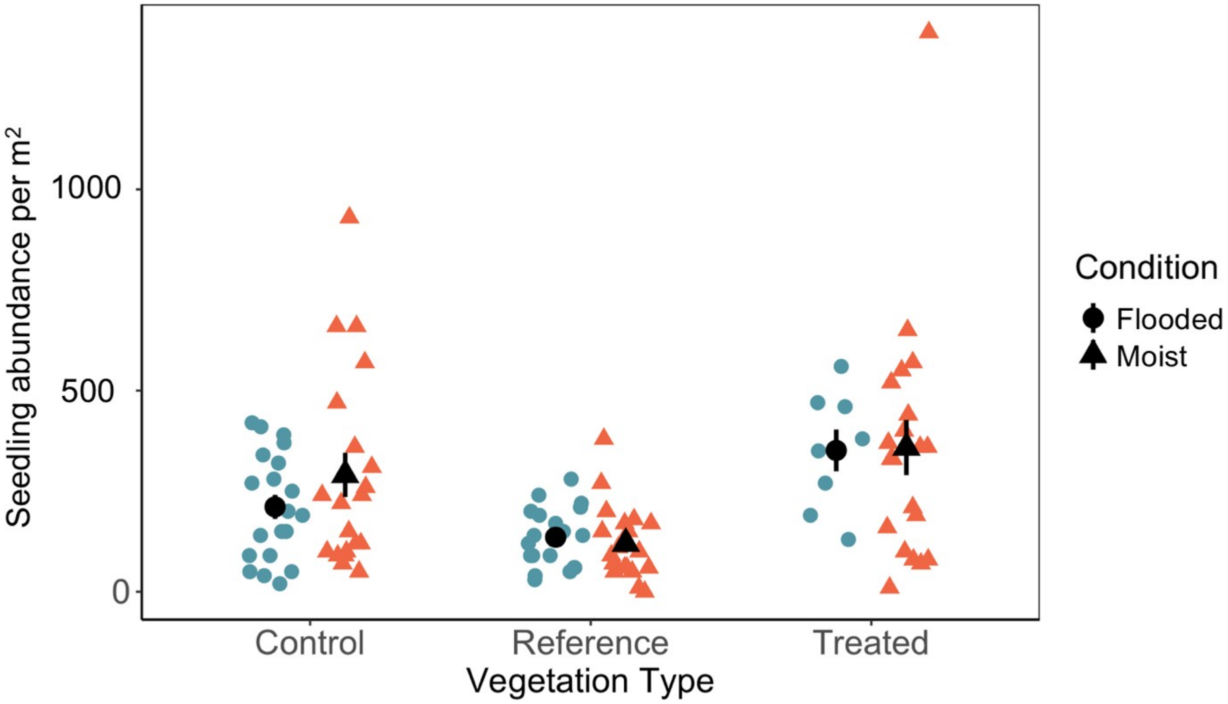


Figure 3-2. Comparison of total seedling densities (per m^2) in control, reference, and treated seedbanks from a 10 cm^2 sediment sample collected 2 cm deep, in the flooded and moist watering regimes. Blue circles represent the samples exposed to the flooded watering regime, and red triangles represent the samples exposed to the moist watering regime. Black symbols represent the mean, and error bars represent standard deviation.

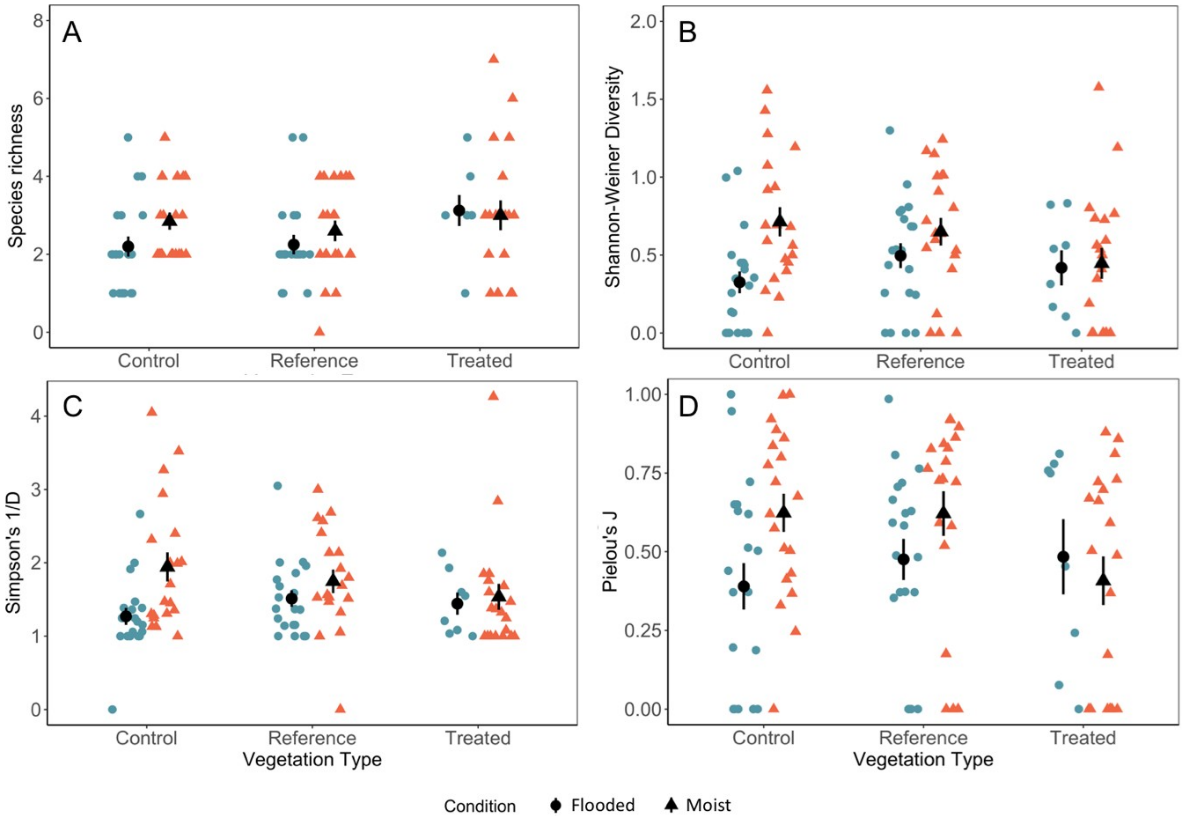


Figure 3-3. Comparison of species richness (A), Shannon-Weiner diversity (*H'*) (B), Simpson's diversity (*1/D*) (C), and Pielou's evenness (*J)* (D) for seedlings that emerged from control, reference, and treated seedbanks, in the flooded and moist watering regimes. Blue circles represent the samples exposed to the flooded watering regime, and red triangles represent the samples exposed to the moist watering regime. Black symbols represent the mean, and error bars represent standard deviation.

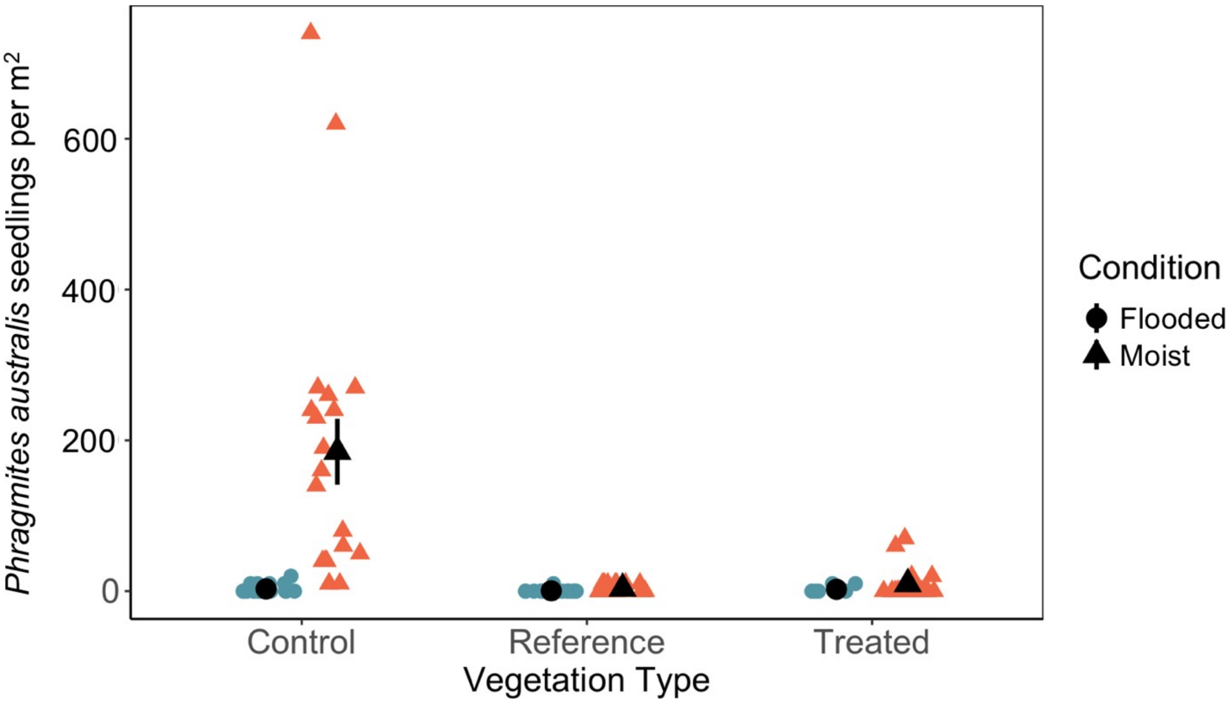


Figure 3-4. Comparison of density (per m^2) of *P. australis* seedlings that emerged from the control, reference, and treated seedbanks from a 10 cm^2 sediment sample collected 2 cm deep, under flooded and moist watering regimes. Blue circles represent the samples exposed to the flooded watering regime, and red triangles represent the samples exposed to the moist watering regime. Black symbols represent the mean, and error bars represent standard deviation.

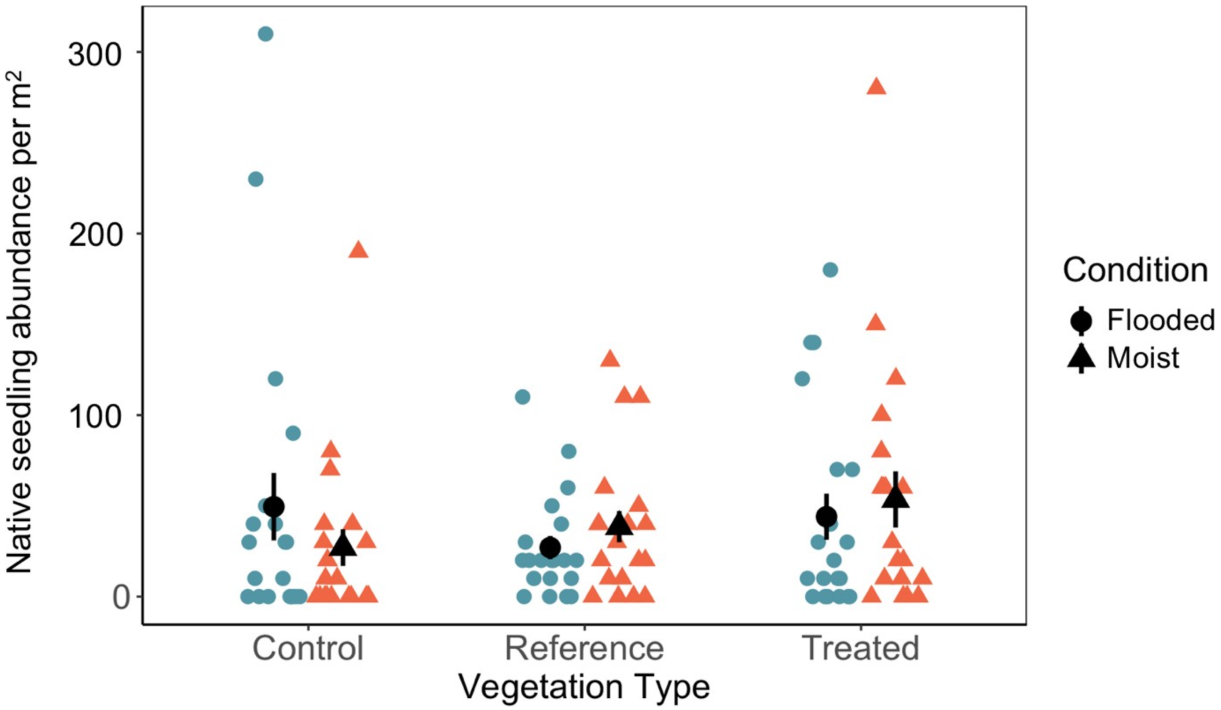


Figure 3-5. Comparison of native seedling density (per m^2) in control, reference, and treated seedbanks from a 10 cm^2 sediment sample collected 2 cm deep, in the flooded and moist watering regimes. Blue circles represent the samples exposed to the flooded watering regime, and red triangles represent the samples exposed to the moist watering regime. Black symbols represent the mean, and error bars represent standard deviation.

Multivariate analyses

At least one species was a significant indicator of each vegetation type in the moist watering regime (Table 3-2). At least one species was also a significant indicator of the control and reference sites in the flooded watering regime, however no species was a significant indicator of herbicide-treated sites in the flooded regime (Table 3-3).

Table 3-2. Results of moist watering regime indicator species analysis. Statistically significant p-values at alpha of 0.05 are indicated in bold font.

Species	Group	Indicator Value	p value
Phragmites australis	**Control**	**81.3**	**0.0002**
Juncus spp.	Control	13.1	0.2635
Carex spp. 2	Control	9.7	0.6159
Eupatorium perfoliatum	Control	2.9	0.7838
Impatiens capensis	Control	4.9	0.8340
Urtica dioica	**Herbicide-treated**	**23.4**	**0.0164**
Calamagrostis canadensis	Herbicide-treated	21.4	0.2196
Barbarea vulgaris	Herbicide-treated	8.3	0.2332
Moist Unknown #15	Herbicide-treated	5.3	0.3217
Lycopus americanus	Herbicide-treated	5.3	0.3337
Persicaria lapathifolia	Herbicide-treated	8.9	0.4395
***Carex* spp. 1**	**Reference**	**35.6**	**0.0012**
***Typha* spp.**	**Reference**	**46.5**	**0.0016**
Carex spp. 3	Reference	15.0	0.0978
Ranunculus sceleratus	Reference	15.0	0.1042
Lythrum salicaria	Reference	5.0	1.0000

Table 3-3. Results of flooded watering regime indicator species analysis. Statistically significant p-values at alpha of 0.05 are indicated in bold font.

Species	Group	Indicator Value	p value
***Juncus* spp.**	**Control**	**5.3**	**0.0386**
Phragmites australis	Control	30.7	0.1620
Najas flexilis	Control	15.6	0.1724
Utricularia spp.	Control	5.0	0.7774
Spirodela polyrhiza	**Herbicide-treated**	**15.9**	**0.0742**
Persicaria lapathifolia	Herbicide-treated	29.2	1.0000
***Typha* spp.**	**Reference**	**7.1**	**0.0010**
Pontederia cordata	**Reference**	**8.9**	**0.0164**
Ranunculus sceleratus	Reference	10.5	0.1022
Potamogeton foliosus	Reference	18.9	0.3103
Hydrocharis morsus-ranae	Reference	43.9	0.3163
Vallisneria americana	Reference	3.3	0.5991

3.4 Discussion

With thousands of hectares of established *P. australis* requiring treatment in Ontario alone (Ontario Phragmites Working Group [OPWG] 2022), the cost of active restoration via seeding or planting native species to repopulate marshes following herbicide-treatment is prohibitive. In most locations land managers will rely on "passive" or "natural" restoration whereby plant colonization of treated areas occurs unassisted by humans. In passive restoration of wetlands, plants are known to be dispersal limited (Kettenring and Tarsa 2020) and reinvasion by *P. australis* from the seedbank or propagule rain (Kettenring and Galatowitch 2011) or secondary invasion by other invasive plant species (Robichaud and Rooney 2021b) can be a major threat to restoration success. Recent research has identified that the quality of the seedbank plays a major role in determining the outcome of *P. australis* suppression efforts (Rohal et al. 2019). Certainly in Long Point, where lake levels are anticipated to decline for the next five years following natural periodicity in water levels (Fig. 1-1), we understand that the seedbank will play an increasingly important part in *P. australis* suppression outcomes. This is because of the long-recognized importance of the seedbank in renewing marsh vegetation following drawdowns (e.g., Smith and Kadlec 1983).

This experiment provides a good indication of the species pool of plants available to colonize newly exposed sediments following drawdown on Lake Erie, as well as successfully providing an indication of the efficacy of *P. australis* herbicide treatment in diminishing the density of *P. australis* seeds in the seedbank.

The vegetation community that emerged under the moist watering regime was both more species-rich and diverse than the communities that emerged from the flooded regime. This is a common outcome of emergence method experiments (e.g., van der Valk and Davis 1978), as meadow marsh communities, which grow under similar conditions to the moist watering regime, tend to have higher richness and diversity than emergent communities (Keddy and Reznicek 1986; Keddy and Campbell 2019). As such, under low Lake Erie water levels, more species-rich and diverse communities should be expected to emerge from the seedbank than the communities capable of germinating under higher water levels.

An average of 185 *P. australis* seedlings/m^2 (± 43.8 std. error) emerged from the seedbanks of control sites under the moist watering regime (n = 20) with a maximum of 740 seedlings/m^2 emerging from one sample. This high abundance of *P. australis* seedlings indicates that viable *P. australis* seeds remain in the seedbank in invaded areas. We found a similar density of viable *P. australis* seedlings to Baldwin et al. (2010), who found 698 viable *P. australis* seeds/m^2 in seedbank samples collected in highly dense *P. australis*. However, the *P. australis* seed density from our Long Point samples is nowhere near as high as the amount of viable *P. australis* seeds found by Elsey-Quick and Leck (2021), who found approximately 6000 viable *P. australis* seeds/m^2 in untreated marsh. Nevertheless, these seeds will continue to propagate *P. australis*, potentially spreading more seeds and vegetative propagules in treated and reference marsh. Elsey-Quick and Leck (2021) note that *P. australis* spread from the seedbank is likely facilitated by anthropogenic modifications that encourage low water levels, such as the creation of roads and landfills. At Long Point, the potential spread of *P. australis* will likely be facilitated not only by these activities, but by predicted low water levels (NOAA 2022; Fig. 1-1)

In contrast, the flooded regime yielded far fewer *P. australis* seedlings on average in the control condition (3 seedlings/m^2 ± 1.28 std. error) (n = 20). These results indicate that flooding as shallow as 2-4 cm deep can effectively suppress *P. australis* germination from the seedbank, although vegetative propagation from adult plants may dominate local expansion of genets, especially in flooded conditions (Belzile et al. 2009; Baldwin et al. 2010). While herbicide treatment effectively controls *P. australis* genets, killing both their above and below ground tissues (Tu et al. 2001), high water levels may effectively constrain *P. australis* dispersal via seed. Interestingly, our results from freshwater Great Lakes coastal marsh agree with those of Baldwin et al. 2010, who studied tidal marshes in the eastern USA and found that *P. australis* seedling emergence was suppressed by 3.5 cm of flooding in a greenhouse experiment. Ekstam and Forseby (1999) suggest that the mechanism preventing *P. australis* from germinating below water is its requirement for a high temperature amplitude to trigger germination, but in the greenhouse flooded and moist soil treatments experienced the same temperature regime. Wijte and Gallagher (1996) additionally suggest that *P. australis* emergence is hindered under lower oxygen levels, which were possibly present in the flooded regime, but not the moist regime, despite the very shallow water depth that our flooded regime maintained.

A much lower density of *P. australis* seedlings emerged from the herbicide-treated sites in comparison to the untreated control sites under moist soil conditions (9.5 seedlings/m^2 ± 4.5 std. error) (n = 20). Thirteen of the 20 herbicide-treated sites were areas where treatment occurred in 2016, 3 y preceding seedbank collection, but the low density of *P. australis* seedlings emerging from the herbicide-treated seedbanks was true even in the 7 Big Creek sites where herbicide-application only occurred in the year preceding seedbank collection (average = 2.9 seedlings/m^2 ± 1.8 std. error in the flooded regime; average = 1.4 seedlings/m^2 ± 1.4 std. error in the moist regime). Recent work by Rohal et al. (2021) similarly found that the number of viable *P. australis* seeds in the seedbank in the Great Salt Lake wetlands decreased rapidly following herbicide treatment. Given that glyphosate is not building up in high concentrations in the sediment in Long Point (Robichaud and Rooney 2021a), it is very unlikely that this post-emergence herbicide is affecting seeds or seed germination in the years following application. Rather, it is likely that the viability of *P. australis* seeds in the seedbank is very short-lived. This should offer land managers hope, as once the source of seeds to the seedbank is eliminated from an area, the stock of viable *P. australis* seed in the seedbank quickly depletes. Potential emergence from the seedbank could be mitigated by maintaining shallow standing water if water level control structures are in place. Even if drawdown occurs within the year following herbicide-based *P. australis* suppression, limited recolonization from the seedbank should be anticipated if treatment occurred before the rametes were able to set seed the preceding fall. These results highlight the urgency of *P. australis* suppression efforts on Lake Erie. With water levels projected to decline, extensive recolonization of *P. australis* should be anticipated in areas with a high density of *P. australis* seeds in the seedbank (i.e., where live *P. australis* stands remain). As we have observed a decrease in *P. australis* seed viability following herbicide treatment and flooding, there is a strict deadline to treat remaining *P. australis* and reduce the abundance of viable seeds at Long Point before dropping water levels facilitate its spread.

Importantly, even if *P. australis* seed viability is effectively reduced via herbicide treatment and flooding, viable seeds of other invasive species were also present in herbicide-treated areas. We detected a high abundance of invasive cattail (*Typha* spp. (L.)) seedlings emerging from all three vegetation types, as well as purple loosestrife (*Lythrum salicaria* (L.)) and wintercress (*Barbarea vulgaris* (W.T. Aiton)), which primarily emerged under the moist watering regime. While *Typha* spp. was commonly observed in the timed survey walkabouts, *L. salicaria* and *B. vulgaris* were not. Interestingly, Frieswyk and Zedler (2006) also detected high densities of *L. salicaria* in their study of the seedbanks in Green Bay coastal wetlands invaded by hybrid cattail (*Typha* × *glauca*), though it was only a low relative densities in the extant vegetation. From this, Frieswyk and Zedler (2006) concluded that wetland resilience was threatened. Despite the low relative abundance of these other invasive plants in the marsh, and the diversity of native species present in the seedbank, secondary invasions have been a common concern following herbicide treatment (e.g., Bonello and Judd 2019; Robichaud and Rooney 2021b; Chapter 2). However, high water levels in the recent past (NOAA 2022) likely helped limit the extent of *B. vulgaris* and *L. salicaria* in our study marshes, as these species prefer meadow marsh habitats to emergent marsh. With lower Lake Erie water levels predicted until 2025 (NOAA 2022), it is possible that these species may germinate from the seedbank in higher abundances in the future, contributing to secondary invasions in the treated areas (Chapter 2). Further monitoring of the area following low water levels would be ideal to ensure that these species do not cause a large-scale secondary invasion.

Despite a high abundance of *P. australis* seeds and other non-native species emerging from the seedbank, the seedbank of all three vegetation types also contained viable native seeds. This has been a frequent observation in *P. australis*-invaded marsh (e.g., Rohal et al. 2021; Hazelton et al. 2018; Baldwin et al. 2010). However, the density of native seedlings that emerged from treated sites (average = 48.8 seedlings/m^2 ± 9.90 std. error) is far less than the density suggested to be required by Robinson (2022) (approximately 6000-10000 seeds/m^2) in order to encourage the growth of native vegetation communities following *P. australis* invasion. Similarly, Rohal et al. (2021) determined that heavily-invaded sites were seedbank-limited following herbicide treatment, resulting in few native seedlings emerging despite the absence of *P. australis*. While the seedbank at Long Point contains native seeds, more “active” restoration techniques following herbicide application, such as seeding areas with native seed mixes, may be necessary to ensure revegetation by native species. Further monitoring of the vegetation communities that emerge under low water levels will indicate if active treatment is required.

While this greenhouse experiment provides a good indication of the types of viable seeds contained in the seedbank (Poiani and Johnson 1988; Brown 1998), the experiment lacks many components that would normally be observed *in-situ*. Namely, herbivory, competition, priority effects, realistic temperatures, varying water levels, and harsh environmental conditions were omitted from this experiment. Additional research that takes these factors into account is needed to determine if these results which plant species are likely to contribute to extant vegetation communities following herbicide-based suppression of *P. australis*.

3.5 Conclusions

The wetland seedbank is an essential tool to aid in determining the vegetation communities that may return following herbicide treatment of *P. australis*. Our results indicate that the vegetation community that emerges from the seedbank at Long Point is both rich and diverse. However, the high abundance of viable *P. australis* seeds and still present in control sites indicates that low water levels may facilitate its spread in areas where marsh remains untreated. However, following herbicide treatment subsequent exposure to flooding, the number of viable *P. australis* seeds present in the seed bank in treated areas decreases significantly. The seedbank of *P. australis*-dense marsh also remains diverse with native seeds. Despite these two findings, we caution that the seedbank contains many viable propagules from non-native species and that additional factors, including priority effects, competition among seedlings, herbivory, as well as fluctuations in environmental conditions like water levels and temperature will influence what components of the seedbank are able to establish *in situ*.

4.0 Conclusions and management implications

4.1 Thesis overview

The Great Lakes coastal wetlands have experienced a large-scale invasion by *Phragmites australis* ssp. *australis* ((Cav.) Trin. ex Steud. (hereafter *P. australis*) since the mid-1990s, which was facilitated by a period of low water levels (Wilcox 2012; Jung et al. 2017). *Phragmites australis* invasions negatively affect native species and species at risk, primarily vascular plants (Bickerton 2015). Without effective measures of treatment, invasion can lead to a decrease in plant diversity and richness (Robichaud and Rooney 2021b), which is of great concern in the Great Lakes coastal wetlands. To protect native species and treat *P. australis* at Long Point Peninsula and Rondeau Provincial Park, both sandspit wetland complexes located on the northern shore of Lake Erie, over 1500 ha of invaded marsh has been treated with a glyphosate-based herbicide since 2016. A re-invasion by *P. australis* and a secondary invasion by *Hydrocharis morsus-ranae* (L.) have been observed at Long Point and Rondeau in the two years post-treatment (Robichaud and Rooney 2021b; Chpt 2). Both re-invasion and secondary invasions are common following *P. australis* herbicide treatment (e.g., Lombard et al. 2012, Quirion et al. 2018, Bonello and Judd 2019), which has caused some researchers to assert that herbicide treatment of *P. australis* is futile (e.g., Lombard et al. 2012, Quirion et al. 2018). However, with Lake Erie water levels naturally oscillating, the vegetation communities returning to Long Point and Rondeau have begun to change. Continued monitoring in Long Point and Rondeau following herbicide treatment is important to assess the long-term trajectory of vegetation communities that return to treated marsh, and to gain an in-depth knowledge of the influence that the seedbank may have on these communities once water levels drop.

The goals of my thesis were 1) to examine the longer-term trajectory (i.e., 5 years post-treatment) of vegetation communities following herbicide treatment of *P. australis*, and 2) to explore the contents of the seedbank to determine the effects of treatment on viable *P. australis* seeds, and to gain insight into the vegetation that establishes following *P. australis* suppression amid dropping water levels.

4.2 Thesis summary

In my first chapter, I outlined the effects that hydrological processes have on wetland plant communities, and how they both facilitate and hinder emergence from the seedbank. I also described the biology of *P. australis*, as well as the many negative impacts it has on native animal and plant communities. In this section I also described the methods through which managers attempt to treat dense *P. australis* stands and the shortcomings of these methods, emphasizing the need for longer-term studies in *P. australis* management. Finally, I outlined the *P. australis* invasion in Lake Erie coastal wetlands, how it has been treated to date, and how its impacts will be exacerbated by falling Lake Erie water levels.

In my second chapter, I examined the long-term trajectory of the vegetation communities that returned to herbicide-treated marsh. I conducted yearly surveys of the vegetation communities in 120 permanent plots at Long Point and Rondeau, in both invaded and treated marsh that were originally established in 2016 and 2017. I determined that herbicide treatment, paired with high water levels following treatment, effectively eradicated *P. australis* in the majority of the plots, leading to higher species richness and diversity. Additionally, while a secondary invasion by *H. morsus-ranae* was observed in the first two years post-treatment, it was short-lived, and a diverse native vegetation community repopulated the area three to five years post-treatment. However, another secondary invasion by *Myriophyllum spicatum* (L.) has begun to occur in some of the treated plots, which will require future monitoring to ensure it does not negatively impact native plant species.

In my third chapter, I examined seedbank samples from invaded, treated, and reference marsh, under two watering regimes. I tested the effects of herbicide treatment on the wetland seedbank to predict the vegetation communities that may return to the marsh following falling water levels. I determined that while a high abundance of viable *P. australis* seeds are present in invaded marsh seedbanks, herbicide treatment followed by flooding is effective in reducing the number of viable *P. australis* seeds that are present in the seedbank. I also determined that viable native seeds remain despite decades of *P. australis* dominance, but seeds of several other non-native species are also present in the seedbank. I concluded that active restoration may be needed to ensure native vegetation recovery.

4.3 Research implications

These results are important for policies regarding the removal of *P. australis* in wetland systems, as future treatment can be tailored to consider these outcomes and maximize treatment efficacy. More specifically, these results are vital for *P. australis* management in the Great Lakes coastal wetlands. It is clear from our results that herbicide treatment combined with the recent period of high water levels encourages native vegetation to recover and prevents recolonization of treated regions from *P. australis* in the seedbank. With decreasing Lake Erie water levels, there is limited time to remove remaining *P. australis* stands in Long Point and Rondeau before drawdown triggers mass germination from the seedbank. Given the apparently short-lived nature of *P. australis* seeds, there is hope that suppression efforts taking place even as the water levels decline will help limit the recolonization of treated areas. It is vital to determine if the native seeds in the seedbank will be able to outcompete the remaining *P. australis* and other invasive species (i.e., *Lythrum salicaria* (L.) and *Barbarea vulgaris* (W.T. Aiton)) present in the seedbank in high densities to re-establish diverse native vegetation.

Our efficacy monitoring (Chapter 2) additionally underscored the need for long-term studies in *P. australis* research. Many wetland processes such as community succession are complex and may take decades to occur (Weller and Spatcher 1965). Changes that take five or more years to occur may therefore be missed in the more common short-term studies (Hazelton et al. 2014). This highlights the importance of long-term studies, which are needed to truly understand the mechanisms behind these processes.

Despite the success in eradicating dense *P. australis* at Long Point and Rondeau and controlling its invasion from the seedbank level, it is also important to note that herbicide treatment does have drawbacks and should not be used as a one-size fits-all solution (Robichaud and Rooney 2021b). Both species richness and diversity indices were suppressed in the years following treatment, facilitating the secondary invasion by *H. morsus-ranae*, which may not have occurred with other forms of treatment. Other forms treatment, or a combination of multiple forms of treatment should continue to be explored to determine the ideal method for controlling *P. australis*, and to ensure that the negative effects of treatment to plant and animal species do not outweigh the benefits.

4.4 Future work

Additional herbicide efficacy monitoring will be useful in determining the future of Long Point and Rondeau vegetation communities, and exploring potential future invasions, such as the invasion by *Myriophyllum spicatum* (L.) currently occurring at Long Point or the potential for *L. salicaria* and *B. vulgaris* to take over from the seedbank. Furthermore, with water levels dropping, the vegetation communities will change, with a higher abundance of seedlings emerging from the seedbank. The balance of vegetative and seed propagules will likely shift and the outcome of competition among propagules for newly available niche space is uncertain. Continued monitoring of the long-term efficacy plots will help determine the adequacy of the seedbank in revegetation and may identify additional factors determining whether vegetation communities remain primarily native-dominated or transition to invasive-dominated.

One form of "active" restoration that may be useful is seeding areas with native seed mixes after *P. australis* suppression efforts. Enhancing the seedbank with additional native seeds may aid native plant communities in outcompeting invasive species, and prevent secondary invasions that are common in *P. australis* treatment studies (e.g., Bonello and Judd 2019). One possibility that is yet to be explored is seeding the area with wild rice (*Zizania palustris* (L.)), also known as *manoomin*, a native plant that is of high cultural significance to Indigenous peoples in North America. Both *Z. palustris* and *P. australis* grow under similar environmental conditions (Biesboer 2019), and *Z. palustris* may therefore offer competition for *P. australis* in treated areas.

Another area of future work pertains to the effects of low water levels on the wetland seedbank. While the greenhouse study examined seedbank emergence under controlled conditions, this experiment does not take realistic *in-situ* environmental conditions into account, nor does it consider priority effects and competition. One experiment that can be used to compensate for these deficiencies is a marsh organ experiment (Morris 2007). This experimental design is named after a pipe organ, as it is made of multiple levels of pipes that emerge below or above the surface of the water. These pipes are filled with sediment, and seedbank samples are placed in the tops of the pipes. The seedbank samples are therefore exposed to lower water levels *in-situ* throughout the growing season, as water only comes up partway through the pipes. A marsh organ experiment was performed with treated, invaded, and reference marsh seedbank samples at Long Point in 2021, but the results are currently unpublished. The results of this experiment, paired with the two experiments detailed in this thesis, will give a good indication of the current and future fate of vegetation communities at Long Point and Rondeau.

Holistically, these studies provide important information that is vital to *P. australis* management moving forward. Both studies fill knowledge gaps that are present in *P. australis* research, including the long-term trajectories of vegetation communities post-treatment, and the effects of herbicide treatment on the seedbank. Additionally, these studies provide additional information regarding the fate of vegetation communities at Long Point and Rondeau. While further monitoring is required to ensure that the vegetation communities of the Lake Erie coastal wetlands remain native-dominated, these results are an important puzzle piece to ensure the success of this treatment project, and *P. australis* treatment projects to come.

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Appendices

Appendix 1.1 Locations of Big Creek NWA, Crown Marsh, and Long Point Provincial Park at Long Point, Ontario.

Source: Google Earth Pro

Appendix 2.1 Site information for permanent monitoring plots established in 2016 and 2017 at Long Point Peninsula and Rondeau Provincial Park.

Site ID	Location	Treatment	Latitude	Longitude
lpc21	Long Point	Control	42.58252448	-80.38841651
lpc22	Long Point	Control	42.58283482	-80.38805844
lpc23	Long Point	Control	42.58246777	-80.3877133
lpc24	Long Point	Control	42.58207445	-80.38804652
lpc25	Long Point	Control	42.58241068	-80.37924376
lpc26	Long Point	Control	42.58324573	-80.38972156
lpc27	Long Point	Control	42.58322705	-80.38915902
lpc28	Long Point	Control	42.58365998	-80.3888969
lpc28	Long Point	Control	42.58367924	-80.38959773
lpc30	Long Point	Control	42.58337764	-80.38675959
lpc31	Long Point	Control	42.58289322	-80.38660068
lpc32	Long Point	Control	42.58282239	-80.38597712
lpc40	Long Point	Control	42.58377394	-80.38252445
lpc39	Long Point	Control	42.58334877	-80.38222818
lpc38	Long Point	Control	42.58374687	-80.38183242
lpc37	Long Point	Control	42.58330873	-80.38451784
lpc36	Long Point	Control	42.58264127	-80.38505933
lpc35	Long Point	Control	42.58285962	-80.38452494
lpc34	Long Point	Control	42.58311924	-80.38395891
lpc33	Long Point	Control	42.58327746	-80.38545521
lpt20	Long Point	Treatment	42.59067132	-80.41275937
lpt19	Long Point	Treatment	42.59037908	-80.41206119
lpt18	Long Point	Treatment	42.59019886	-80.412846
lpt05	Long Point	Treatment	42.58921065	-80.41966182
lpt06	Long Point	Treatment	42.58922261	-80.42038795
lpt07	Long Point	Treatment	42.58974238	-80.42063146
lpt08	Long Point	Treatment	42.58992786	-80.41980283
lpt09	Long Point	Treatment	42.58985083	-80.42326082
lpt10	Long Point	Treatment	42.59192265	-80.41719617
lpt11	Long Point	Treatment	42.59141286	-80.41726777
lpt12	Long Point	Treatment	42.59155959	-80.41651334
lpt13	Long Point	Treatment	42.59241118	-80.41669268
lpt14	Long Point	Treatment	42.59303337	-80.41785595
lpt15	Long Point	Treatment	42.59320973	-80.41710689
lpt16	Long Point	Treatment	42.59363582	-80.41696548
lpt17	Long Point	Treatment	42.59391175	-80.41780617
lpt41	Long Point	Treatment	42.59241689	-80.41365981
lpt42	Long Point	Treatment	42.5933967	-80.41264734
lpt43	Long Point	Treatment	42.59313527	-80.41350413
lpt44	Long Point	Treatment	42.59408159	-80.41333169

Site ID	Location	Treatment	Latitude	Longitude
rpc2	Rondeau	Control	42.26117888	-81.87627526
rpc3	Rondeau	Control	42.26163494	-81.87593758
rpc4	Rondeau	Control	42.26147158	-81.87565245
rpc5	Rondeau	Control	42.26178163	-81.87570987
rpc6	Rondeau	Control	42.26137522	-81.87617662
rpc7	Rondeau	Control	42.28735681	-81.86401693
rpc8	Rondeau	Control	42.28758795	-81.8638892
rpc9	Rondeau	Control	42.28794121	-81.86353419
rpc10	Rondeau	Control	42.28823814	-81.86329954
rpc11	Rondeau	Control	42.28848272	-81.86336171
rpc12	Rondeau	Control	42.28888886	-81.86314904
rpc13	Rondeau	Control	42.28911383	-81.86298009
rpc14	Rondeau	Control	42.28974278	-81.86291845
rpc15	Rondeau	Control	42.28953281	-81.86272512
rpc16	Rondeau	Control	42.28958022	-81.86239103
rpc17	Rondeau	Control	42.27580966	-81.87917225
rpc18	Rondeau	Control	42.27575962	-81.87945741
rpc20	Rondeau	Control	42.27536621	-81.87950537
rpc19	Rondeau	Control	42.27554855	-81.87929779
rpc41	Rondeau	Control	42.27452248	-81.88152405
rpt21	Rondeau	Treatment	42.2585652	-81.87899404
rpt22	Rondeau	Treatment	42.25901376	-81.87889669
rpt23	Rondeau	Treatment	42.25963058	-81.87867672
rpt24	Rondeau	Treatment	42.26179077	-81.87777494
rpt25	Rondeau	Treatment	42.26454802	-81.87523916
rpt26	Rondeau	Treatment	42.26515658	-81.87480833
rpt27	Rondeau	Treatment	42.26413497	-81.87547435
rpt28	Rondeau	Treatment	42.26350276	-81.87544809
rpt29	Rondeau	Treatment	42.27534553	-81.87325857
rpt30	Rondeau	Treatment	42.27485296	-81.87349819
rpt31	Rondeau	Treatment	42.27445085	-81.87393444
rpt32	Rondeau	Treatment	42.27395333	-81.87383164
rpt33	Rondeau	Treatment	42.2713003	-81.8702627
rpt34	Rondeau	Treatment	42.27121537	-81.8708876
rpt35	Rondeau	Treatment	42.27202312	-81.87006294
rpt36	Rondeau	Treatment	42.2726443	-81.8699608
rpt37	Rondeau	Treatment	42.27606641	-81.86657321
rpt38	Rondeau	Treatment	42.27757184	-81.86762264
rpt39	Rondeau	Treatment	42.27769802	-81.86668559
rpt40	Rondeau	Treatment	42.27847327	-81.86715289
LPGR1	Long Point	Ground	42.57829111	-80.37293827
LPGR2	Long Point	Ground	42.57881962	-80.37242118
LPGR3	Long Point	Ground	42.57954797	-80.3722189
LPGR4	Long Point	Ground	42.57860122	-80.37365412
LPGR5	Long Point	Ground	42.58292184	-80.42119924

Site ID	Location	Treatment	Latitude	Longitude
LPGR6	Long Point	Ground	42.58338698	-80.42057342
LPGR7	Long Point	Ground	42.58346451	-80.41987806
LPGR8	Long Point	Ground	42.58294886	-80.41941976
LPGR9	Long Point	Ground	42.59092444	-80.40124643
LPGR10	Long Point	Ground	42.59048236	-80.40109222
LPGR11	Long Point	Ground	42.58884203	-80.40257045
LPGR12	Long Point	Ground	42.58840192	-80.40279403
LPGR13	Long Point	Ground	42.58786902	-80.40249443
LPGR14	Long Point	Ground	42.58760425	-80.40180227
LPGR15	Long Point	Ground	42.58785302	-80.40115396
LPGR16	Long Point	Ground	42.58931101	-80.40270003
LPGR17	Long Point	Ground	42.58477096	-80.39220172
LPGR18	Long Point	Ground	42.5839234	-80.39370892
LPGR19	Long Point	Ground	42.58302223	-80.39359579
LPGR20	Long Point	Ground	42.58337372	-80.39194719
LPAE1	Long Point	Aerial	42.59210616	-80.42268655
LPAE2	Long Point	Aerial	42.59212949	-80.42195504
LPAE3	Long Point	Aerial	42.5945807	-80.40642824
LPAE4	Long Point	Aerial	42.59479998	-80.4070356
LPAE5	Long Point	Aerial	42.59276896	-80.40785929
LPAE6	Long Point	Aerial	42.59249244	-80.40837382
LPAE7	Long Point	Aerial	42.59200193	-80.40930477
LPAE8	Long Point	Aerial	42.58914997	-80.40637008
LPAE9	Long Point	Aerial	42.59048745	-80.4055286
LPAE10	Long Point	Aerial	42.5918577	-80.40579591
LPAE11	Long Point	Aerial	42.59056973	-80.40057949
LPAE12	Long Point	Aerial	42.59044022	-80.39992258
LPAE13	Long Point	Aerial	42.59090676	-80.39958902
LPAE14	Long Point	Aerial	42.59045491	-80.39928866
LPAE15	Long Point	Aerial	42.58997207	-80.39995144
LPAE16	Long Point	Aerial	42.58983374	-80.39933119
LPAE17	Long Point	Aerial	42.5894209	-80.39960329
LPAE18	Long Point	Aerial	37.72474974	-80.44709104
LPAE19	Long Point	Aerial	42.58519521	-80.40961278
LPAE20	Long Point	Aerial	42.58529828	-80.41039177

Appendix 2.2 List of species identified in the permanent experimental plots at Long Point and Rondeau between the years 2016 and 2021. Taxonomic authorities were obtained via the International Taxonomic Information System (ITIS 2022), native/non-native designations were obtained from the USDA Plants Database (USDA 2022), and coefficients of conservatism were obtained from the University of Michigan Herbarium Database (University of Michigan 2022).

Species	**Taxonomic authority**	**Common Name**	**Native/Non-Native/Possibly non-native**	**Coefficient of conservatism**
Bryophyte spp.		Moss		N/A
Achillea millefolium	L.	Common yarrow	Possibly non-native	1
Boehmeria cylindrica	(L.) Sw.	False nettle	Native	5
Bolboschoenus fluviatilis	(Torr.) Soják	River bulrush	Native	6
Brasenia schreberi	J.F. Gmel.	Water shield	Native	6
Calamagrostis canadensis	(Michx.) P. Beauv.	Bluejoint	Native	3
Calystegia sepium	(L.) R. Br.	Hedge false bindweed	Native	2
Calystegia spp.	R. Br.	Bindweed	Possibly non-native	7
Campanula aparinoides	Pursh	Marsh bellflower	Native	2
Carex aquatilis	Wahlenb.	Water sedge	Native	7
Carex buxbaumii	Wahlenb.	Buxbaum's sedge	Native	10
Carex crawfordii	Fernald	Crawford's sedge	Native	4
Carex comosa	Boott	Longhair sedge	Native	5
Carex cryptolepis	Mack.	Northeastern sedge	Native	8
Carex lacustris	Willd.	Hairy Sedge	Native	6
Carex pellita	Muhl. Ex Willd.	Wooly sedge	Native	2
Carex lasiocarpa	Ehrh.	Woolyfruit sedge	Native	8
Carex sartwelli	Dewey	Sartwell's sedge	Native	5
Carex spp.	L.	Sedge	Possibly non-native	7
Ceratophyllum demersum	L.	Coon's-tail	Native	1
Chara spp.	L.	Stonewort	Native	N/A

Species	**Taxonomic authority**	**Common Name**	**Native/Non-Native/Possibly non-native**	**Coefficient of conservatism**
Cladium mariscoides	(Muhl.) Torr.	Smooth sawgrass	Native	10
Cornus spp.	L.	Dogwood	Native	2
Decodon verticillatus	(L.) Elliott	Swamp loosestrife	Native	7
Dulichium arundinaceum	(L.) Britton	Three-way sedge	Native	8
Eleocharis palustris	(L.) Roem. & Schult.	Common spikerush	Native	5
Elodea canadensis	Michx.	Canadian waterweed	Native	1
Eleocharis spp.	R. Br.	Spikerush	Native	7
Equisetum fluviatile	L.	Water horsetail	Native	7
Eupatorium spp.	L.	Thoroughwort	Possibly non-native	4
Fern spp.		Fern		N/A
Fontinalis spp.	Hedw.	Aquatic Moss	Native	N/A
Fragaria virginiana	Duchesne	Virginia strawberry	Native	2
Galium labradoricum	(Wiegand) Wiegand	Northern bog bedstraw	Native	8
Galium trifidum	L.	Three-petal bedstraw	Native	6
Hydrocharis morsus-ranae	L.	European frogbit	Non-native	0
Hypericum kalmianum	L.	Kalm's St. Johnswort	Native	10
Impatiens capensis	Meerb.	Spotted touch-me-not	Native	2
Iris versicolor	L.	Harlequin blueflag	Native	5
Juncus balticus ssp. *littoralis*	(Engelm.) Snogerup	Mountain rush	Native	3
Juncus brevicaudatus	(Engelm.) Fernald	Narrowpanicle rush	Native	8
Juncus spp.	L.	Rush	Possibly non-native	5
Leersia oryzoides	(L.) Sw.	Rice cutgrass	Native	3
Lemna minor	L.	Common duckweed	Native	5
Lycopus americanus	Muhl. Ex W.P.C. Bartram	Water horehound	Native	2
Lysimachia thyrsiflora	L.	Tufted loosestrife	Native	6
Lythrum spp.	L.	Loosestrife	Possibly non-native	0
Lythrum salicaria	L.	Purple loosestrife	Non-native	0

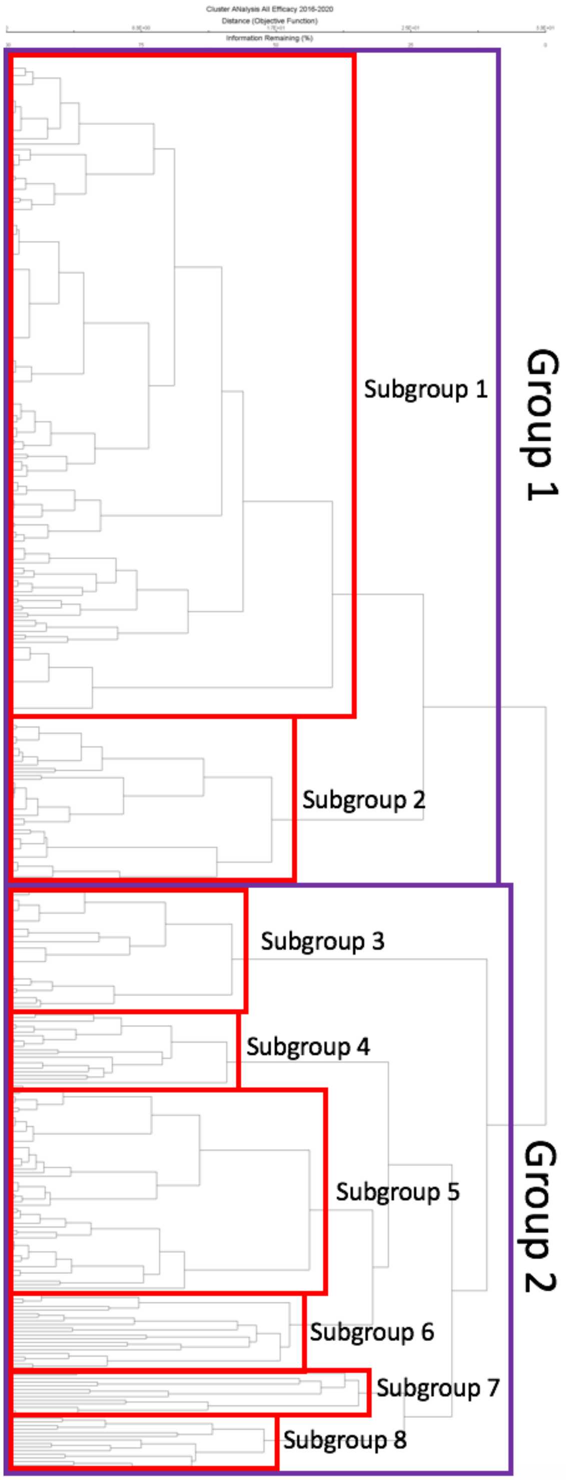
Species	**Taxonomic authority**	**Common Name**	**Native/Non-Native/Possibly non-native**	**Coefficient of conservatism**
Myriophyllum spp.	L.	Milfoil	Possibly non-native	5
Myriophyllum sibiricum	Kom.	Northern watermilfoil	Native	10
Myriophyllum spicatum	L.	Eurasian watermilfoil	Non-native	0
Najas flexilis	(Willd.) Rostk. & W.L.E. Schmidt	Water nymph	Native	5
Nitella spp.		Algae	Native	N/A
Nuphar lutea	(L.) Sm.	Yellow pond lily	Native	7
Nymphaea odorata	Aiton	American white water-lily	Native	6
Phragmites australis	(Trin.) ex	Common reed	Non-native	0
Persicaria amphibia	(L.) Delbare	Water smartweed	Native	6
Polygonum spp.	(L.) Mill	Knotweed	Native	6
Pontederia cordata	L.	Pickerelweed	Native	8
Potamogeton foliosus	Raf.	Leafy pondweed	Native	4
Potamogeton gramineus	L.	Variableleaf pondweed	Native	5
Potamogeton natans	L.	Floating pondweed	Native	5
Potamogeton praelongus	Wulfen	Whitestem pondweed	Native	8
Potamogeton richardsonii	(A. Benn.) Rydb.	Richardson's pondweed	Native	5
Potamogeton zosteriformis	Fernald	Flat-stem pondweed	Native	5
Potamogeton spp.	L.	pondweed	Possibly non-native	5
Rosa spp.	L.	Rose	Possibly non-native	5
Sagittaria spp.	L.	Arrowhead	Native	5
Sagittaria cuneata	E. Sheld.	Arumleaf arrowhead	Native	6
Sagittaria latifolia	Willd.	Broadleaf arrowhead	Native	4
Schoenoplectus pungens	(Vahl) Palla	Three-square bulrush	Native	5
Schoenoplectus acutus	(Muhl. ex Bigelow) Á. Löve & D. Löve	Hard-stem bulrush	Native	4

Species	**Taxonomic authority**	**Common Name**	**Native/Non-Native/Possibly non-native**	**Coefficient of conservatism**
Schoenoplectus tabernaemontani	(C.C. Gmel.) Palla	Soft-stem bulrush	Native	5
Solanum spp.	L.	Nightshade	Possibly non-native	0
Sparganium spp.	L.	Bur-reed	Native	6
Sparganium emersum	Rehmann	European bur-reed	Native	6
Sparganium eurycarpum	Engelm.	Broadfruit bur-reed	Native	5
Spirodela polyrhiza	(L.) Schleid.	Greater duckweed	Native	6
Stuckenia pectinata	(L.) Börner	Sago pondweed	Native	3
Thelypteris palustris	Schott	Eastern marsh fern	Native	2
Typha spp.	L.	Cattail	Possibly non-native	0
Utricularia gibba	L.	Humped bladderwort	Native	8
Utricularia intermedia	Hayne	Flat-leaved bladderwort	Native	10
Utricularia minor	L.	Lesser bladderwort	Native	10
Utricularia vulgaris	L.	Common bladderwort	Native	6
Vallisneria americana	Michx.	Eelgrass	Native	7
Vicia spp.	L.	Vetch	Possibly non-native	0
Zizania palustris	L.	Northern wild rice	Native	8

Appendix 2.3 List of species with fewer than 4 occurrences removed from the hierarchal cluster analysis and indicator species analysis.

Species	**Common Name**
Achillea millefolium	Common yarrow
Bolboschoenus fluviatilis	River bulrush
Calystegia sepium	Hedge false bindweed
Carex buxbaumii	Buxbaum's sedge
Carex crawfordii	Crawford's sedge
Carex cryptolepis	Northeastern sedge
Carex lacustris	Hairy Sedge
Carex pellita	Wooly sedge
Carex sartwelli	Sartwell's sedge
Carex spp.	Sedge
Ceratophyllum demersum	Coontail
Cladium mariscoides	Smooth sawgrass
Cornus spp.	Dogwood
Eleocharis spp.	Spikerush
Fern spp.	Fern
Fontinalis spp.	Aquatic Moss
Fragaria virginiana	Virginia strawberry
Galium labradoricum	Northern bog bedstraw
Galium trifidum	Three-petal bedstraw
Hydrilla verticillata	Waterthyme
Hypericum kalmianum	Kalm's St. Johnswort
Iris versicolor	Harlequin blueflag
Juncus articus ssp. *littoralis*	Mountain rush
Juncus brevicaudatus	Narrowpanicle rush
Juncus spp.	Rush
Leersia oryzoides	Rice cutgrass
Lythrum spp.	Loosestrife
Lythrum salicaria	Purple loosestrife
Potamogeton gramineus	Variableleaf pondweed
Potamogeton natans	Floating pondweed
Rosa spp.	Rose
Sagittaria cuneata	Arumleaf arrowhead
Schoenoplectus pungens	Three-square bulrush
Schoenoplectus tabernaemontani	Soft-stem bulrush
Solanum spp.	Nightshade
Sparganium emersum	European bur-reed
Stuckenia pectinata	Sago pondweed
Thelypteris palustris	Eastern marsh fern
Utricularia minor	Lesser bladderwort
Vallisneria americana	Eelgrass

Appendix 2.4 Full dendrogram for all 2016 aerially-treated and control permanent monitoring plots at Long Point and Rondeau (n = 80), both pre-treatment (2016), and post-treatment (2017-2020). Control site data was not collected in 2021.



Appendix 3.1 List of species identified in the timed surveys carried out at the field sites from where seedbank samples were collected. Taxonomic authorities were obtained via the International Taxonomic Information System (ITIS 2022), and native/non-native designations were obtained from the USDA Plants Database (USDA 2022).

Species Name	**Taxonomic authority**	**Common Name**	**Native/Non-native/Possibly non-native**
Boehmeria cylindrica	(L.) Sw.	Smallspike false nettle	Native
Calamagrostis canadensis	(Michx.) P. Beauv.	Canada bluejoint	Native
Carex aquatilis	Wahlenb.	Water sedge	Native
Carex spp.	L.	Sedge	Possibly non-native
Ceratophyllum demersum	L.	Coon's tail	Native
Cornus stolonifera	Michx.	Redosier dogwood	Native
Decodon verticillatus	(L.) Elliott	Swamp loosestrife	Native
Dulichium arundinaceum	(L.) Britton	Three-way sedge	Native
Elodea canadensis	Michx.	Canadian waterweed	Native
Eleocharis quadrangulata	(Michx.) Roem. & Schult.	Squarestem spikerush	Native
Equisetum fluviatile	L.	Water horsetail	Native
Equisetum spp.	L.	Horsetail	Native
Galium trifidum	L.	Threepetal bedstraw	Native
Heteranthera dubia	(Jacq.) MacMill.	Grassleaf mudplantain	Native
Hydrocharis morsus-ranae	L.	European frogbit	Non-native
Juncus spp.	L.	Rush	Native
Lemna minor	L.	Common duckweed	Native
Lycopus americanus	Muhl. Ex W.P.C. Bartram	American water horehound	Native
Lythrum salicaria	L.	Purple loosestrife	Non-native
Mimulus ringens	L.	Allegheny monkeyflower	Native
Myriophyllum spicatum	L.	Eurasian watermilfoil	Non-native
Myriophyllum spp.	L.	Watermilfoil	Possibly non-native
Najas flexilis	(Willd.) Rostk. & W.L.E. Schmidt	Nodding Waternymph	Native

Species Name	**Taxonomic authority**	**Common Name**	**Native/Non-native/Possibly non-native**
Nuphar lutea	(L.) Sm.	Yellow pond-lily	Native
Nymphaea odorata	Aiton	American white waterlily	Native
Phragmites australis ssp. *australis*	(Cav.) Trin. ex Strued.	Common reed	Non-native
Polygonum amphibia	(L.) Delbare	Water smartweed	Native
Pontederia cordata	L.	Pickerelweed	Native
Populus deltoides	W. Bartram ex Marshall	Eastern cottonwood	Native
Potamogeton crispus	L.	Curly pondweed	Non-native
Potamogeton epihydrus	Raf.	Ribbonleaf pondweed	Native
Potamogeton foliosus	Raf.	Leafy pondweed	Native
Potamogeton gramineus	L.	Variableleaf pondweed	Native
Potamogeton natans	L.	Floating pondweed	Native
Potamogeton richardsonii	(A. Benn.) Rydb.	Richardson's pondweed	Native
Potamogeton zosteriformis	Fernald	Flatstem pondweed	Native
Potamogeton spp.	L.	Pondweed	Native
Sagittaria cuneata	E. Sheld.	Arumleaf arrowhead	Native
Sagittaria spp.	L.	Arrowhead	Native
Salix spp.	L.	Willow	Native
Schoenoplectus tabernaemontani	(C.C. Gmel.) Palla	Softstem bulrush	Native
Schoenoplectus acutus	(Muhl. ex Bigelow) Á. Löve & D. Löve	Hardstem bulrush	Native
Sium suave	Walter	Hemlock waterparsnip	Native
Solanum dulcamara	L.	Climbing nightshade	Non-native
Sparganium emersum	Rehmann	European bur-reed	Native
Sparganium eurycarpum	Engelm.	Broadfruit bur-reed	Native
Sparganium spp.	L.	Bur-reed	Native
Spirodela polyrhiza	(L.) Schleid.	Greater duckweed	Native
Stuckenia pectinata	(L.) Börner	Sago pondweed	Native
Typha spp.	L.	Cattail	Possibly non-native

Species Name	**Taxonomic authority**	**Common Name**	**Native/Non-native/Possibly non-native**
Utricularia minor	L.	Lesser bladderwort	Native
Utricularia vulgaris	L.	Common bladderwort	Native
Vallisneria americana	Michx.	American eelgrass	Native

Appendix 3.2 List of species identified in the greenhouse experiment in the moist and flooded watering regimes. Taxonomic authorities were obtained via the International Taxonomic Information System (ITIS 2022), and native/non-native designations were obtained from the USDA Plants Database (USDA 2022).

Species Name	**Taxonomic authority**	**Common Name**	**Native/Non-native/Possibly non-native**	**Moist regime/Flooded regime/Both**	**Observed/Not observed in timed survey**
Barbarea vulgaris	W.T. Aiton	Wintercress	Non-native	Moist	Not observed
Calamagrostis canadensis	(Michx.) P. Beauv.	Canada bluejoint	Native	Moist	Observed
Carex spp. 1	L.	Sedge	Possibly non-native	Moist	Observed
Carex spp. 2	L.	Sedge	Possibly non-native	Moist	Not observed
Carex spp. 3	L.	Sedge	Possibly non-native	Moist	Not observed
Eupatorium perfoliatum	L.	Common boneset	Native	Moist	Not observed
Hydrocharis morsus-ranae	L.	European frogbit	Non-native	Flooded	Observed
Impatiens capensis	Meerb.	Jewelweed	Possibly non-native	Moist	Not observed
Juncus spp.	L.	Rush	Native	Both	Observed
Lycopus americanus	Muhl. ex W.P.C. Bartram	Water horehound	Native	Moist	Observed
Lythrum salicaria	L.	Purple loosestrife	Non-native	Moist	Observed
Najas flexilis	(Willd.) Rostk. & W.L.E. Schmidt	Water nymph	Native	Flooded	Observed
Persicaria lapathifolia	(L.) Gray	Pale smartweed	Non-native	Both	Not observed
Phragmites australis ssp. *australis*	(Cav.) Trin. ex Strued.	Common reed	Non-native	Both	Observed
Pontederia cordata	L.	Pickerelweed	Native	Flooded	Observed
Potamogeton foliosus	Raf.	Leafy pondweed	Native	Flooded	Observed
Ranunculus sceleratus	L.	Celery-leaved buttercup	Possibly non-native	Both	Not observed

Species Name	**Taxonomic authority**	**Common Name**	**Native/Non-native/Possibly non-native**	**Moist regime/Flooded regime/Both**	**Observed/Not observed in timed survey**
Spirodela polyrhiza	(L.) Schleid.	Greater duckweed	Native	Flooded	Observed
Typha spp.	L.	Cattail	Possibly non-native	Both	Observed
Urtica dioica	L.	Stinging nettle	Native	Moist	Not observed
Utricularia spp.	L.	Bladderwort	Native	Flooded	Observed
Vallisneria americana	Michx.	Eelgrass	Native	Flooded	Observed