Best Management Practices for Invasive Phragmites *Control*

by

Graham Michael B Howell

A thesis
presented to the University of Waterloo
in fulfilment of the
thesis requirement for the degree of
Master of Science
in
Biology

Waterloo, Ontario, Canada, 2017
© Graham Michael B Howell 2017

Author's declaration

I hereby declare that I am the sole author of this thesis.

This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

The invasion of European *Phragmites australis* in North America has altered resident species plant assemblages in wetlands and created large monotypic patches. As a response, North American land managers control this invasive species through a combination of herbicide (often glyphosate-based) and mechanical treatments. The impact of glyphosate herbicides and the density of *P. australis* patches on wetland seedbanks remains unclear, and the mechanical removal of *P. australis* biomass requires appropriate disposal to avoid further spreading the invasion. I tested the effect of the glyphosate herbicide WeatherMAX® and examined the effect of *P. australis* stem density on the number and richness of germinating seeds in wetland seedbanks. I also examined the utility of burial as a simple disposal method for *P. australis* biomass during excavation projects. I found that neither the use of glyphosate herbicide nor the density of *P. australis* stems significantly affected the number or richness of germinated seeds. Additionally, I observed that the application of herbicide prior to *P. australis* seed set can reduce the number of viable *P. australis* seeds added to the local seedbank. After testing the burial method with a mesocosm study, no regrowth of *P. australis* was observed in units buried 0.7 m or more. My results indicate that a viable seedbank survives herbicide application and high density *P. australis* invasions. Although my study suggests that 0.7 m is a sufficient burial depth, I recommend 1 m be the minimum burial depth in practice to provide a margin of safety that reflects the invasive potential of *P. australis*. My research contributes to the body of work related to the control and disposal of *P. australis,* and the restoration of areas that *P. australis* has invaded.

Acknowledgements

The last few years have brought me to a variety of places and situations-often involving chest waders, ticks, a Tilley hat, and appalling grass allergies. Those few who assisted me along the way will forever have my many thanks and gratitude for helping me through one of the greatest challenges of my life (so far).

First, I thank my supervisor Dr. Rebecca Rooney for her unending mentorship through these past two years. You have taught me how to collect, analyze, and communicate precious research and knowledge-skills will surely help me down the road of life. During this degree you have shown me true leadership and demonstrated not only how to succeed but also challenged me to strive for that same excellence. I thank you for the time and patience you have invested in me during my time with Rooney Lab.

I have many thanks for Drs. Kirsten Müller and Janice Gilbert for your support and guidance during my degree, both through moral and academic support when I required it. Your wisdom and support throughout this adventure has been greatly appreciated, and I hope this thesis meets your expectations.

To Courtney Robichaud and Sarah Yuckin: you helped me count the merry flowers of June, July and August for hours in the hot sun, and words do not describe how grateful I am for your help. To Adam Kraft, Matt Bolding, Daina Anderson, Jenny Gleason, Heather Polan, Nicole Meyers, and Jody Daniel, thank you for your fellowship during my time in Rooney Lab.

I am greatly appreciative of the support from Danny Bernard (EC-CWS), Denby Sadler (EC-CWS), Eric Cleland (MNRF), Claire Paller (MNRF), Lynn Hoyles (University of Waterloo Greenhouse), and Anne and Scott Hammond for helping me along the road to my thesis defense.

To my Mum, Dad, brother and Grandma, thank you for helping me succeed and allowing me the time I needed to accomplish this degree. I hope that in the coming years I can make more time for my family and the important things in life.

To my fiancé, Lisa: I do not think that I could have continued through the last two years without your unending encouragement, positive attitude, and the reminder to "just chill" when I needed it. Your love and commitment have been a constant reminder of why we are such an awesome team. I hope that our adventures lead us down streams that never find the sea, over rock and under tree, to look at last on meadows green.

Table of contents

List of figures

List of tables

1.0 Literature review

The invasive European lineage of *Phragmites australis* (Cav.) Trin. *ex* Steud (hereafter referred to as *P. australis*) has the potential to radically alter wetlands in Canada, earning it the title "Canada's #1 Invasive" (Catling 2005a). European *P. australis* was likely brought to the east coast of North America in ship ballast in the 19th century (Saltonstall 2002); however, the first documented example in Canada was in Nova Scotia in 1910 (Catling and Mitrow 2011). Preferring wet areas such as ditches and wetlands (Haslam 1972), *P. australis* is highly effective at out-competing resident plants (Minchinton and Bertness 2003; White et al. 2017), resulting in large monotypic stands (Holdredge and Bertness 2011). As a result of the aggressive nature of *P. australis*, a significant amount of time, funding, and resources are allocated to controlling this invasive species

The most common treatment for the control of *P. australis* is the application of a glyphosate-based herbicide (Derr 2008a; Hazelton et al. 2014; Mozdzer et al. 2008; Rapp et al. 2012). The effectiveness of herbicide treatment can be enhanced with mechanical control methods such as mowing or burning (Ailstock et al. 2001; Carlson et al. 2009; Hazelton et al. 2014). Glyphosate herbicides are widely considered post-emergent and so should not affect seeds before they germinate (Franz et al. 1997), and such herbicides are often used in agriculture to halt seed maturation and encourage plant death prior to harvest (reviewed by Blackburn and Boutin 2003). Recent research has suggested that some glyphosate-based herbicide mixtures that include a surfactant may impact un-germinated seeds (Gomes et al. 2017); however, research that directly examines if these herbicides affect the germination of seeds resting in the seedbank is limited. In Chapter 2 of my thesis I examine if the use of a glyphosate herbicide had a measurable effect on the seedbank using a germinability assay.

When mechanical control methods are used instead of herbicides, *P. australis* biomass can require safe disposal. Living *P. australis* tissue fragments have the ability to reproduce vegetatively (Ailstock et al. 2001; Bart and Hartman 2003; Haslam 1969a), thus *P. australis* biomass that is alive when removed using mechanical means must be disposed of in a manner that does not risk spreading propagules (including vegetation fragments and seeds) to uninvaded areas. Government agencies and private organizations recommend that *P. australis* tissues be either dried in the sun on an impermeable surface, buried (California Invasive Plant Council 2012; New Hampshire Department of Transportation 2008), or composted at high temperatures (California Invasive Plant Council 2012; Ontario *Phragmites* Working Group 2015). Some agencies have discouraged the use of composting and desiccation due the need for specialized equipment and the possibility that viable *P. australis* seeds may survive the composting process (Michigan Department of Environmental Quality 2007; Ontario Ministry of Natural Resources and Forestry 2011), and will require transportation to a suitable location and supervision during drying (California Invasive Plant Council 2012). Burial of *P. australis* tissues using 0.91 m (3 feet) of clean fill is suggested by several organizations (California Invasive Plant Council 2012; New Hampshire Department of Transportation 2008; New York State Department of Transportation 2004; Ontario *Phragmites* Working Group 2015); however, the recommended burial depth is arbitrary and does not appear to be based on field trials. This recommendation is possibly inadequate, since *P. australis* rhizomes are reported growing at depths > 1 m in the soil (Haslam 1972). Given that living *P. australis* tissues are routinely excavated during construction and road maintenance projects, it is important to ascertain whether the best management practice guidelines on how to bury these tissues to prevent the spread of *P. australis* are adequate. In Chapter 3, I

quantify the burial depth at which *P. australis* regrowth is prevented to determine if burial is a suitable option for the disposal of living *P. australis* tissues.

1.1 Geographic distribution of Phragmites australis

1.1.1 Global distribution of Phragmites australis

The genus *Phragmites* has four species, namely: *Phragmites karka* (Retz) Trin. *ex* Steud (Polynesia), *Phragmites mauritianus* Kunth (Africa), *Phragmites japonicus* Steud (Japan and China) and *Phragmites australis* (Cav.) Trin. *ex* Steud (Clevering and Lissner 1999). *Phragmites australis* has been called one of the most widely distributed species in the world (Clevering and Lissner 1999; Meyerson et al. 2016), and members of this species can be found on every continent except Antarctica (Clevering and Lissner 1999). A European lineage of *Phragmites australis* was introduced to the east coast of the United States within the last 200 years (Chambers et al. 1999), and has since expanded across most of the continent (Saltonstall 2002). There are two lineages of *Phragmites australis* in North America that differ from the invasive European lineage: the Gulf Coast lineage (found in the southern U.S. and South America) and the native North American lineage (Saltonstall 2002, 2016), which is present in Canada and found in my study area. Given that the appropriate taxonomic status of this species is currently undergoing revision, I will follow Saltonstall (2016) and refer to the native as the North American lineage and the introduced as the European lineage when there might be confusion around the lineage in question. Throughout most of my thesis, however, I simply refer to the European linage as *P. australis,* as it is this introduced lineage that is the subject of investigation here and that has come to dominate my study area.

1.1.2 European Phragmites australis *introduction and spread in Canada*

Examination of herbarium specimens from the Canadian Museum of Nature has indicated that European *P. australis* was in Canada as early as 1910 on the coast of Nova Scotia, and was collected again in 1916 and 1929 near Quebec city and Montreal, respectively (Catling and Mitrow 2011). In 1948, European *P. australis* was collected at Walpole Island, Ontario, and continued to spread locally via roads within these regions until the 1990s when the European lineage expanded dramatically in coastal areas of Lake Ontario, Lake Erie, and Lake Huron (Catling and Mitrow 2011). Low water levels in the Great Lakes during this period, especially on Lake Erie, are thought responsible for the exponential increase in abundance of European *P. australis* during this period, as the newly exposed shoreline provided favourable habitat for its establishment and spread (Wilcox et al. 2003). By 2010, *P. australis* had spread following the road networks in previously invaded provinces (Jodoin et al. 2008), and had been detected in Newfoundland, New Brunswick, Manitoba and British Columbia (Catling and Mitrow 2011).

1.2 Impacts of European Phragmites australis *invasion*

Aggressive competition by European *P. australis* typically displaces resident plant species, consequently decreasing plant species diversity (Hazelton et al. 2014; Minchinton et al. 2006; White et al. 2017; Whyte et al. 2008) and ultimately replacing resident wetland plant species with large monotypic stands of *P. australis* (Meyerson et al. 2000; Wilcox et al. 2003). These monocultures alter the habitat value of invaded marshes for many animal species (Meyerson et al. 2000), with recent research concluding that invasion has negative consequences for marsh nesting

birds (Benoit and Askins 1999; Robichaud and Rooney 2017; Tozer 2016), Fowler's toads (Greenberg and Green 2013), turtles (Bolton and Brooks 2010) and fish species (Fell et al. 2003).

1.3 Biology of European Phragmites australis

1.3.1 Morphology of European Phragmites australis

Phragmites australis produces cane-like stems with yellow-brown internodes (Catling and Mitrow 2011). The stems typically grow up to 4 m tall (Melchior and Weaver 2016) but can grow up to 6 m (Mal and Narine 2004) and may remain standing for more than two years after they senesce (Meyerson et al. 2000). *Phragmites australis* monocultures are therefore characterized by high densities of living and dead stems and a thick detrital layer of slowly decomposing biomass (Ekstam 1995; Meyerson et al. 1999). Leaves produced by *P. australis* are large, with a <0.1-0.9 mm ligule (Catling and Mitrow 2011; Swearingen and Saltonstall 2010), and seed heads are a dense panicle, 30 cm long and dark purple or blonde (Haslam 1972; Ontario *Phragmites* Working Group 2015; Swearingen and Saltonstall 2010). Aboveground stolons are often produced by *P. australis,* which extend along the substrate or water's surface and produce shoots at nodes (Haslam 1969b). Although the aboveground biomass is formidable, the belowground tissues of *P. australis* are key to this plant's invasive abilities.

The belowground network of roots and rhizomes in established *P. australis* stands can account for 60-70% of the total biomass (Mal and Narine 2004) and may extend 0.95 m (Moore et al. 2012) to >1 m below the surface (Haslam 1972). Rhizomes are used to store resources, support aboveground tissues, transport oxygen through aerenchyma to the roots, and for vegetative reproduction (Granéli et al. 1992; Mal and Narine 2004; Weisner and Strand 1996).

1.3.2 Vegetative reproduction

Vegetative reproduction was long thought to be the primary means of European *P. australis* reproduction (Brisson et al. 2008; Mal and Narine 2004). This mode of reproduction uses rhizomes and stolons for the local horizontal expansion of existing stands (Albert et al. 2015; Haslam 1972; Kettenring et al. 2016; Minchinton and Bertness 2003). Horizontal rhizomes have the ability to extend 20 m from the original source (Holm et al. 1977), giving the plant flexibility within a wide range of abiotic conditions even if a clonal stand does not have high genet richness (Honnay and Bossuyt 2005; Kettenring et al. 2016). Stolons and vertical rhizomes produce buds that develop into vertical shoots (Granéli et al. 1992), and stolons have the ability to terminate in aerial shoots or respond to drought conditions by becoming belowground horizontal rhizomes (Haslam 1969b). Living stems that fall in water may develop stolons (Haslam 1969b) and/or axillary buds (Ontario *Phragmites* Working Group 2015). In addition to new buds from stems, fragments of rhizomes with three nodes that are >20 cm in length have been shown to produce axillary buds and shoots within three weeks of cutting (Haslam 1969a). Haslam (1969a) noted that ploughing *P. australis* patches in Europe releases dormancy in rhizomes, and these fragmented rhizomes produce smaller and more dense shoots than would be expected had the patch been left in tact. The production of short and dense shoots following ploughing is likely due to the lower resources available in the small and fragmented rhizomes than would be the case in a larger intact rhizome network (Haslam 1969a). The combination of shoot production from stolons, rhizomes and fallen shoots contributes to the rapid expansion of *P. australis* under suitable habitat conditions and its capacity to persist under periods of unfavourable conditions. Apart from vegetative reproduction, the reproductive success of *P. australis* is further increased through sexual reproduction and seed dispersal.

1.3.3 Sexual reproduction

The size of a *P. australis* inflorescence is a function of the size of the stem, with larger panicles borne on larger (presumably thicker and taller) stems (Haslam 1972). In Britain, the density of flowering stems has been recorded as high as 90% (Haslam 1970); however, poor growing conditions may reduce the flowering density and rametes from young rhizomes and support lower flowering density (Haslam 1970, 1972). Individual panicles are capable of producing 500-2000 seeds/seed head (Wijte and Gallagher 1996); however, not all spikelets on a panicle open at once (Meyerson et al. 2010). Research by Karin Kettenring has shown that patches of *P. australis* that have several unique genotypes within them can create the genetic diversity necessary for cross pollination, leading to increased seed viability (Kettenring et al. 2010, 2011, 2016), which would otherwise be reduced where cross-pollination occurs in stands of low genet diversity (Kettenring et al. 2016). After examining genet relatedness amongst *P. australis* patches, McCormick et al. (2016) found that most seed dispersal by wind occurred under 100 m from the source patch, and did not disperse further than 500 m. Other authors, however, suggest that longer distance dispersal is common. For example, Fér and Hroudová (2009) have found evidence for a maximum of 10 km dispersal of seeds in the Czech Republic, and it is speculated that the seeds may have travelled ~40 km to colonize Krakatoa island after it was destroyed by a volcano (Ridley 1930). Similar genetic analysis suggests that seeds may also disperse as far as 200 km along waterways (Kirk et al. 2011). Vehicles are also likely spread vectors along roads, capable of transporting seeds long distances (Brisson et al. 2008; Lippe and Kowarik 2007). It is therefore possible for sexual reproduction to contribute to the expansion of *P. australis,* particularly along human roadways. Sexual reproduction and dispersal is therefore dependent on several factors, and the successful germination and establishment of these seeds is highly dependent on site abiotic conditions.

1.3.4 Seedling establishment and germination

Dispersal and establishment by seed depends on site conditions and genet richness; however, in some cases seeds can constitute a large proportion of stand propagation alongside vegetative reproduction (Belzile et al. 2010; Kettenring et al. 2016). Seedling establishment and germination require specific habitat conditions that differ from those of adult plants, namely ample light, moist (not inundated) soils, and temperatures >10 °C (Ekstam and Foreseby 1999; 1971). Some *P. australis* seeds are able to germinate right after maturity, while others are dormant and remain so until a cold treatment is applied (Kettenring and Whigham 2009). It is possible that an individual *P. australis* stand may produce viable seeds, but site conditions where they fall (including established *P. australis* stands) may not allow successful germination (Albert et al. 2015; Kettenring et al. 2016; Ter Heerdt and Drost 1994). Although *P. australis* seeds can contribute to the seedbank (Baldwin et al. 2010; Kettenring et al. 2010), it is speculated that seeds may not remain viable in the soil for long (Hazelton et al. 2014). Natural and human-related disturbance can alter site conditions in such a way that *P. australis* seedling establishment is facilitated (Albert et al. 2015; Fant et al. 2016; Kettenring et al. 2011; Minchinton and Bertness 2003; Wilcox et al. 2003). Even if they establish during ideal summer conditions, seedlings are susceptible to cold damage during the winter (Brisson et al. 2008; Haslam 1970; Thompson and Shay 1985), which may limit the geographic spread of *P. australis* by non-vegetative means of establishment (i.e. seed dispersal). Seedling establishment is dependent on a variety of factors that may allow or discourage germination, and subtle abiotic factors will either prohibit or encourage widespread germination.

1.3.5 Habitat requirements for Phragmites australis

Unlike seedlings, mature *P. australis* plants are able to tolerate a wide moisture gradient from moist to permanently inundated substrates (Melchior and Weaver 2016) and expand vegetatively into water depths of 1 m (Holm et al. 1977) to 4 m in warmer climates (Haslam 1972). Because *P. australis* flourishes in brackish wetlands (Meyerson et al. 2000), ditches with road salt inputs provide ideal conditions for *P. australis* (Baldwin et al. 2010; Meyerson et al. 2000). *Phragmites australis* is tolerant of heavy metals (Bonanno and Giudice 2010) and benefits from increased nutrient levels that enhance growth and reproduction (Kettenring et al. 2011; Minchinton and Bertness 2003). Shading by tall trees or dense shrubs has been shown to exclude *P. australis* (Brisson et al. 2010; Havens et al. 2003), although in Ontario *P. australis* can successfully dominate dense groves of *Cephalanthus occidentalis* (Howell, personal observation). Once the obstacle of establishment by seed or vegetation fragment has been passed, *P. australis* has the remarkable ability to survive and become entrenched in a wide range of abiotic conditions.

1.4 Best management practices to prevent Phragmites australis *spread*

Given the ability of *P. australis* to disperse and thrive in a variety of environments, the adoption of best management practices (BMPs) for working with this species are critical to prevent further spread. The invasion of roadsides is increasingly attributed to the transport of *P. australis* plant fragments, seeds, and soil between sites (Ailstock et al. 2001; Bart and Hartman 2003; Brisson et al. 2010; Catling and Mitrow 2011). To discourage further spread of *P. australis,* BMPs for work in or around this invasive species describe equipment cleaning protocols to be conducted before leaving a site. These protocols range from visual inspections and removal of any plant material on

clothing, equipment, and machinery (New York State Department of Transportation 2004), to the precautionary washing and brushing off all machinery and equipment following visual inspections (New Hampshire Department of Transportation 2008; Ontario Ministry of Natural Resources and Forestry 2011; Ontario Invasive Plant Council 2016; Ontario *Phragmites* Working Group 2015). If transportation of soil containing *P. australis* propagules or harvested biomass between sites is necessary, these materials must be covered to prevent them being blown away (California Invasive Plant Council 2012; New Hampshire Department of Transportation 2008) or otherwise contained in an appropriate manner (Ontario *Phragmites* Working Group 2015). Best management practices involving work in and around *P. australis* exist in many parts of North America, however wider adoption by local governments is required, particularly when control treatments are applied.

1.5 Control treatments for Phragmites australis

1.5.1 Chemical control

The extensive belowground perennial rhizomes of *P. australis* make this plant a challenge to effectively manage and control. The use of non-selective post-emergent herbicides is the most commonly chosen treatment for *P. australis* control (Martin and Blossey 2013; Hazelton et al. 2014), as successful control requires that the perennial rhizome tissues be killed, and not just the annual aboveground tissues (Derr 2008a; Knezevic et al. 2013; Mozdzer et al. 2008). When used appropriately, glyphosate is able to eradicate 82% of *P. australis* after one year (Derr 2008). As high as 94% U.S. land managers involved in controlling *P. australis* use herbicide, and products containing the active ingredient glyphosate are the most commonly applied to control *P. australis* in North America (Hazelton et al. 2014). This popularity is owed to glyphosate's proven success

in killing both above and belowground plant tissues and it offers efficient control for large infestations of *P. australis*.

Glyphosate (N-(phosphonomethyl)glycine) was first developed by Swiss chemist Henri Martin in 1950 (Benbrook 2016). When glyphosate was developed as a herbicide in 1970 by Monsanto chemist John Franz, the low toxicity to non-plant life and remarkable ability to kill plant species proved to be ground breaking (Franz et al. 1997). In plants, glyphosate inhibits the enzyme 5-enolpyruvyl-shikimate-3-phospate synthase (EPSPS) within the shikimate pathway, thus impairing plant growth and ultimately leading to plant death (Duke and Powles 2008). Glyphosate can be produced in the form of an isopropylamine salt, potassium salt, ammonium salt, diammonium salt, or dimethylammonium salt (Dill et al. 2010) with the potassium salt form included in herbicides licensed to control *P. australis* in Canada. Glyphosate herbicides are presently used in agriculture, roadside management, pipeline management, powerline management, and the mass consumer market (Benbrook 2016). Techniques of application range from hand wicking to helicopter spraying, thus requiring specialized equipment and licensing to purchase and apply these products.

In Canada, the herbicides that can be applied on *P. australis* are limited to three products: Monsanto's glyphosate-based Roundup WeatherMAX® With Transorb 2 Technology Liquid Herbicide (Monsanto Canada Inc. 2016), Monsanto's glyphosate-based Roundup VisionMAX® (Monsanto Canada Inc. 2015), and BASF's imazapyr-based ARSENAL® PowerLine (BASF Canada Inc. 2017). All three products contain additive chemicals and formulating ingredients that are toxic to aquatic life and therefore can only legally be applied to *P. australis* that is not in standing water. WeatherMAX® and VisionMAX® are chemically identical and contain the same potassium salt form of glyphosate and additive surfactants (Michael Cunningham, Forestry Lead,

Engage Agro, pers. comm.) and only differ in name and label instructions. A greater variety of herbicides are licensed for use in the U.S. than in Canada, most notably a product produced by Dow AgroSciences called Rodeo® that is rated for over-water use. Rodeo® does not have additive chemical surfactants that are highly toxic to aquatic life (Tu et al. 2001), though neither Rodeo® nor an equivalent product appropriate to control *P. australis* is available for over-water use in Canada. Thus, in Canada the active ingredient glyphosate in a pure form (i.e. not in a blend combining adjuvants and surfactants or with non-toxic additives) cannot be purchased commercially for *P. australis* control.

The Canadian Council of Ministers of the Environment states that in order to protect freshwater aquatic life, glyphosate concentrations should not exceed 0.027 g/L in the short term and 0.0008 g/L or 0.8 ppm in the long term (Canadian Council of Ministers of the Environment 2012). This organization also states that glyphosate concentrations in livestock drinking water should never exceed concentrations of 0.00028 g/L (Canadian Council of Ministers of the Environment 1989). The toxicity of differing glyphosate herbicides is set by determining the dose required to kill 50% of animals within a study population. For terrestrial animals, the dose of a chemical administered orally (or by other means) required to kill 50% of the test subjects is referred to as LD50, and is standardized as the mass of the substance per unit mass of the animal. The LD50 method would be difficult for aquatic animals, thus the ambient concentration of the chemical in local environment required to kill 50% of the test subjects is called LC50. When measured the LD50 value of a substance, the chemical is administered as a function of the weight of the animal. Following testing during the licensing procedure, glyphosate is considered one of the lowest-risk herbicides registered with the U.S. Environmental Protection Agency.

Technical grade glyphosate is often referred to as glyphosate acid. Glyphosate acid has low toxicity to animals (Franz et al. 1997; Benbrook 2016), with an LD50 of glyphosate for rats, Bobwhite quail, rabbits and goats is >5, >3.851, 3.800, and >3.500 g/kg, respectively (Franz et al. 1997). These dosage levels are far higher than could be expected following the normal application of a glyphosate herbicide. VisionMAX® and WeatherMAX® both have 540 g/L of glyphosate acid equivalent when sold as a product, which if diluted to a 5% solution for spraying *P. australis* would consist of 27 g/L of glyphosate acid equivalent in solution.

Despite the known low toxicity to animals, the effect of glyphosate on humans is controversial. Extensive literature reviews have concluded that there is no evidence that glyphosate is carcinogenic or that it presents a long-term health risk to humans (Dill et al. 2010; Mink et al. 2012; Williams et al. 2000). Despite this, in 2015 the International Agency for Research on Cancer (IARC) officially classified glyphosate as "possibly carcinogenic" and advised that it may be linked to non-Hodgkin lymphoma (International Agency for Research on Cancer 2015). A definitive answer requires more publicly available data on the regional and global use of herbicides and the consequences for human health (Benbrook 2016).

The harmfulness of glyphosate becomes more nuanced when considering the toxicity of different forms of glyphosate, and the fact that this toxicity is variable with each form. Although glyphosate acid is relatively non-toxic to animals, some glyphosate herbicide forms can be toxic to aquatic life (Tu et al. 2001). Glyphosate acid is reported to dissipate and photodegrade quickly in surface waters (Maqueda et al. 2017), and have a half-life in water that varies from 12-70 days (Tu et al. 2001). Under experimental conditions glyphosate acid was toxic to fish, with the 96 hour LC50 of 120 mg/L for bluegill sunfish (Tu et al. 2001), and carp exposed to glyphosate for two weeks at levels of >5 mg/L exhibited gill damage and liver damage (Nešković et al. 1996). Two

hours after spraying *P. australis,* Glyphosate has been seen to have a maximum of 0.26 ppm in the water two hours after application, which is well below the 0.8 ppm guideline from the Canadian Council for Ministers of the Environment (Rooney, unpublished data). The acid form of glyphosate has an inherent low acidity and is not used in many commercially available herbicides (other salt forms are commonly used), thus its use should be avoided in toxicity studies seeking to realistically test the effect of herbicides on the environment (Tsui and Chu 2003). Bullfrog tadpoles exposed to 1 mg/L concentrations of potassium salt glyphosate for 96 hours experienced thickened epidermises (Rissoli et al. 2016). In contrast, the Dow AgroSciences glyphosate product Rodeo® can have LC50 values of >900 mg/L for some aquatic species (Tu et al. 2001). Created with the isopropylene salt form of glyphosate, Rodeo® is regarded as being non-toxic to aquatic life (Henry et al. 1994; Fell et al. 2006), and Fell et al. (2006) found that the use of Rodeo® combined with mowing to control *P. australis* did not negatively affect fish or invertebrates. Thus, the toxicity of any glyphosate containing formulation will depend not only on the concentration, but also the form of glyphosate used.

In contrast to aquatic environments, in terrestrial settings glyphosate is immobile, typically sorbs to soil particles (Duke and Powles 2008), and has a half-life that ranges from 10-100 days (average 47 days) (Piotrowicz-Cieslak et al. 2010; Tu et al. 2001). Although the sorption of glyphosate to soil particles is pH dependent (Blackburn and Boutin 2003; Koskinen et al. 2016): a high pH and inorganic phosphate concentration (typical in fertilized farm fields) can cause the chemical to become mobile (Franz et al. 1997). Glyphosate is regarded as having few to no fungicidal or bactericidal properties, and is degraded by microflora in both aerobic and anaerobic conditions (Dill et al. 2010). The application of Roundup PowerMAX® (containing a surfactant) and technical grade glyphosate acid has been observed to cause soil respiration to increase, and

although microbial communities in previously sprayed sites may not change considerably, soils that had never been previously sprayed with herbicide may see a shift in the soil microbial community (Lane et al. 2012; Zabaloy et al. 2012). Thus, the use of glyphosate in terrestrial environments typically has few negative impacts, but the presence of additive surfactants plays a factor in the overall toxicity.

When commercial herbicides that are used for *P. australis* control consist of glyphosate and an added surfactant, the toxicity of the solution can be increased. A surfactant is a chemical that broadly enhances the effect of a herbicide by breaking surface tension in droplets. This facilitates the movement of chemical through the leaf's waxy membrane (Dill et al. 2010), and may enhance the toxic effects of the herbicide on the plant (Franz et al. 1997). The actual formulation of the surfactant varies between products and companies and is sometimes considered a trade secret, therefore generalizing across all glyphosate-based herbicides should be avoided unless they are chemically similar. The surfactant polyethoxylated tallow amine (POEA) that is often associated with terrestrial herbicides (i.e. Monsanto's Roundup® brand) is known to be toxic to fish (Tu et al. 2001), amphibians (Relyea and Jones 2009; Rissoli et al. 2016), and to bacteria, protozoa, crustaceans (Tsui and Chu 2003). However, a variety of surfactants can be used and added by the applicator, from alcohol ethoxylates to methylated soybean oil. Due to the wide list of possible formulations and application rates (resulting in differing toxicities), investigation into the toxicity of products to different at realistic application rates is important to understanding the true impact of these herbicides not only to wildlife, but also to non-target plants and seeds.

The immobility of glyphosate in the soil is the basis for considering this herbicide to have no pre-emergence seed properties (Duke and Powles 2008) even if applied in high volumes (Franz et al. 1997). Despite this consensus, very little research has directly tested if glyphosate herbicides

affect seeds. If the herbicide is in anyway affecting the seedbank, this could impede the recovery of treated wetlands significantly

Of the research that has examined the effect of glyphosate on seeds, studies have used both technical grade and commercially available herbicides containing a salt-form of glyphosate. Glyphosate used in agricultural settings has been shown to reduce seed viability and germination when applied to crop plants before seed maturation (Blackburn and Boutin 2003). Although Egley and Williams (1978) found that single applications of 30, 125, or 250 mg/L of glyphosate directly on the seeds of 5 weed species (including the grasses *Sorghum halepense* and *Echinochloa crus-galli*) did not significantly affect germination rates. In this study, glyphosate application encouraged higher germination of *Amaranthus retroflexus* at all three application concentrations relative to the control, yet the seedling length of this species decreased as the concentration of glyphosate increased (Egley and Williams 1978). The authors noted that typical agricultural application rates are unlikely to affect seed germination of weed species; however, it is not specified if this study used a technical grade of glyphosate or a commercial product (Egley and Williams 1978). Glyphosate (isopropylamine salt form) concentrations ranging from 0.20 to 455.84 mg/L have been shown to have no effect on the seed germination of an array of crop and ruderal species (Piotrowicz-Cieslak et al. 2010). Although germination was not affected, herbicide application to seeds was associated with shorter root lengths in some species (Piotrowicz-Cieslak et al. 2010). In a similar study, Gomes et al. (2017) found that daily exposure of tree seeds to 5-50 mg/L of technical grade glyphosate or Roundup® reduced seed germination regardless of concentration, with lower germination where Roundup® was applied. Like several aquatic studies, Gomes et al. (2017) attributed the lower seed germination from Roundup® to the toxicity of the POEA surfactant used in the product.

The impact of a non-selective glyphosate herbicide on germinated resident species guides when application takes place. When used according to commonly accepted procedures, glyphosate herbicides are typically applied in late summer or early fall (Derr 2008a) after most resident plants species have senesced (Mozdzer et al. 2008; Ontario *Phragmites* Working Group 2015). This practice results in application occurring after *P. australis* seed set, which permits the production and release of many thousands of seeds from even a single *P. australis* patch (review by Hazelton et al. 2014; Kettenring et al. 2011). Controlling *P. australis* with herbicide typically requires repeated treatments over several years (Derr 2008a; Lombard et al. 2012; Mozdzer et al. 2008), and several reviews of U.S. *P. australis* management have found one-time applications may achieve only short term reductions in *P. australis* abundance (Breen et al. 2014; Hazelton et al. 2014). If this herbicide indeed has "essentially no pre-emergent or residual soil activity" (Franz et al. 1997), then resident species and *P. australis* seeds that are viable in the seedbank could germinate and re-establish the invasion (Kettenring and Mock 2012). Repeated applications of glyphosate herbicides to control recolonizing *P. australis* same stands have the potential to develop glyphosate-resistant populations, and thus requires the use of diverse array of herbicides (Powles 2008) that may include products with the active ingredient imazapyr.

Imazapyr is an alternative chemical to glyphosate, and was recently licensed to control *P. australis* in Canada in the product ARSENAL® Powerline where no standing water is present (BASF Canada Inc. 2017). Many studies report that imazapyr can have greater success in controlling *P. australis* than glyphosate (Cheshier et al. 2012; Derr 2008a; Knezevic et al. 2013; Mozdzer et al. 2008). Mozdzer et al. (2008) found that an application of either 2 or 5% imazapyr herbicide was most effective at controlling *P. australis* when applied in June, but this treatment also coincided with the lowest recovery of resident species in their study. In a review of North

17

American *P. australis* control measures, Hazelton et al. (2014) recommended future research into the potential negative impacts of imazapyr on resident species recovery and the seedbank.

1.5.2 Mechanical control

The use of hand tools, mowing, machine rolling, burning or flooding are mechanical means to control *P. australis,* though burning, machine rolling, and cutting are the most widely used (Hazelton et al. 2014). Hand tools involve the manual removal of *P. australis* shoots or roots, and can be used to target either specific areas of the plant to cause rhizome mortality (Short 2015) or for mowing aboveground shoots using bladed power tools (Derr 2008b). Machines may also be used to mow on large scales using a bladed attachment; however, use of this method can be limited by the presence of water (Ontario Ministry of Natural Resources and Forestry 2011). Machine rolling involves pulling a cylindrical attachment behind a vehicle that compresses standing stems, and may be used to prepare a site for a controlled burn (Ontario Ministry of Natural Resources and Forestry 2011). Controlled burning involves the planned combustion of *P. australis* biomass by trained professionals, and in Ontario requires an application six months prior to the intended burn date (Ontario Ministry of Natural Resources and Forestry 2014). Finally, flooding pertains to the use of water to damage the perennial tissues of *P. australis*, and involves either the manipulation of water levels or the mechanical removal of aboveground stems (Ontario *Phragmites* Working Group 2015). Flooding can be effective at controlling *P. australis* by preventing snorkeling; a process whereby living stems convey oxygen to the submerged rhizomes to aerate them (Weisner and Strand 1996) and thus makes flooding more effective when combined with mowing.

If timed appropriately, mowing can prevent the transfer of photosynthates from or cutting stems to the rhizomes and thereby starve them (Greet and Rees 2015; Rolletschek et al. 2000). The harvested tissues must be carefully disposed of to prevent spreading *P. australis* to the disposal site or recolonizing the mowed area. In North America, mowing at two week intervals over four months has been shown to reduce regrowth by 55% in the following growing season (Derr 2008b). Mowing can be successful on a small scale, but is not a realistic method to control *P. australis* over large areas because it may take several years of continuous effort and site an wildlife disturbance to achieve eradication.

The success of mowing is improved when the cutting takes place underwater or where mowed stems can then be flooded (Greet and Rees 2015; Rolletschek et al. 2000). Breaking *P. australis* stems at substrate level in ~30 cm deep water has been shown to attain 59-100% control of *P. australis,* lasting 1.5 years after treatment (Smith 2005), and cutting twice in 5 cm deep fresh or brackish water has also been observed to reduce *P. australis* density after one year (Hellings and Gallagher 1992). Greet and Rees (2015) concluded that slashing and flooding *P. australis* stubble at a minimum depth of 20 cm resulted in a ~50% reduction of *P. australis.* The authors endorsing this approach report that, with the stems removed, the flooded rhizomes are cut off from oxygen and perish. While cutting can be effective at severing the aboveground stems of *P. australis*, a similar method called spading can have improved results.

Spading is a mechanical control method whereby a sharpened spade is inserted into the soil to sever the *P. australis* shoots about 5 cm belowground without disturbing the soil. Harvested shoots are then removed from the site and disposed of safely to prevent the spread of *P. australis*. The technique eliminates the photosynthetic tissues, and thereby slowly starves the rhizomes of photosynthates in a manner analogous to but more effective than mowing (Short 2015).

Preliminary results from Dr. Short indicate that spading twice during the growing season can significantly reduce belowground biomass (Short pers. comm.). Thus, spading may be effective in controlling small patches of *P. australis* or when herbicide application is not permitted. Techniques such as spading and mowing do not require extensive training, licensing, or expensive equipment, but can be labour intensive and can require a significant time commitment. The mechanical removal of living *P. australis* tissues also necessitates the proper handling and disposal of these materials in a manner that will not further spread the invasion.

A mechanical treatment significantly more complex than spading is controlled burning. Thompson and Shay (1985) examined controlled burn treatments on *P. australis* in either the spring, summer, or fall seasons. Burning in any of the three seasons resulted in higher stem density, thinner shoots and the removal of 90% of dead biomass compared to control plots (Thompson and Shay 1985). Burns in the spring and fall resulted in an increase in *P. australis* biomass in subsequent years compared to controls, but summer burning led to a decrease in biomass in subsequent years (Thompson and Shay 1985), thus the timing of controlled burns is vital to their success as a control strategy. Similarly, summer and fall burns resulted in lower flowering density compared to control plots, but spring burning led to higher flowering density (Thompson and Shay 1985). The improvements in flowering and biomass following burns at certain times are attributed to improved light penetration due to the removal of litter and improving the growing conditions for new shoots. Self-shading by dense *P. australis* monocultures has been shown to decrease spring shoot production, and can be reversed by litter and biomass removal (Ekstam 1995; Thompson and Shay 1985). Overall burning was effective at diminishing rhizome starch reserves, but failed to eradicate *P. australis* (Thompson and Shay 1985). Consequently, burning is not recommended as a stand-alone *P. australis* control method, although it is often used to clear standing dead

biomass in advance of or following other control methods during the winter (Ailstock et al. 2001; Breen et al. 2014; Carlson et al. 2009).

The reason that mowing and burning fail to achieve long term *P. australis* eradication are that they affect only the aboveground tissues and the perennial belowground tissues are usually unharmed. In its native range in Europe, mowing and burning are advocated as a management technique to encourage the rejuvenation of *P. australis* reed beds by removing accumulated litter and standing dead stems, which leads to increased live stem density (Rolletschek et al. 2000; Valkama et al. 2008). It is therefore not surprising that these treatment approaches are not effective at controlling the plant where it has invaded North America. Moreover, care must be taken when using mowing or burning, as these methods disturb the marsh and leave vacant niches that do not necessarily recolonize with desirable species (Ailstock et al. 2001). When methods such as mowing or spading are used, large amounts of *P. australis* biomass are created that require safe disposal.

Of the many disposal options available, composting has been recommended only if temperatures of 57°C can be reached to destroy stem and leaf material (Ontario *Phragmites* Working Group 2015). Although the California Invasive Plant Council (2012) suggests on-site composting by containing biomass in plastic bags/tarps and exposing to the sun; this agency cautions that this method should only be used if appropriate to the reproductive biology of the plant. Desiccation in the sun can be an effective and inexpensive method to render *P. australis* shoots non-viable, and could be combined with composting. The New Hampshire Department of Transportation recommends placing invasive plant tissues on unshaded asphalt, covering with a tarp and exposing to the sun for at least one month (New Hampshire Department of Transportation 2008). Unfortunately, both composting and desiccation may not affect *P. australis* seeds

(California Invasive Plant Council 2012; Michigan Department of Environmental Quality 2007; Ontario *Phragmites* Working Group 2015), and during transportation to a site suitable for desiccation and composting there is a risk that propagules can be spread (California Invasive Plant Council 2012). Consequently, some sources advise against conventional composting (Michigan Department of Environmental Quality 2007; Ontario Ministry of Natural Resources and Forestry 2011). Therefore, if desiccation or composting is used, tissues should be bagged and disposed of at a landfill to minimize the risk of spread (California Invasive Plant Council 2012; Ontario Ministry of Natural Resources and Forestry 2011). An alternative disposal option for harvested tissues without the need to transport materials to a drying or composting facility is to bury them.

Burial is a particularly appealing option for control projects that already include some excavation, for example, where pond creation or restoration of interspersion with open water is planned. However, burying at a shallow depth is unlikely to be effective at preventing recolonization or spread from the harvested tissues as the plants may simply re-sprout from the burial site. Several U.S. state governments (California Invasive Plant Council 2012; New Hampshire Department of Transportation 2008; New York State Department of Transportation 2004) have published guidelines recommending 0.91 m (3 feet) as an adequate burial depth to prevent the spread of *P. australis*, though the basis for this guideline is unclear. In agreement with U.S. sources, the Ontario *Phragmites* Working Group (OPWG) also recommends burying *P. australis* biomass with at least 0.91 m of clean fill (Ontario *Phragmites* Working Group 2015). However, *P. australis* roots and rhizomes can grow at soil depths of 0.95 m (Moore et al. 2012) to over 1 m (Haslam 1972), raising concerns that 0.91 m may not be deep enough to effectively kill buried materials. Despite the ubiquity of this 0.91 m guideline, to the best of my knowledge it has

never been formally tested. A field trial is required to determine the minimum effective burial depth for disposing of *P. australis.*

1.5.3 Biocontrol

In the early 1990s, European *Lythrum salicaria* monocultures threatened North American wetlands (Malecki et al. 1993; Blossey 1999). Mechanical methods such as mowing and flooding proved to only be successful on small infestations, and although Rodeo® was highly effective as a control measure in the U.S., its use was cautioned against due to detrimental effects to non-target plant species (Malecki et al. 1993; Skinner et al. 1994). Malecki et al. (1993) investigated European herbivorous insects specific to *L. salicaria* that would not be detrimental to North American species of loosestrife. Following field trials, the weevil *Hylobius transversovittatus* was released in North America in 1992 (Malecki et al. 1993) and 1994 (Blossey 1999; Blossey et al. 1994). The introduction of this weevil has suppressed *L. salicaria* expansion and allowed invaded wetlands to recover (Blossey 1999). The same process is currently being used to seek effective and specific biocontrols for *P. australis* (Blossey and Casagrande 2016). There are concerns that it will be impossible to find a biocontrol agent that will target only the invasive European lineage and not the North American lineage of *P. australis,* as the two belong to the same species (Bhattarai et al. 2016, 2017; Cronin et al. 2016). Yet a recent survey of U.S. managers involved in *P. australis* control found that 91% of the respondents (260 of 285) approve of a biocontrol that only affects European *P. australis,* and 46% approve of biocontrol agents that would also impact the North American *P. australis* lineage (Martin and Blossey 2013). Given the openness of managers and need for a diversity of tools to control *P. australis*, research into the use of insects as biocontrol agents that would feed on *P. australis* is progressing.

Tewksbury et al. (2002) identified 201 insects in Europe that feed on *P. australis*, and noted that of the 21 that have accidentally been introduced to North America already, of which 10 are *P. australis* specialists (Blossey and Casagrande 2016). The list of suitable biocontrol insects has since been narrowed down to two stem feeding moths: *Archanara geminipuncta* (Häfliger et al. 2006a) and *Archanara neurica* (Häfliger et al. 2006b) that utilize almost exclusively the European lineage of *P. australis* and often disregard the North American lineage (Blossey and Casagrande 2016).

Archanara geminipuncta is currently the most effective candidate for reducing *P. australis* height and biomass production (Häfliger et al. 2006a), but the result will likely only impair the competitive ability of *P. australis* and site recovery would likely depend on resident species regaining dominance via competition with moth-impaired *P. australis* (Häfliger et al. 2006a). Although the use of insects to control *P. australis* has potential, early research is also being conducted to examine the impact of fungal pathogens to impair *P. australis*.

Shearer and Harms (2012) have begun investigation into the use of fungal pathogens present in North America that could attack *P. australis* as a means of biocontrol. Twenty species of fungus were identified growing on European *P. australis*, with five of these found on both North American and European lineages (Shearer and Harms 2012). The use of fungal pathogens as a biocontrol is still in its infancy, and research continues examining the interactions between European *P. australis* and fungal species.

Not all fungal associations are detrimental to *P. australis*, as fungal endophytes may also facilitate plant growth. Clay et al. (2016) found that a diversity of fungal endophytes positively associate with European *P. australis* tissues. In fact, the use of fungicides to disrupt symbiosis between European *P. australis* and beneficial endophytic fungi is also being investigated as a

mechanism for European *P. australis* control. Identification and disruption of abundant endophytes on European *P. australis* could reduce its competitive ability and give resident species an opportunity to regain dominance (Clay et al. 2016). Researchers with the U.S. Geological Survey's (USGS) Great Lakes Restoration Initiative have shown that fungicides can disrupt the fungal endophytes of European *P. australis,* resulting in a reduction in the number of new shoots compared to a control where fungicides were not applied (USGS Great Lakes Science Centre 2013). However, the susceptibility of European *P. australis* fungal endophytes to fungicide may vary by endophytic species (Fischer and Rodriguez 2013). Although still in the early stages, research into the possibility of fungal endophyte disruption or the use of fungal pathogens may present another tool for European *P. australis* management. The disruption of fungal interactions and many other forms of biocontrol represent an emerging field of *P. australis* management, and may all prove useful to control this prolific invasive species.

1.6 Conclusion

Research into the biology of *P. australis* and potential control options is an ever-expanding body of work. Once a *P. australis* patch is established, resident plant species are gradually out-competed and a dense monoculture is created. The impact of invasion and dominance by *P. australis* on the seedbank has not been extensively examined, particularly in high density *P. australis* patches where inputs of resident plant propagules are likely limited. The use of glyphosate as a post-emergent herbicide to kill *P. australis* perennial tissues is currently the most effective and widely applied management option. It has been suggested that seeds exposed to glyphosate herbicide could be affected by the herbicide during seed development or germination, which could ultimately

impact the resident species seedbank. Damage to the seedbank is undesirable given the crucial role of the seedbank in repopulating areas where *P. australis* has been chemically controlled. If resident species do not quickly colonize treated areas, these areas are susceptible to reinvasion. Despite a widelyheld belief that glyphosate does not affect the seedbank, some literature suggests the herbicide may have a negative effect.

The ability of *P. australis* to occupy a variety of site conditions and to reproduce through both vegetative and sexual propagation has made it one of the most successful invasive species in North America. Living vegetation fragments of *P. australis* rhizomes and stems can produce shoots and root into the soil, and the viability of seeds produced by *P. australis* flowers are increasingly proven to germinate under suitable conditions. Consequently, managers involved with road maintenance, excavation, or *P. australis* control projects are required to safely dispose of any harvested *P. australis* tissues by either composting, desiccation, or burial. Concerns have been raised that composting and desiccation may not destroy viable *P. australis* seeds. Moreover, both desiccation and composting typically require transportation to a drying/composting site, monitoring, and final disposal once drying is complete. Burial of tissues under 0.91 m of clean fill has been recommended by several North American agencies as sufficient to prevent the regrowth of *P. australis* and other invasive species, although there are no scientific grounds for this burial depth guideline. Research is required to confirm safe burial depths for the disposal of *P. australis* tissues.

1.7 Thesis outline

In Chapter 2, I investigate if wetland seedbanks are affected by glyphosate herbicide application for *P. australis* control, and further whether the density of *P. australis* stems impacts the seedbank by intercepting propagules before they reach the soil or by intercepting the herbicide applied and limiting its contact with the sediment. I use a germinability assay to determine if spraying glyphosate herbicide has a measurable effect on the seedbank composition or number of germinating seeds in Long Point wetlands, and whether this response differs between areas where the density of *P. australis* stems is low or high. Glyphosate is known to target areas of growth and development within a germinated plant (Duke and Powles 2008), and has strong chelating properties and fast degradation within terrestrial environments (Franz et al. 1997). Given these characteristics, I hypothesize that 1) spraying glyphosate herbicide will not affect the number or richness of germinated seeds of resident species and thus that I will observe similar germination in seedbank samples collected from sprayed and unsprayed plots. The invasive *P. australis* outcompetes resident species, creates a buildup of litter and standing dead stems, and invasion often results in the creation of a dense monotypic stand (Ailstock et al. 2001; Chambers et al. 1999). The physical barrier created by *P. australis* shoots and litter may block resident species propagules from replenishing the seedbank and intercept applied herbicide. Given the potential influence of *P. australis* biomass, I hypothesize that 2) the number of resident species seeds that germinate will be lower in high density *P. australis* stands compared to those in low density *P. australis* stands.

In Chapter 3, I examine the relationship between *P. australis* tissue burial depth and survival. The objective of this chapter is to empirically determine the burial depth required to prevent the regrowth and re-establishment of *P. australis* tissues from harvested fragments. I

employ a mesocosm study to address this research question. Given the finite energy reserves held within *P. australis* tissue fragments, I hypothesize that 1) there will be a significant negative relationship between burial depth and the number of *P. australis* stems that emerge from each mesocosm. With the common recommendation of burial in 0.91 m of clean fill that is issued by several agencies (California Invasive Plant Council 2012; New Hampshire Department of Transportation 2008; New York State Department of Transportation 2004; Ontario *Phragmites* Working Group 2015), I hypothesize that 2) a threshold of roughly 1 m of overburden will exist, beyond which no *P. australis* stems will grow from buried rhizomes. This research will support the work of natural resource and government authorities who must dispose of *P. australis* tissues in a responsible manner.

In Chapter 4, I summarize my results and additions to the body of research related to *P. australis* control and management, and provide recommendations for resource managers.

2.0 The effect of glyphosate herbicide and Phragmites australis *stem density on the seedbank*

2.1 Introduction

Common Reed (*Phragmites australis* [Cav.] Trin. *ex* Steud; Poacea, is a perennial grass distributed across much of the world (reviewed by Mal and Narine 2004; Saltonstall 2016). A European lineage of *Phragmites australis* (hereafter referred to as '*P. australis*') was introduced to the east coast of North America within the last 200 years, and has since expanded across most of the continent (Chambers et al. 1999; Saltonstall 2002). *Phragmites australis* occupies a moisture gradient from moist to permanently inundated substrates such as ditches or wetlands (Melchior and Weaver 2016) and capitalizes on anthropogenic (Lelong et al. 2007) and natural disturbance to invade and create dense monocultures (Ailstock et al. 2001; Minchinton and Bertness 2003). *Phragmites australis* reproduces through sexual and vegetative means; however, the relative importance of these two methods varies with the stage of invasion, site conditions (Saltonstall et al. 2010) and the genetic diversity of a *P. australis* patch (Kettenring et al. 2010). In freshwater systems *P. australis* culms may grow up to 6 m tall (Mal and Narine 2004) and invasion generally leads to increased litter material and standing dead culms (Meyerson et al. 2000; Minchinton et al. 2006).

After *P. australis* has become established, aggressive competition decreases plant species diversity (Hazelton et al. 2014; Minchinton et al. 2006; White et al. 2017; Whyte et al. 2008). A consequence of established *P. australis* monocultures is the alteration of a wetland's utility as habitat for many animal species (Meyerson et al. 2000), which may negatively impact some marsh nesting bird species (Robichaud and Rooney 2017; Tozer 2016) and has been shown to reduce

habitat quality for Fowler's toads (Greenberg and Green 2013) and turtles (Bolton and Brooks 2010) in Long Point, Ontario.

Due to the negative impacts of invasion, *P. australis* is heavily managed in both the U.S. and Canada. Control typically involves the use of mechanical (burning, machine rolling, cutting) or chemical treatments (the application of imazapyr or glyphosate-based herbicides) to remove *P. australis* stands (Ailstock et al. 2001; Knezevic et al. 2013; Mozdzer et al. 2008; Rapp et al. 2012). Glyphosate-based herbicides are more commonly applied to control *P. australis* in Canada and the U.S. than imazapyr equivalents (Hazelton et al. 2014; Mozdzer et al. 2008). Glyphosate herbicides are non-selective, post-emergent systemic pesticides employed across a variety of industries (Duke and Powles 2008; Franz et al. 1997). As a post-emergent herbicide, glyphosate is designed to act on germinated plants (Mateos-Naranjo and Perez-Martin 2013), as opposed to pre-emergent herbicides designed to kill seeds (Franz et al. 1997). After being applied to living plant tissues, glyphosate inhibits the enzyme 5-enolpyruvyl-shikimate-3-phospate synthase (EPSPS) within the shikimate pathway which leads to plant death (Duke and Powles 2008). Glyphosate readily adsorbs to soil particles and is considered generally immobile in terrestrial environments (Duke and Powles 2008) and short-lived as soil microbes break down glyphosate after an average of 47 days (Tu et al. 2001). Following monitoring of a large scale glyphosate application in Crown Marsh in September 2016, concentrations of glyphosate and AMPA had both returned to baseline levels within 30 days of herbicide application (unpublished data), suggesting that in my study system the half-life is quite a bit less than this published average.

Franz et al. (1997) state that the nature of glyphosate as a chelating agent allows the chemical to bind to soil particles, causing glyphosate herbicides to have "essentially no pre-emergence or residual soil activity," even when applied in high volumes. Many sources in the

literature agree that these herbicides are post-emergent (Blackburn and Boutin 2003; Franz et al. 1997; Koskinen et al. 2016), and although such herbicides are often used in agriculture to stop seed maturation and cause plant death to quicken harvest (reviewed by Blackburn and Boutin 2003), very few studies have examined if glyphosate herbicides affect seed viability.

Many researchers have stressed the importance of a viable seedbank as a resource for successful restoration following *P. australis* control (Ailstock et al. 2001; Baldwin et al. 2010; Carlson et al. 2009; Hallinger and Shisler 2009). Ailstock et al. (2001) examined the recovery of wetlands following *P. australis* control treatments, and stated: "By maintaining a viable seedbank, these wetlands can respond to vegetation loss and disturbance, including herbicide application, with a diverse plant community in the next growing season." Given the prevalence of glyphosate use in restoration settings and the scarce literature characterizing the direct effect of glyphosate on seed germination, research examining how glyphosate could affect the seedbank and seedling germination is required. Further, if indeed glyphosate herbicide does not affect seeds, any *P. australis* seeds that are viable in the seedbank could germinate in the spring after herbicide application to re-establish the invasion (Kettenring and Mock 2012). The goal of this research was to determine if the application of glyphosate herbicide has a measurable effect on the seedbank in Long Point marshes.

I predicted that the effect of glyphosate application on the seedbank might vary, depending on the stem density of *P. australis* being treated. In high density patches, more of the applied herbicide should be intercepted by plant leaves and less should reach the sediment directly, thus the exposure of the seedbank should be lower in high density patches. Further, in high density patches, I expect that the diversity and abundance of viable seeds from plant species other than *P. australis* should be lower, as the dense biomass would also intercept incoming seeds (Minchinton

et al. 2006). I therefore anticipate that the density of *P. australis* will affect both the risk that herbicide treatment presents to the seedbank and the seedbank itself. A secondary aim of my thesis chapter is therefore to contrast the effects of herbicide treatment in a high-density patch of *P. australis* and in a low-density patch of *P. australis*.

2.2 Methods

2.2.1 Seedbank sampling

To achieve my thesis objectives, I surveyed two locations in Long Point, Ontario that have been invaded by *P. australis*: the Big Creek National Wildlife Area and the Crown Marsh waterfowl management unit (see Appendix A). The study area in Big Creek was a constructed impoundment dyke, and in Crown Marsh a former meadow invaded by *P. australis*. *Phragmites australis* control projects that included treatment with a glyphosate-based herbicide (5% Roundup WeatherMAX® with a 1% soybean methylated seed oil adjuvant) at both locations provided an opportunity to contrast the seedbank response in treated and adjacent un-treated (control) areas. In Big Creek, this also afforded me the opportunity to contrast the seedbank in both treated and control locations that were situated in low and high density *P. australis* stands. In Big Creek, herbicide application took place on July 6th, 2015, which is early compared to standard best management practices for *P. australis* control (Ontario Ministry of Natural Resources and Forestry 2011; Ontario *Phragmites* Working Group 2015). In Crown Marsh, herbicide application took place on September 29th, 2015, which is in keeping with the recommended practice (Ontario Ministry of Natural Resources and Forestry 2011; Ontario *Phragmites* Working Group 2015).

I collected seedbank samples from a standard area of 0.044 m^2 that comprised the upper 2 cm of soil. In Big Creek, I collected 20 samples on July 20th, 2015; these 20 samples consisted of 5 replicates from each of four treatments: 1) herbicide-treated, high density plots (>60 live stems per m^2); 2) herbicide-treated, low density plots (<20 live stems per m^2); 3) control, high density; and 4) control, low density plots. In Crown Marsh, I collected 15 seedbank samples on November 19th, 2015, comprising 5 replicates from an area that was treated with herbicide and 10 control replicates, five each from two adjacent control sites that were not treated. As a stage of the ongoing *P. australis* control project in Crown Marsh, the sampling sites were machine rolled on March 11 & 12, 2016. This is in accordance with the recommendations of the Ontario *Phragmites* Working Group (2015).

Vegetation surveys conducted at both seedbank sampling locations provided a species list to help with the identification of seedlings. Further, I referred to the species list in the Flora of Long Point (Reznicek and Catling 1989). To compare seedbank emergence with the vegetation that grew in treated and control plots the following field season, I surveyed the Crown Marsh on June 28th, 2016. To characterize the vegetation that was growing in sites where seedbank samples were collected in Crown Marsh, I used 1 m^2 quadrats (one replicate for each seedbank sample). This could not be conducted in Big Creek because the herbicide treatment was part of a dike reconstruction and the original sampling sites had been heavily disturbed.

2.2.2 Greenhouse methods

Soil samples from both Big Creek and Crown Marsh were washed with high pressure water through a 4 mm sieve, followed by a 0.212 mm sieve to collect seeds and an organic slurry.

Greenhouse trays were prepared with a 5 cm base layer of Sunshine® Mix #4, and capped by a 3 mm layer of sterilized sand (Ter Heerdt et al. 1996). The sand was intended to stop seeds from migrating down into the soil to encourage maximum germination. On top of the sand layer, the organic seed slurry was quantitatively transferred into growth tray cells as a 4 mm thick layer. Sufficient cells were used to accommodate all the slurry at this standard layer thickness. The planted cells were placed in the University of Waterloo Biology Greenhouse, in Waterloo Ontario, and watered every 1-2 days to maintain a moist soil. The Big Creek assay was conducted during the winter season, so samples were lit for a standard 12-hour photoperiod with Sunlux Ultra Ace pressure sodium lamp. The Crown Marsh assay was conducted during the summer season, and samples were exposed to the natural photoperiod.

Trays were checked daily and surveyed weekly. When identifiable, seedlings were counted and removed from the tray. The assays continued until a week passed without new germination (following Ter Heerdt et al. 1996). Trays were then left to desiccate for one week, before I mixed the seed slurry layer to trigger dormant seeds to emerge. The survey process was then repeated until germination terminated once more (about five to six weeks).

2.2.3 Data analysis

All analysis and graphing was completed using R studio (RStudio Team 2015). The R studio packages used included: "e1071" (Meyer et al. 2015), "Rcpp" (Eddelbuettel and Francois 2011), "vegan" (Oksanen et al. 2016), "BiodiversityR" (Kindt and Coe 2005), and "ggplot2" (Wickham 2009). In the Big Creek Study, I tested for the effect of *P. australis* stem density (low vs. high),

herbicide application, as well as their interaction using two-factor model 1 ANOVAs with an interaction term. In the Crown Marsh study, I tested for the effect of treatment (application of herbicide and vs. control) using Welch's t-tests. In both studies, I applied these tests to determine if factors predicted: 1) the total richness of seedlings emerged, 2) the total stem density of seedlings emerged, 3) the stem density of *P. australis* seedlings that emerged, 4) and the stem density of non-*P. australis* resident species that emerged. Two datasets from the Big Creek study were square root transformed to meet parametric assumptions when plots of the residuals vs estimated values and variance showed a departure from normality (Gotelli and Ellison 2012).

To test if species community composition differed between treatments, I used the multi-response permutation procedure (MRPP) on Sørenson (Bray-Curtis) distance matrices (McCune and Grace 2002). To visualize the community composition compared to experimental units, relativized data was used to perform non-metric multidimensional scaling (NMDS) procedures on Sørenson (Bray-Curtis) distance matrices (McCune and Grace 2002). These analyses were carried out for Big Creek seedling stem density, Crown Marsh seedling stem density, and Crown Marsh species presence/absence to compare 2016 field surveys to seedling presence-absence in the greenhouse. In addition, the Big Creek seedling stem density data was square-root transformed to reduce the influence of dominant species (Peck 2010) without changing the rank of species contributions to total density (McCune and Grace 2002).

2.3 Results

2.3.1 Big Creek univariate analysis: seedling germination of Phragmites australis and resident species

Throughout the seven-month growing period for this assay, the Big Creek greenhouse seedbank samples yielded 1606 seedlings from 21 species. The six most abundant species were *Urtica diocia, Carex* spp., *Barbarea vulgaris, P. australis, Typha* spp.*,* and *Solidago canadensis* (see Appendix B for a full species list). Five species could not be identified because seedlings did not reach maturity. These were numbered Species #12, Species #17, Species #18, Species #19, and Species #22.

Results of two-way ANOVAs on square-root transformed seedling stem density and richness from Big Creek are presented in Table 2-1. The interaction terms and density of *P. australis* stems were never a significant predictor of the response variables. Treatment with herbicide, however, was a significant predictor of *P. australis* seedling stem density, with average *P. australis* seedling stem density higher in the control sites where no herbicide was applied (Mean = 8.7 seedlings/sample, Standard Error = 1.91 seedlings/sample) than in herbicide-treated sites (Mean = 3 seedlings/sample, Standard Error = 0.67 seedlings/sample) (Fig. 2-1).

2.3.2 Big Creek multivariate analysis: species stem density

I selected a two dimensional NMDS ordination solution to depict the relative abundance patterns in seedlings germinated from the Big Creek seedbank samples. A scree plot with the final stress of the optimal ordination solution plotted against the number of dimensions revealed that the addition of a third dimension resulted in only a marginal reduction of stress (see Appendix C for

scree plots). Furthermore, the gradients in relative seedling abundance depicted in the first two axes of the optimal three dimensional solution appeared to be very similar to the first two axes of the optimal two dimensional solution (see Appendix D for three dimensional ordinations) and the community gradient associated with the third dimension was not something I could relate to my covariate data. Therefore, I selected a two dimensional NMDS ordination to characterize variation in the abundance of seedling stem densities, with a final stress of 0.21 after 20 iterations (Fig. 2-2a,b). The seedbank composition in sites treated with herbicide was visibly distinct from the seedbank in sites that were not treated, with separation along axis 1 (Fig. 2-2a). Sites where herbicide was applied in July produced more *Barbarea vulgarus, Carex* spp. and *Typha* spp. seedlings, whereas sites that were not treated with herbicide produced more *P. australis, Solidago canadensis, Alliaria petiolata,* and *Persicaria lapathifolia* seedlings (Fig. 2-2a). Of the species that were associated with non-sprayed sites, all except for *Alliaria petiolata* flower in late August/early September. In contrast, there was substantial overlap in the community structure of the seedbank samples collected from areas of high and low *P. australis* stem density (Fig. 2-2b).

The patterns I observed visually with these ordinations were supported by my MRPP results, where seedbank composition did not differ significantly among low and high *P. australis* stem densities (chance-corrected $A = -0.02$, $p = 0.879$). In contrast, the seedbank composition differences between herbicide-treated and control sites were statistically significant (chance-corrected $A = 0.05$, $p = 0.008$).

2.3.3 Crown Marsh univariate analysis: seedling germination of Phragmites australis *and resident*

species

The Crown Marsh seedbank assay lasted nine months, during which time 975 seedlings were

observed from nine distinct species. The four most abundant species were: *Juncus brevicaudatus,*

Typha spp*., Scirpus pungens,* and *P. australis* (see Appendix E for a full species list)*.* Two species

could not be identified and did not reach maturity. These were numbered Species #6 and Species

#8.

Results of a Welch's independent sample t-test on each response variable are presented in

Table 2-2. Unlike the results from Big Creek, treatment with herbicide was not a significant

predictor for any of the response variables (Table 2-2).

2.3.4 Crown Marsh multivariate analysis: species stem density

As with the ordination of Big Creek seedbank samples, when choosing the optimal number of

dimensions for the final NMDS solution based on the decay in final stress (see scree plots in

Appendix F), I observed only a marginal reduction in stress when accepting a third dimension.

Further, the community gradient depicted on the third dimension was not something I could relate

to covariate data (see Appendix G for three dimensional ordinations). Therefore, I present the

optimal two dimensional NMDS ordination solution to dissect variation in the relative abundance

of seedlings, with a final stress of 0.17 after 20 iterations. In this ordination, sites appear

moderately separated by herbicide treatment along axis 1 and 2 (Fig. 2-3). The control sites (those

that were not sprayed with herbicide) spanned a wide range on axis 1 and score higher on axis 2,

with higher abundances of Species #8, Species #6, and *P. australis*, and occasionally *Lythrum*

salicaria, and *Juncus brevicaudatus* (Fig. 2-3). Sites that were treated with herbicide were associated with higher *Scirpus pungens* and *Typha* spp., compared to the control sites. Despite the visual representation in ordination space, an MRPP showed that the relative abundance of different seedling species did not differ significantly between herbicide-treated and control seedbank samples (chance-corrected $A = 0.03$, $p = 0.087$). *2.3.5 Crown Marsh univariate analysis: comparison between greenhouse and field germination*

Results of a Welch's independent sample t-test comparing the species richness observed in the greenhouse to 2016 field surveys are summarized in Table 2-3. There was a significant effect of location, $t_{17} = 2.12$, $p = 0.049$, with higher average richness in the Greenhouse (Mean = 5.33 species/sample, Standard Error = 0.29 species/sample than the species richness in from the Crown Marsh field site (Mean = 3.47 species/sample, Standard Error = 0.83 species/sample) (Fig. 2-4). *Phragmites australis* seedlings were not observed growing in the field in Crown Marsh; however, many were observed in the greenhouse assay in seedbank samples collected from both the herbicide-treated and control plots.

2.3.6 Crown Marsh multivariate analysis: comparison of greenhouse and field site presence-absence

The optimal NMDS solution to examine the patterns in the occurrence of species between the Greenhouse and field site was two dimensional, with a final stress of 0.08 after 20 iterations. When symbolized according to location, there was a clear separation of sites in the field and their associated greenhouse-grown seedbank samples along axis 1 with little overlap (Fig. 2-5).

The tight clustering of greenhouse samples in Fig. 2-5 indicated that the occurrence of species in the seedbank samples grown in the greenhouse was highly homogenous compared to the diversity of species identified in the field survey, regardless of whether the seedbank samples were collected from an area treated with herbicide or not. In comparison, the heterogeneity of field sites surveyed in Crown Marsh indicates that field sites support a more variable plant assemblage (higher beta diversity) compared to greenhouse seedbank samples grown in the greenhouse. This is despite finding a higher average per sample richness (mean alpha diversity) in the seedbank samples through univariate analysis (Section 2.3.5), compared to the field surveys, as described in the section above. A total of 18 species were observed across the field sites, in contrast to the nine-species seen in the greenhouse seedbank samples taken from Crown Marsh. Several species observed in the greenhouse were absent from the field, and likewise with species observed in the field being absent from the greenhouse species list (see Appendix E). This effect is demonstrated by the fact that 33.3% of species that germinated in the greenhouse were observed in the field, and 16.7% of species observed in the field germinated from seeds in the greenhouse.

The vectors indicating the strength and direction of associations between the occurrence of different species and the ordination axes emphasize how the greenhouse seedbank samples differ from the diversity of field plot surveys. Note that *P. australis* that was not strongly associated with either location. The observed patterns in the NMDS were confirmed by MRPP results, which supported my conclusion that the greenhouse and field survey results were significantly different in terms of the presence-absence of plant species (chance-corrected $A = 0.31$, $p = 0.001$).

2.4 Discussion

Working alongside ongoing *P. australis* control projects in Long Point, Ontario, I sought to determine if the stem count and richness of seedlings emerging from the seedbank was affected by herbicide treatments and *P. australis* stem density. Although herbicides containing the chemical glyphosate are considered post-emergence herbicides (Franz et al. 1997), the time of application could conceivably alter the composition of the seedbank by killing late flowering species before they set seed. The knowledge of a viable *P. australis* seedbank would enable restoration practitioners to avoid creating conditions which favour *P. australis* germination and encourage resident species recolonization. I observed *P. australis* seedling germination in nearly all seedbank samples collected as part of my study, but notably did not detect seedlings growing in herbicide treated areas in the field when I surveyed sites post-treatment. Herbicide treatment did not have a significant effect on the species richness of seedlings that emerged from seedbank samples, and was only a significant predictor of seedling stem density in Big Creek, where *P. australis* stem density was significantly higher in the sites that were not treated with herbicide compared to those that were treated. In contrast, the stem density, richness, and composition of the seedbank did not differ between areas of high and low *P. australis* stem density.

2.4.1 Seedbank response in Big Creek and Crown Marsh

Given the success of glyphosate-based herbicides in killing mature plants without impacting the seeds (Duke and Powles 2008) I did not expect the application of herbicide would affect the seedbank. Yet in the Big Creek germinability assay, I detected significant differences in *P. australis* seedling stem densities between seedbank samples collected from herbicide-treated and

untreated plots. The stem density of *P. australis* seedlings in Big Creek was significantly higher in control sites than sites treated with herbicide, immediately raising questions about the impacts of herbicide treatment on seeds.

One possible explanation for this surprising result is that the glyphosate application in 2015 may have impaired the viability of some *P. australis* seeds in the treated areas. Blackburn and Boutin (2003) reviewed several studies that demonstrate reduced germination for seeds collected from plants treated with glyphosate herbicide, and observed that herbicide application to plants in the late stage of seed production caused reduced viability and maturation of the seeds (Blackburn and Boutin 2003). In contrast, Piotrowicz-Cieślak et al. (2010) found no effect on seedling germination, and a similar result was also found by Egley and Williams (1978). Despite finding no effect of glyphosate on seed germination, Piotrowicz-Cieślak et al. (2010) detected root length inhibition amongst some of the seedlings that grew from seeds treated with 0.20 to 455.84 mg/L of isopropylamine salt form glyphosate. Gomes et al. (2017) examined the effect of two glyphosate formulations that were applied to seeds of the Brazilian tree *Dimorphandra wilsonii*. These authors scored germination as successful if a seed was capable of forming a root at least 2 mm in length, and found that daily applications of 5-50 mg/L resulted in reduced germination of *D. wilsonii* seeds. The reduction in seed germination took place regardless of which formulation was applied, but germination was lowest in samples treated with the formulation including a surfactant. This reduced germination was attributed to the disruption of enzymes related to mitochondrial metabolism, which negatively impacted respiration of the seeds (Gomes et al. 2017). The studies that found an effect of glyphosate on seeds exposed the herbicide directly to the seeds, whereas this is unlikely to occur in the field. When applied to *P. australis,* glyphosate would be intercepted by living and dead shoots and litter before it could reach the soil. Therefore, the

exposure of seeds during field projects will likely to differ from research that directly exposed seeds to glyphosate during laboratory conditions.

Although it is a possibility that glyphosate treatment reduced seed viability in my seedbank samples collected from the area of Big Creek that was treated with herbicide, I do not believe that this is what was in action in my study. First, if the herbicide were affecting mitochondrial metabolism, I would expect to see reductions in the stem density of most or all seedling species, and not only *P. australis* seedlings. A shift in the community composition was observed in the ordination between herbicide treated sites and control sites, such that most of the species associated with non-sprayed sites flower late in the season. Second, I would also expect to also see higher *P. australis* germination in control sites (relative to sites treated with herbicide) in Crown Marsh. I observed no significant reduction in total stem density with the herbicide treatment in either study location, nor was *P. australis* stem density higher in control plots in the samples collected from Crown Marsh. These findings suggest that herbicide treatment was not directly reducing the viability of seeds in the seedbank.

A more probable explanation for the observed reduction in *P. australis* seedling emergence in Big Creek is the early timing of herbicide application. Herbicide was applied to the Big Creek impoundment on July 6th 2015, and the collection of seedbank samples occurred two weeks later on July 20th, 2015. This is an atypical treatment time, as the best management guidelines for Ontario recommend herbicide application for the control of *P. australis* take place in the fall to take advantage of the late senescence of invasive *P. australis* relative to most resident species (Ontario Ministry of Natural Resources and Forestry 2011). By applying herbicide in the early fall, the treatment should not harm resident plants that have already senesced but will harm *P. australis* as it is translocating nutrients from the shoots to the rhizomes in preparation to senesce (Ontario

Ministry of Natural Resources and Forestry 2011; Ontario *Phragmites* Working Group 2015). The recommended practice of fall application was not followed in Big Creek because this impoundment was being treated in preparation for repair work that would likely destroy all native plants (Erling Armson, Invasive Species and Northern Projects, Ducks Unlimited Canada, pers. comm.). The unusual July application may have prevented the treated *P. australis* from setting seed while the untreated *P. australis* rametes were permitted time for their seeds to mature and disperse. Thus, I believe that the control plots had higher densities of *P. australis* seed germination than the herbicide-treated plots not because the herbicide application damaged *P. australis* seeds already in the seedbank, but because it killed mature plants before they could set seed and contribute that seed to the seedbank. Given that nearly all of the plants associated with non-sprayed sites also flower in late August/early September, individuals of these species may have been affected by July herbicide in treated areas and were thus prevented from flowering.

The flowering and seed set period for *P. australis* varies across North America. In Manitoba and the east coast of the U.S., *P. australis* is reported to flower between July and September (Saltonstall et al. 2010; Thompson and Shay 1985), whereas seed set may occur between September and November (Marks et al. 1993; Saltonstall et al. 2010). Observations of *P. australis* phenology at Long Point in 2014 suggested that flowering of *P. australis* began in early July (Courtney Robichaud, University of Waterloo, pers. comm.). We timed our seedbank sample collection to occur two weeks after the July 6th 2015 herbicide application to give it time to effectively kill mature plants. It is possible that this two-week window after the herbicide treatment was sufficient for some *P. australis* growing in the control locations to set seed and add propagules to the seedbank, given the timing of flowering that year. Furthermore, it is speculated that *P. australis* seeds do not persist long in the seedbank (Hazelton et al. 2014), which increases the

likelihood that an input of seeds during the sampling year resulted in the increase of *P. australis* seedlings where herbicide was not applied. The newly deposited seeds would therefore be responsible for my observation of greater *P. australis* stem density in the control sites.

As seen in Fig. 2-1, the number of *P. australis* seedlings in herbicide treated sites is on average far lower than the control sites. The absence of an annual input at sprayed sites shows the impact of one year's input of viable *P. australis* seeds in the seedbank. Given that *P. australis* has been present in the Big Creek sampling area for several years, one would expect a large accumulation of viable seeds if the seeds are long lived, however this does not seem to be the case. The literature is unsure of the exact longevity of *P. australis* seeds, however my research indicates that even after a long-lived invasion and the formation of a monoculture, large numbers of *P. australis* seeds may not remain viable in the seed bank longer than 1-2 years. These findings support predictions from other authors that *P. australis* seeds may not remain viable over a period of several years.

One difference between Crown Marsh and Big Creek is that in Crown Marsh both the control and herbicide-treated samples had all standing stalks flattened by machine rolling in March 2016; however, this occurred after seedbank sampling. Another important difference is that in Crown Marsh, the herbicide application took place in September, as recommended by the Ontario *Phragmites* Working Group (Ontario *Phragmites* Working Group 2015). In contrast to Big Creek, I observed no effect of the application of herbicide on any response variable relating to the diversity or density of seeds in the seedbank from Crown Marsh, where these control sites were situated adjacent to the herbicide-treated site (see Appendix H for location of controls and sprayed sites). In summary, herbicide treatment did not significantly affect either total or resident species stem density or diversity in Crown Marsh or Big Creek, and further did not affect the abundance of *P.*

australis seedlings emerging from the treated seedbank samples from Crown Marsh. Therefore, I conclude that the herbicide treatment did not affect seeds directly, but rather the timing of treatment affected the contribution of seeds from treated *P. australis* plants in the July-treated Big Creek marsh.

2.4.2 Effect of Phragmites australis *stem density on Big Creek seedbank*

A typical *P. australis* stand that has been established for several years is characterized by high stem densities, a deep litter layer of slowly decomposing material, and an enclosed canopy that reduces light penetration (Meyerson et al. 2000; Windham 2001). Given these characteristics, I expected that the exclusion of resident plant species from high density stands and the dense *P. australis* biomass would act as a barrier to resident species propagules. However, I determined that there was no difference in seedbank abundance, diversity or community composition between high and low density patches of *P. australis* in Big Creek. Thus, I conclude that propagules from resident species reached the soil, even in dense *P. australis* stands in Big Creek, and it is likely that the density of *P. australis* did not affect on the seedbank stored within the upper 2 cm of the soil horizon. Limited dispersal of seeds can occur at the edge of thin *P. australis* stands such as the sample site in Big Creek (Minchinton et al. 2006) however this likely will not apply to larger *P. australis* patches that are not thin linear features. In the case of a larger patch, a thick litter layer would be built up over time that resident species propagules are unlikely to penetrate. Therefore, restoration following the control of a larger patch is likely to be reliant on the existing seedbank and recent propagule input, and this must be considered during project planning. The presence of a viable seedbank despite the invasion of *P. australis* stresses the importance of removing *P.*

australis biomass to prevent suppression of seedbank species during a restoration project (Ailstock et al. 2001).

2.4.3 Comparison of species richness and occurrence between the greenhouse and field site

My study was designed to determine whether the seedbank in marshes dominated by *P. australis* is affected by herbicide application compared to the seedbank where herbicide was not applied. For the Crown Marsh site, however, I was also able to return the year after the herbicide was applied to compare what grew in the field with what I collected from the seedbank samples the preceding year.

It does not make sense to compare raw abundances between greenhouse grown seedlings and the field surveys because the greenhouse assay took a concentrated slurry of seeds and strove to give each seed an opportunity to germinate by spreading them thinly on appropriate substrate and exposing them to favourable conditions for emergence. The greenhouse approach minimizes any effect of competition on seedling germination, which is in stark contrast with the reality of seedling emergence in the field where shading from plants, fluctuations in water depth, and variation in temperature all likely constrain seed germination.

Though raw abundance comparisons between greenhouse and field survey results are not advisable, it is appropriate to compare the occurrence of species in the greenhouse trial with those in the field to assess similarities in community diversity. Generally, the average species richness per sample (alpha diversity) was significantly greater in the greenhouse samples than in the field surveys. This I attribute to the more favourable greenhouse conditions, which were selected to maximize the germination of seeds. In contrast, field conditions in the *P. australis* invaded

meadow were variable and water depths likely limited what species could emerge from the seedbank following herbicide treatment. Furthermore, this measure averages across the control and treatment plots, which I expect would reduce the average richness in field sites where competition from untreated *P. australis* would likely reduce diversity compared to the herbicide-treated sites where *P. australis* adults were eradicated. However, in terms of gamma diversity, the overall number of species that grew from the seedbank in the greenhouse was only a subset of the number of species I detected during my field survey. This difference between the trend in alpha diversity (species richness is higher in total in the greenhouse experiment) and gamma diversity (species richness is higher on average in the field) is reconciled by a difference in beta diversity, whereby the greenhouse samples all supported the same species, albeit in differing relative abundances. In contrast, the variation in species occurrence among individual replicates was much higher for the field surveys. This trend in beta diversity is equally after averaging across treatments, meaning that this is true for sites treated by herbicide and the control sites.

I believe this trend in beta diversity is due to the spatial heterogeneity of environmental conditions present in the field, which means that one site will have conditions that favor the emergence of a certain subset of the seedbank whereas an adjacent site will have different environmental conditions that favour the germination of a distinct subset of the seedbank. In the greenhouse, germinability assay conditions favoured germination of a broader subset of the seedbank on average, with ample light, moist but not submerged soils, and almost no competition for resources. Because of variation in these environmental conditions in the field and the fact that the control plots were still dominated by adult *P. australis* whereas the herbicide-treated sites were free of adult *P. australis*, an overall larger subset of the seedbank could germinate when the whole suite of survey sites are considered collectively.

The observation of many *P. australis* seeds in the greenhouse but a conspicuous absence from the field surveys is also easily explained by a difference in abiotic conditions.

2.4.4 Conclusions and management implications

My research found that the use of 5% WeatherMAX® glyphosate herbicide combined with a 1% soy bean MSO adjuvant did not impact the overall stem density or richness of seedlings that emerged from seedbank samples in herbicide-treated plots, compared with control plots. The literature has several examples of glyphosate-based herbicides affecting seed viability; however, the role of surfactants and other chemicals is not always clearly separated from the impact of glyphosate itself. The standard fall application of such herbicides to control *P. australis* prior to senescence is well-timed to avoid unnecessary harm to wildlife and resident plant species (Ontario *Phragmites* Working Group 2015); however, this practice likely falls after seed set for many *P. australis* plants in Ontario. Given that each *P. australis* plant may produce 500-2,000 seeds per seed head (Wijte and Gallagher 1996), a considerable number of viable *P. australis* seeds can be added annually to the seedbank, allowing potential recolonization from the seedbank in the following summer if environmental conditions support seed germination. If herbicide application takes place prior to seed set, my research shows that it may significantly reduce the abundance of viable seeds in the seedbank, and therefore reduce the risk of recolonization in recently treated areas. When aiming to control a stand of *P. australis* with herbicide it would be prudent to monitor the flowering status of the *P. australis* culms and treat plants prior to seed set.

The argument against earlier application is that it may harm non-target species, but in areas of high density *P. australis*, where resident plant species are already excluded, I argue that the risk

of harming them is low and could be offset by seeding with desirable species following herbicide application. In my study, conditions in the field following herbicide treatment did not favour *P. australis* seed germination, but my greenhouse assays demonstrate that viable *P. australis* seeds are present. With several recent publications highlighting the ability of *P. australis* to use sexual reproduction to expand its dominance of invaded marshes and to colonize across long distances (Kettenring and Mock 2012; Kettenring and Whigham 2009; Saltonstall et al. 2010), the importance of follow-up monitoring for *P. australis* seedlings following herbicide-based control efforts cannot be overemphasized and an earlier treatment that arrests seed set should be considered where the risk to non-target plant species is low.

In the greenhouse, I exposed seedbank samples to a consistent set of conditions including moist soil, full light exposure, stable temperatures, and minimal competition. This is standard practice for seed germinability assays (Ter Heerdt et al. 1999), though it is not very representative of the dynamic conditions that seeds experience in the field. The greenhouse conditions supported the germination of numerous *P. australis* seeds from my seedbank samples from both Big Creek and Crown Marsh, so it is clear that viable seeds were present in these locations. The absence of *P. australis* seedlings in the field survey of Crown Marsh thus suggests that abiotic field conditions in 2016 differed sufficiently from the conditions maintained in my greenhouse experiments, such that many of the *P. australis* seeds in the seedbank were not able to germinate. The greenhouse conditions of moist but not inundated soils, stable temperature, and full light clearly support *P. australis* germination, and I expect that if field conditions more closely resembled greenhouse conditions, e.g., if there were low water levels that exposed the soil, that *P. australis* seedling emergence would be triggered in the field too. Unfortunately, predicting springtime conditions at the time of herbicide application in late summer or early autumn is highly uncertain, so the risk of

seedling emergence cannot be factored into the timing of herbicide treatment. However, low water conditions in the year or two following herbicide treatment should trigger thorough post-treatment monitoring to determine whether *P. australis* seedlings germinate and begin recolonizing treated areas.

The unfavourable abiotic conditions in Crown Marsh and Big Creek that prevented *P. australis* seedlings from germinating could be incorporated into restoration planning. By encouraging rapid colonization of the site by desireable plant species, *P. australis* seeds can be shaded and denied the ample light and warm temperatures they require. Given the intolerance of *P. australis* seedlings of standing water, encouraging restoration of a site during naturally manipulated high water levels could add additional prevention to ensure abiotic conditions prevent the recolonization of *P. australis.* A study that examines the longevity of *P. australis* seeds following *P. australis* control would offer insight into the timeframe necessary to monitor for *P. australis* seedlings after a patch is controlled.

Disparities in abiotic conditions between the greenhouse germinability assay and field site conditions meant that my greenhouse experiment was not predictive of what would emerge following herbicide treatment of an invaded marsh. As described above, I attribute this discrepancy to differences in spatial heterogeneity of conditions in the field compared to the greenhouse and the variable resource requirements of different plant species. To estimate the actual richness of resident species in the seedbank to inform herbicide-control and restoration of *P. australis* invaded marshes, I recommend the use of a tiered germinability assay that divides seedbank samples into subsamples and exposes different subsamples to different moisture levels. This approach may more accurately capture the range of germination conditions required by the

diversity of seeds in the seedbank and therefore better predict the potential diversity of the site following *P. australis* removal (Galinato and Van Der Valk 1986; Ter Heerdt et al. 1999).

Despite invasion by *P. australis*, my study results from both Big Creek and Crown Marsh indicate that the viable seedbank can contain a diversity of species, even in areas where the *P. australis* stem density is high and the invasion is decades old. Given the heterogeneity and spatial variation in field conditions, it is difficult to draw conclusions from a germination assay regarding what might emerge from the seedbank in any specific location; however, I conclude that the seedbank can serve as an important source of colonists able to revegetate areas where *P. australis* was chemically controlled. Indeed, the seedbank is crucial to site restoration following *P. australis* control treatments, and it is imperative that control treatments and restoration activities are chosen to create favourable conditions for recolonization by resident species. Practitioners must also be on alert for reinvasion by *P. australis* seeds from the seedbank, especially in low water years where conditions may favour their germination. Those considering herbicide treatment of high density patches of *P. australis* where few resident species could be harmed might consider an earlier application period to help insure against reinvasion by reducing the number of viable *P. australis* seeds that enter the seedbank immediately prior to treatment. This recommendation would be a deviation from typical best management practices, and should be thoroughly assessed for potential non-target effects.

2.5 Figures and tables
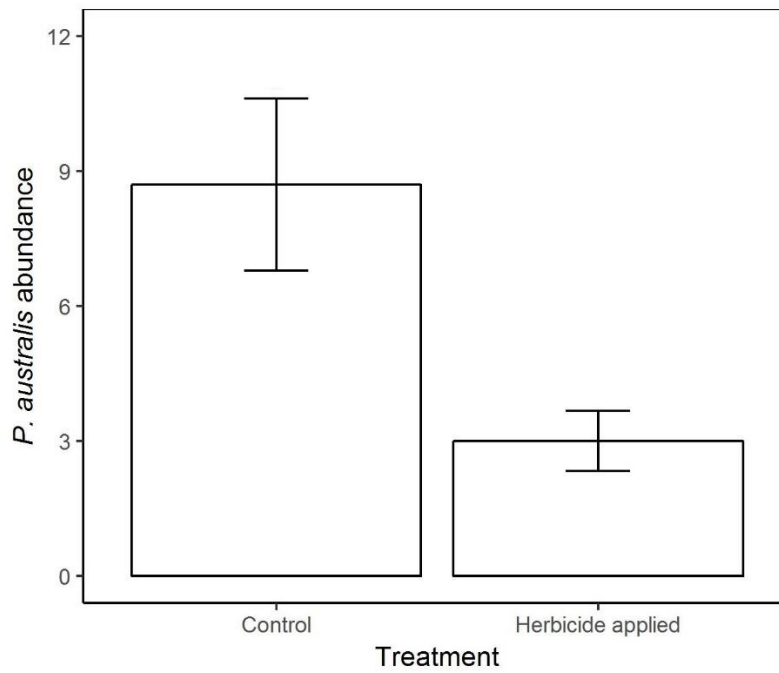
Figure 2-1. Average values of *P. australis* stem density by treatment in Big Creek, ON. Error bars depict standard error. The mean *P. australis* stem density in the control sites is significantly higher than in the herbicide-treated sites at α = 0.05.
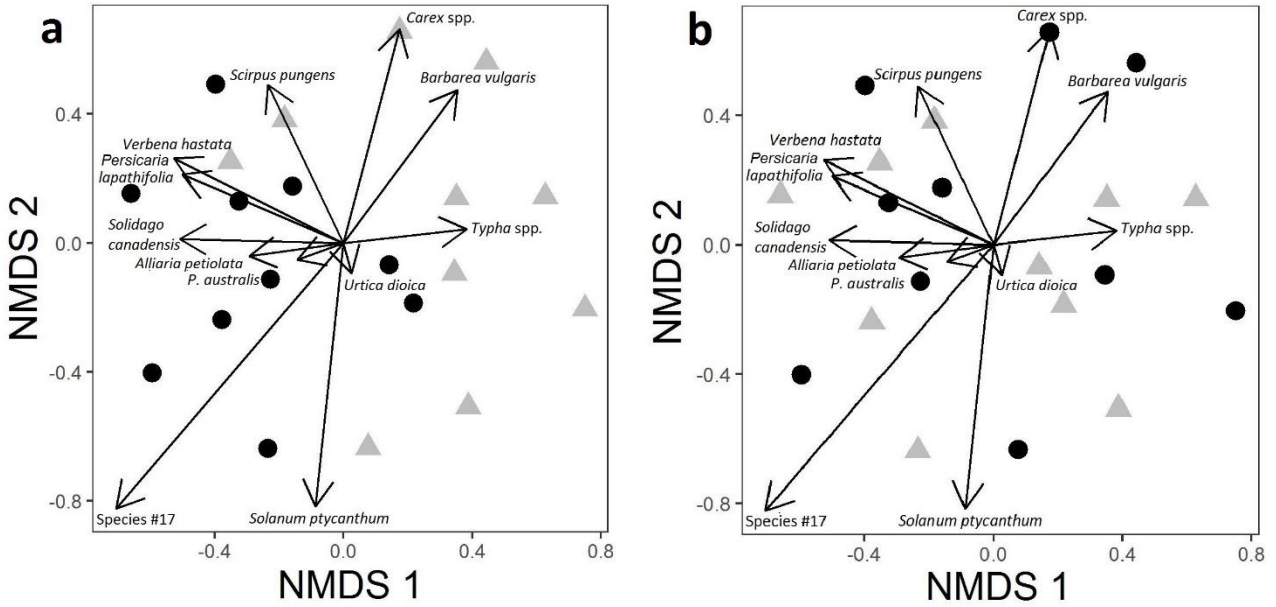
Figure 2-2. 2D NMDS ordination solution of a Bray-Curtis dissimilarity matrix using relativized and square-root transformed stem density values for species that germinated from the Big Creek seedbank samples (final stress of 0.21, Procrustes RMSE = 0.0001, maximum residuals = 0.0003) with plant species overlaid as vectors. The symbology in panel A compares the herbicide-treated (grey triangles) and control (back circles). The symbology in panel B contrasts the sites with high *P. australis* density (black circles) with the sites with low *P. australis* density (grey triangles).

Figure 2-3. 2D NMDS ordination of a Bray-Curtis dissimilarity matrix using relativized Crown Marsh species stem density values (final stress of 0.17, Procrustes RMSE = 0.00002, max residuals = 0.00004) with seedling species relative abundances overlaid as vectors. Control sites that were not sprayed (black circles) and the herbicide-treated sites (grey triangles) are displayed according to the factor of treatment.

Figure 2-4. Average plant community richness contrasting field survey results from Crown Marsh with associated seedbank samples collected from the same locations but grown in the greenhouse. Error bars depict standard error. The mean value for total richness of seedlings that emerged in the greenhouse is significantly higher than the total richness of plants surveyed in Crown Marsh at an $\alpha = 0.05$.

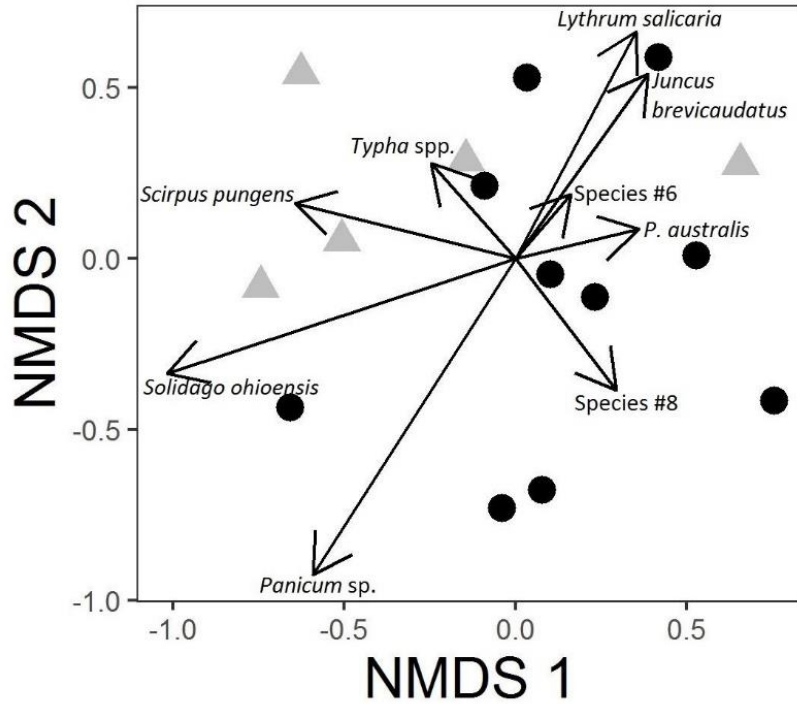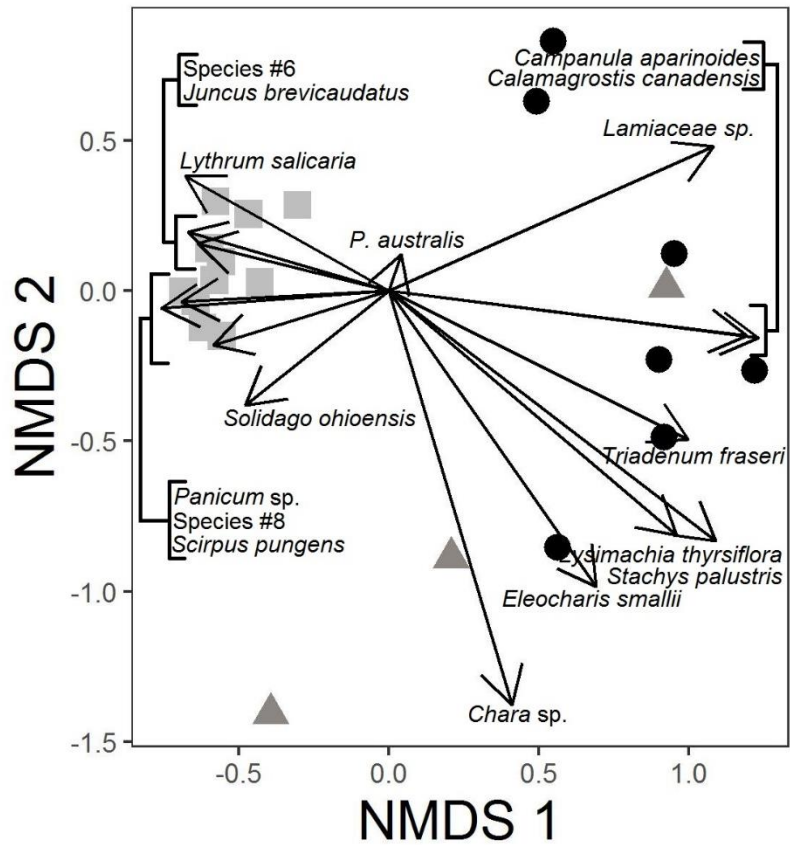*Figure 2-5.* 2D NMDS of a Bray-Curtis dissimilarity matrix using relativized Crown Marsh presence/absence values (final stress of 0.08, Procrustes RMSE = 0. 00001, max residuals = 0.00005) with plant species overlaid as vectors. The 2016 field survey of control sampling sites (black circles), herbicide-sprayed sampling sites (dark grey triangles) and the Greenhouse sites across both treatments (grey squares) are displayed per location.

Table 2-1. Results of Two-Way ANOVA (with interaction) with the effects of *P. australis* density and herbicide application in Big Creek. Bold font indicates a statistically significant difference in that factor among treatments.

Response variable	Model Df	Factor 1: *P. australis* density		Factor 2: Herbicide application		Interaction	
		F	p	F	p	F	p
Total richness	1, 16	0.500	0.490	1.235	0.283	0.827	0.377
Square-root transformed total stem density	1, 16	0.860	0.368	0.077	0.785	1.892	0.188
P. australis stem density	1, 16	0.002	0.963	7.063	**0.017**	0.002	0.963
Square-root transformed resident species stem density	1, 16	0.828	0.376	0.001	0.978	1.961	0.181

Table 2-2. Summary information from Welch's independent t-tests on Crown Marsh variables with the single factor of treatment.

Variable	Df	t	p
Total stem density	11	2.02	0.069
Total richness	5	-0.24	0.820
Resident species stem density	13	2.00	0.067
P. australis stem density	5	-0.30	0.774

Table 2-3. Summary information from a Welch's independent sample t-test tests comparing the factor of location in Crown Marsh. Bold font indicates a statistically significant difference in that factor among treatments.

Response variable	Df	t	p
Location	17	2.12	**0.049**

3.0 Burial depth to prevent regrowth of Phragmites australis

3.1 Introduction

The European lineage of Common Reed, *Phragmites australis* (Cav.) Trin. Ex Steudel (hereafter referred to as *P. australis*), has been termed Canada's worst invasive plant species by Agriculture and Agri-food Canada (Catling 2005b). This designation is due in large part to its negative impacts on wetland ecological integrity, accessibility and value for recreational users (Braun et al. 2016; Lathrop et al. 2003). *Phragmites australis* has been present in North America since the 19th century after being brought to Atlantic coastal ports of the U.S. in the ballast of ships (Saltonstall 2002), but the first confirmed example of the European lineage was in Nova Scotia in 1910 (Catling and Mitrow 2011). This perennial grass can reproduce effectively by seed (Belzile et al. 2010; Kettenring and Whigham 2009) but also vegetatively using stolons and rhizomes, and can create new shoots if living biomass falls on moist soils (Kettenring et al. 2016). The ability of *P. australis* to grow new shoots from plant fragments makes the disposal of plant biomass a major concern when remediating invaded areas. When work is undertaken where *P. australis* is present, all equipment and vehicles that come into contact with *P. australis* need to be cleaned thoroughly to avoid transferring plant fragments and seeds to new territory, especially along highways (Brisson et al. 2010; Halloran et al. 2013). How best to dispose of these potential propagules remains an important question for natural resource managers confronted by *P. australis*.

Public and private organizations in North America have published protocols for managing *P. australis*, as well as equipment cleaning and recommendations for final disposal of *P. australis* biomass (Halloran et al. 2013). As the reuse of soil containing *P. australis* propagules will only serve as a spread vector (Keller 2000), these materials must first be composted at very high temperatures (California Invasive Plant Council 2012; Ontario *Phragmites* Working Group 2015)

or dried in the sun or buried (California Invasive Plant Council 2012; New Hampshire Department of Transportation 2008). Some authorities advise against composting as it may not guarantee 100% destruction of viable seeds (Michigan Department of Environmental Quality 2007; Ontario Ministry of Natural Resources and Forestry 2011) and to do so responsibly requires special equipment to achieve appropriate temperatures. To sufficiently dry propagules and plant biomass, they must be placed on unshaded asphalt and covered with a tarp for one month (New Hampshire Department of Transportation 2008). Regardless of space and time constraints, desiccation and composting presents the possibility of propagule spread both during transportation to the appropriate site and during the desiccation or composting process (California Invasive Plant Council 2012). Desiccation requires monitoring until plant materials are no longer viable propagules (New Hampshire Department of Transportation 2008). The last option, burial, does not require special composting equipment or large unshaded areas for desiccation; however, burial does present the danger that vegetative propagules may sprout if not buried deeply enough. Guidelines published by numerous government and not for profit organizations in the United States (California Invasive Plant Council 2012; New Hampshire Department of Transportation 2008; New York State Department of Transportation 2004), which typically recommend 0.91 m as an adequate burial depth, are likely successful in preventing the spread of *P. australis*. In agreement with U.S. sources, the Ontario *Phragmites* Working Group (OPWG) also recommends burying *P. australis* biomass with at least 0.91 m of clean fill (Ontario *Phragmites* Working Group 2015). Furthermore, *P. australis* is known to grow at soil depths over 1 m (Haslam 1971; Moore et al. 2012), raising concerns that 0.91 m may not be deep enough to prevent its spread. It does not appear that the appropriate capping depth for *P. australis* has been tested in the field, necessitating a field trial of this disposal method.

In Long Point, Ontario where waterfowl habitat creation was undertaken via excavation to create open water patches (Schummer et al. 2012), it was observed that the sand spoils in some areas were sufficient to impede the regrowth of buried *P. australis*, but in other areas regrowth was rapid and vigorous. I aimed to empirically determine the burial depth necessary to prevent regrowth and re-establishment of *P. australis* to support the work of municipalities, natural resource managers, and transportation authorities tasked with managing *P. australis* within their jurisdictions.

3.2 Materials and methods

3.2.1 Field site establishment

Plastic waste bins were used to construct ten mesocosms ranging in height. These were deployed along the sand berm created when a pond was recently excavated by the Ontario Ministry of Natural Resources and Forestry in the Crown Marsh Waterfowl Management Unit. This site was chosen because clean sand could be used as fill, and adjacent to the sand spoil was a large patch of *P. australis* which served as a source of rhizomes. A combination of peat and *P. australis* rhizomes was harvested from this patch in August of 2015. *Phragmites australis* rhizomes ranging from 15 to 30 cm in length were prepared sorted and mixed with the harvested peat. Rhizomes of this size were chosen to mimic the process of excavating an area invaded by *P. australis* and because prior research has shown that this is the minimum size required for shoot production (Haslam 1969a). Rhizome survival in *P. australis* is known to be related to biomass, so it was important that this aspect be standardized among treatments (Juneau and Tarasoff 2013). A 20 cm deep layer of this homogenous peat and *P. australis* rhizome mix was placed at the bottom of each

mesocosm. Peat was included along with rhizomes to mimic the situation where a wetland manager would have excavated material containing *P. australis* biomass that requires disposal. Sand was taken from the exposed berm and screened to remove any propagules or debris and then added to the mesocosms, burying the peat-rhizome mix at a depth of either 0, 10, 20, 30, 50, 70, 90, 110, 130, or 150 cm. These mesocosms were then buried in the sand berm in a random arrangement, such that their tops were at a common elevation. I ensured that no mesocosms were buried to a depth below the water table so that inundation was not a confounding factor. Piezometers constructed from 3.81 cm diameter ABS pipe were installed to monitor the ground water level adjacent to each mesocosm to confirm that none were submerged (Appendix I).

3.2.2 Field site sampling

The mesocosms over wintered in place, then during the summer of 2016 field surveying of environmental and covariate data was undertaken. Environmental data was collected six times between May and July 2016 to study the response of the plant propagules to the sand cap treatment: stem counts of all species present, percent cover of all species present, and average canopy height of plants present for each mesocosm. During the same six site visits, the following covariates were monitored to assess whether edaphic conditions within the mesocosms diverged during the study: soil electrical conductivity (WET-2 Sensor, Delta-T), soil temperature (WET-2 Sensor, Delta-T), and soil moisture content (WET-2 Sensor, Delta-T). I measured light penetration as the percentage of incident photosynthetically active radiation (PAR) reaching the soil surface, measured with a (LI-1500 Light Sensor Logger, LI-COR). These measurements were taken three times between May and July on sunny days as close to noon as possible to maximize comparability among

measurements. Readings for light penetration were taken at the top of the plant canopy and soil level simultaneously.

3.2.3 Statistical analysis

Statistical analysis was completed to test the hypothesis that a strong, negative relationship existed between sand cap depth and the density and canopy height of *P. australis* in the mesocosms and to discover if a significant threshold existed that might serve as an optimal sand cap depth. The following data required square root transformation to meet parametric requirements: *P. australis* stem count, average canopy height, total richness, and soil moisture. The following data did not require square root transformation to meet parametric requirements: *P. australis* percent cover, percent light penetration, soil electrical conductivity and soil temperature. The same analysis was used to determine if a significant relationship existed between covariate data and sand cap depth.

The following environmental covariates were averaged across the six sampling events: soil electrical conductivity, soil moisture, and soil temperature. The mean readings of this covariate data were calculated to gain insight into the average growing conditions throughout the 2016 field season. To calculate percent light penetration, raw data was taken from the last sampling event as that point in time represented as close to peak biomass as possible. The percentage of light penetration was calculated by dividing the soil level readings by the paired canopy level readings and multiplying by 100.

During analysis of environmental data, the 0 cm sand cap control was excluded from analysis because no *P. australis* grew in this exposed treatment, suggesting it was more a test of

the efficacy of desiccation than a control for a test of burial depth. The 0 cm control was included in all covariate analyses to examine if the absence of a sand cap resulted in a difference of growing conditions or other covariate parameters during the sampling period. To determine if mesocosm depth impacted environmental and covariate parameters, I used R Studio to perform simple linear regressions. The packages used in R Studio (RStudio Team 2015) were: "ggplot2" (Wickham 2009), "Rcpp" (Eddelbuettel and Francois 2011), "e1071" (Meyer et al. 2015).

3.3 Results

3.3.1 Stem counts

After excluding the 0 cm cap, sand cap depth was a significant predictor of *P. australis* stem count in July, ($F_{1,7} = 6.26$, $p = 0.041$), with an R^2 value of 0.397 (Fig. 3-1). The relationship between *P. australis* stem count and mesocosm capping depth was best described by the equation

$$\sqrt{\text{Stem count (m}^2)} = -0.02(\text{Sand cap depth cm}) + 2.31$$

Phragmites australis growth from tissue fragments was observed in mesocosms with sand cap depths of less than 50 cm, except for the 30 cm sand cap mesocosm, where no *P. australis* emerged (Fig. 3-1). In mesocosms with sand caps of 70 cm or deeper, no *P. australis* regrowth was observed.

Additional resident plant species also grew in mesocosms with 0 – 50 cm deep sand caps (Table 3-3). Those that could be identified were species capable of vegetative reproduction in sympatry with *P. australis* and were also observed growing in the same location where *P. australis*

rhizomes were harvested for the experiment. No resident species growth was observed where sand cap depths were >70 cm (Table 3-3).

3.3.2 Canopy height

Canopy height varied significantly with sand cap depth ($F_{1,7}$ = 7.06, p = 0.033, R^2 = 0.431) (Fig. 3-2). This was best explained by the equation

$$\sqrt{\text{Canopy height (cm)}} = -0.04(\text{Sand cap depth cm}) + 4.93$$

Although no *P. australis* grew in the exposed peat-rhizome mix (0 cm sand cap mesocosm), other plant species were able to grow, yielding a canopy shorter than I observed in the *P. australis* dominated growth from the 20 cm deep cap treatment, but on par with what I observed in the 10 cm deep cap treatment (Fig. 3-2).

3.3.3 Total richness

Total richness was seen to vary significantly with sand cap depth ($F_{1,7}$ = 7.084, p = 0.032, R^2 =0.432). This was best explained by the equation

$$\sqrt{\text{Total richness}} = -0.01(\text{Sand cap depth cm}) + 1.098$$

3.3.4 Covariate data

Covariate data collected from each station indicated that the environmental conditions were similar among the ten mesocosms (Table 3-2), however soil moisture was found to be significant: $F_{1,8}$ =

7.16, $p = 0.033$, $R^2 = 0.406$. The relationship between sand cap depth and soil moisture was best explained by the model

$$\sqrt{\text{Soil Moisture (\%)}} = -0.01(\text{Sand cap depth cm}) + 3.98$$

Soil moisture values were on average higher in the 0 cm sand cap mesocosm than the other nine (Table 3-2). Depth to the water table, monitored in wells installed beside each mesocosm, was greater than the depth of any buried *P. australis* rhizomes, and thus none of the mesocosms had their *P. australis* tissue submerged. Soil temperature, soil electrical conductivity and *P. australis* percent cover were not significantly impacted by sand cap depth (Table 3-1).

3.4 Discussion

Although *P. australis* did not emerge beyond the 70 cm treatment, I recommend a minimum capping depth of 100 cm (1 m) to prevent harvested *P. australis* rhizomes from colonizing the disposal area. In risk management, it is important to incorporate a margin of safety to protect against variation in environmental and climate conditions and to ensure that shoots from rhizomes with large energy reserves cannot breach the burial depth.

The lack of *P. australis* emergence from the mesocosm with no sand cap (0 cm deep) I attribute to its exposure. The exposed peat-rhizome mix in this mesocosm was not protected from freezing through the winter between mesocosm placement and sampling, which may have resulted in mortality. Although horizontal rhizomes are resistant to frost, *P. australis* seedlings and vertical buds are sensitive to freezing (Haslam 1971; Thompson and Shay 1985). It is possible that the

shallowly buried treatments, including the 30 cm deep cap treatment, may also have experienced some mortality due to freezing.

Further, the propagules in the unburied peat-rhizome mix were likely exposed to warmer temperatures and desiccation because of their exposure during the summer and the albedo differences between white sand and dark peat. Indeed, spreading out harvested propagules to dry is one of the recommended disposal practices I encountered in my review of published best management practices (New Hampshire Department of Transportation 2008). Certainly *P. australis* performance is affected by the availability of moisture and the water table depth (Haslam 1971). On average, the temperature in the 0 cm treatment was only negligibly higher than in the surface soils of the other mesocosms (Table 3-2) and moisture content at the soil surface was higher (about 27% on average compared with 8 to 13% in the other mesocoms); however, this data could be misleading as it represents conditions at the surface, not the conditions in the peat-rhizome mix which was buried in all the other treatments. It is likely that the moisture content of buried peat-rhizome mix was higher than the sand it was buried in.

I used coarse sand for my burial experiment as it is the common substrate in the 35 km Long Point sand spit. Sand is a highly permeable material, with high hydraulic conductivity that is unlikely to retain much moisture (Domenico and Schwartz 1990). Burial in sand may reduce regrowth if it limits the availability of water, as young *P. australis* grows best in moist soils (Haslam 1971). Inversely, I observed *P. australis* plants growing from seeds on the pure sand surface of one mesocosm (Table 3-3), showing that viable seeds of this invasive plant may be highly adaptable. Future research should explore the effect of the overburden soil texture on capping depth thresholds, as finer textured soils may require burial in deeper soils than coarse

sand, simply because of the added stress of desiccation that sand capping exposes plant fragments to.

The lack of *P. australis* emergence in the 30 cm deep treatment is less easily explained. In trials examining the success of *P. australis* fragment survival under varying conditions, Bart and Hartman (2003) found that small rhizome fragments (2 g in weight) were less successful in establishing new clonal stems than large (>4 g) rhizomes. Similarly, Juneau and Tarasoff (2013) found that rhizome survival was size-dependent. I selected rhizome fragments in the 15-30 cm length range not only to mimic excavation practices, but also to ensure that rhizomes had the nodes necessary for sprouting shoots. Due to my regression design, I lack replication of the treatments. It is therefore possible that despite my efforts to homogenize the peat-rhizome mix and to standardize rhizome size, the 0 and 30 cm sand cap depth treatments may not have had as large or as many viable *P. australis* propagules as the other mesocosms.

Because the different mesocosms were all buried in a sand berm to differing depths, we were concerned that some treatments could position the *P. australis* rhizomes beneath the water table, and thus yield a difference in regrowth related more to the inundation of rhizomes than the depth of sand cap. In my experiment, all *P. australis* biomass remained above the water table, so flooding was not a confounding variable in my design. In practice, I expect that burial of *P. australis* rhizomes beneath the water table would reduce the regrowth risk, as associated anoxic conditions are known to harm *P. australis* rhizomes by cutting off air and depleting energy supplies (Greet and Rees 2015; Hellings and Gallagher 1992; Ostendorp 1991). Future research could examine whether inundation alters the depth of sand cap necessary to prevent *P. australis* regrowth.

Finally, this study did not use differing tissue types or sizes, and selected only rhizomes between 15 and 30 cm in length. I judged that rhizomes (with their energy stores) would be the more capable of growing through different burial depths than stem or stolon material, thus representing the greatest test of the burial method. Furthermore, the use of 15-30 cm rhizomes was intended to simulate the fragmented rhizomes created during an excavation project that was ongoing in Crown Marsh. Future research should explore the effect of burial using other *P. australis* tissue types and sizes in order to further explore the utility of burial as a disposal method for *P. australis* biomass.

3.5 Conclusions and management implications

My findings provide empirical support for existing recommendations of a 0.91 m burial depth recommended by the New Hampshire Department of Transportation, The California Invasive Plant Council, the New York State Department of Transportation, and the Ontario *Phragmites* Working Group for the safe disposal of *P. australis* biomass. Due to the invasive potential of *P. australis*, I recommend land managers utilize a 1 m standard when burying *P. australis* biomass as a means of disposal only, as the use of burial as a primary *P. australis* control method would not be sensible. The use of the burial method will necessitate the use of heavy machinery, and thus will require following a clean equipment protocol that ensures that *P. australis* propagules are not spread between sites. Replication of my study in additional years, under flooded conditions and using different capping soil textures would ensure that this guideline is broadly applicable. Additional risk management steps could include chipping the plant biomass into fragments less than 2 g in weight, although my results suggest this is not necessary providing the capping depth is adequate.

Adoption of *P. australis* burial guidelines by Canadian and Great Lakes stewards and managers would add the growing body of resources related to the management and control of *P. australis* and benefit managers by recommending disposal options related to common place excavation projects.
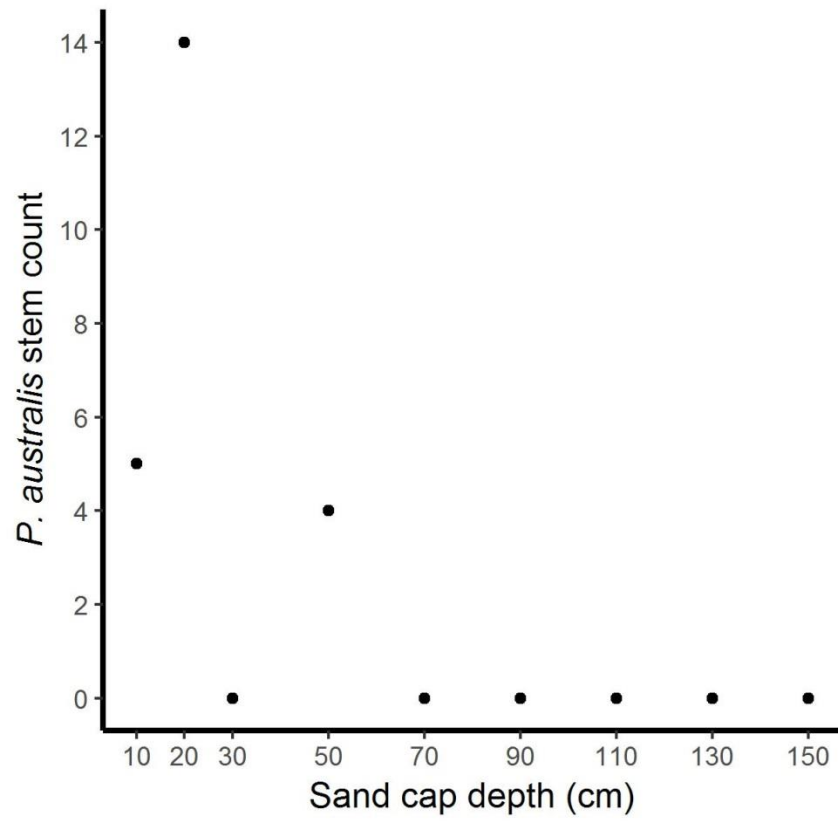
3.6 Figures and tables

Figure 3-1: *P. australis* stem counts across sand cap depth treatments.
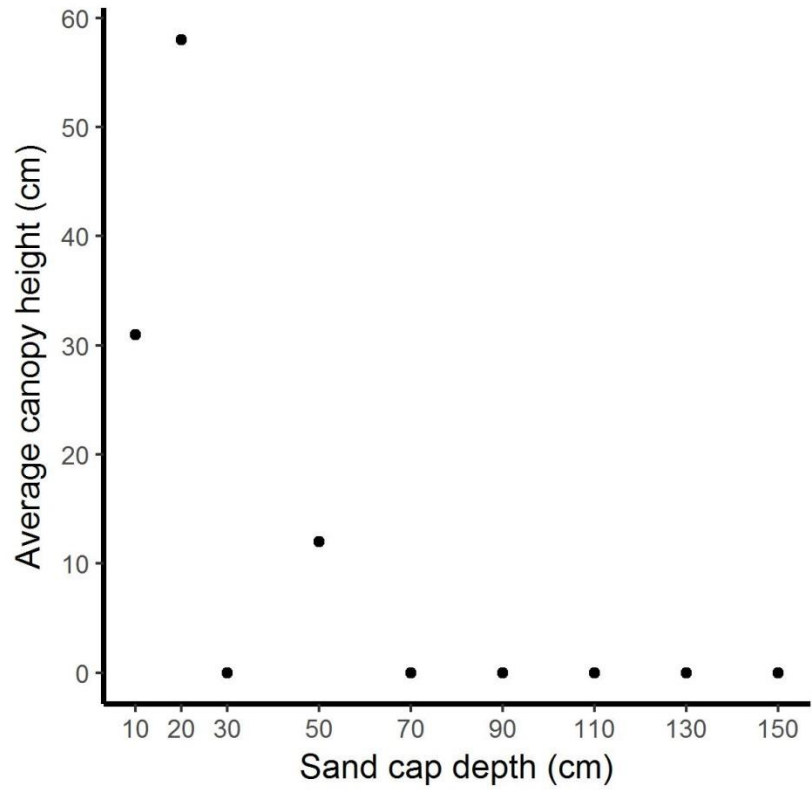
Figure 3-2: Average canopy height of plants across sand cap depth treatments, including *P. australis* and resident species.

Table 3-1: Results of simple linear regressions on environmental and covariate data. Environmental data was analyzed with the 0 cm sand cap control removed, while the covariate data was analyzed with the 0 cm sand cap control included. Bold font indicates a statistically significant difference in that factor among treatments.

Variable	Adj. R square	DF	F	p
Square-root transformed *P. australis* stem count	0.397	1, 7	6.262	**0.041**
P. australis percent cover	0.305	1, 7	4.503	0.072
Square-root transformed average canopy	0.431	1, 7	7.058	**0.039**
Square-root transformed total richness	0.432	1, 7	7.084	**0.032**
Percent light penetration	0.038	1, 8	1.356	0.278
Soil electrical conductivity	0.047	1, 8	1.447	0.263
Square-root transformed soil moisture	0.406	1, 8	7.160	**0.033**
Soil temperature	0.094	1, 8	1.938	0.201

Table 3-2: Raw covariate data collected between May and July 2016. The last sampling date in July was used to determine the percent of light penetration. Mean and standard error (SE) values of soil electrical conductivity, soil moisture, and soil temperature were calculated using six sampling dates throughout 2016.

Sand cap depth (cm)	Light Penetration (at last survey date) Intensity (%)	Soil electrical conductivity (N=6) (mS/c)	+/- SE	Soil moisture (N=6) Moisture (%)	+/- SE	Soil temperature (N=6) Temp. (˚C)	+/- SE
0.00	8.55	0.12	0.03	27.62	0.65	20.97	2.13
10.00	89.17	0.00	0.00	12.03	0.80	20.47	2.07
20.00	72.58	0.00	0.00	10.32	0.68	20.55	2.20
30.00	89.19	0.00	0.00	13.32	1.53	20.53	2.18
50.00	84.88	0.00	0.00	11.88	0.84	20.45	2.15
70.00	86.03	0.00	0.00	8.03	1.23	20.43	2.29
90.00	91.68	0.01	0.01	8.27	0.84	20.18	2.18
110.00	61.62	0.00	0.00	7.67	1.04	20.15	2.35
130.00	86.50	0.00	0.00	8.12	1.00	20.52	2.17
150.00	86	0.00	0.00	8.78	1.01	20.58	2.31

Table 3-3: List of plant species observed in the sand mesocosm study. Common and scientific names of species surveyed in the mesocosms during 2016 are shown here. Species which likely arose from rhizomes in the harvested peat samples are marked with an asterisk. It is also noted that "Sedge sp." is likely a rhizomatous species, however this could not be confirmed through species identification. Burial depth indicates the depth of sand overburden that the species successfully grew through. Note that *P. australis* seedlings were observed on the surface of the mesocosm with a 20 cm sand cap, however a lack of connective tissue indicated that these seedlings did not arise from buried peat. Identification of plants at both the sampling sites and greenhouse was done using wetland field guides.

Common Name	Species	Capable of Vegetative Reproduction (*)	Burial depth (cm)
Sedge sp.	Unknown sedge	Likely	0, 10
Common Reed	*P. australis*	*	10, 20, 50
Common Reed	*P. australis* seedlings	*	20
Common threesquare	*Scirpus pungens*	*	0, 10

4.0 Conclusions and policy implications

4.1 General summary

For decades, the invasive European lineage of *P. australis* has threatened North American wetlands by outcompeting resident plant species and creating large monotypic stands (Ailstock et al. 2001; Minchinton and Bertness 2003). The biology of *P. australis* and the ecological impacts of its invasion are well studied; e.g., see special issues in Biological Invasions (2016) and AoB Plants (2014). Despite this, relatively little has been published that validates existing guidance on *P. australis* management. My aim in this thesis was to inform the best management practices surrounding the control and disposal of *P. australis*. In Chapter 1, I described the biology of *P. australis,* the subsequent impacts of invasion, and explained the uncertainty regarding the use of glyphosate herbicide to control *P. australis*. This chapter also outlines the benefits and drawbacks of several disposal options for harvested *P. australis* biomass, thus providing the background for my 2nd and 3rd chapters.

A 2009 survey of U.S. land managers found that 94% of respondents used herbicide to control *P. australis* (Martin and Blossey 2013), and a recent review of U.S. *P. australis* management found that herbicides containing glyphosate are more commonly used than any other variety (Hazelton et al. 2014). The common application of glyphosate is predicated in part on the assumption that it should have no effect on exposed seeds (Franz et al. 1997). However, some research has suggested that formulations of glyphosate herbicide with added surfactants may impact seed viability (Gomes et al. 2017). The sensitivity of the seedbank to glyphosate treatment as part of *P. australis* control therefore requires direct study. Given the prevalence of glyphosate use to control *P. australis* in North America, the possibility of glyphosate inhibiting resident species seeds would be a major consideration for the future use of this herbicide. To address

knowledge gaps related to the potential effect of glyphosate on the seedbank, in Chapter 2 I tested if the glyphosate herbicide formulation sold under the commercial name WeatherMAX® impacted the germination of seeds and sought to determine if glyphosate exposure on the seedbank might interact with the *P. australis* stem density. I hypothesized that the use of a glyphosate herbicide would not have a measurable effect on the number of resident species or abundance of individual seeds that germinate; however, I expected to observe fewer seedlings where *P. australis* was growing in high densities compared to low densities because of its ability to intercept seeds and prevent them reaching the seedbank.

I found that the use of herbicide did not significantly affect the number of seedlings or richness of resident species seedlings that emerged; however, at one location I did find significantly higher numbers of *P. australis* seedlings grew from the seedbank in my control plots that were not exposed to the herbicide. This significant result is likely due to the application of herbicide immediately prior to seed set in July, while plants not treated with herbicide reached seed set before I sampled the seedbank. Despite the physical barrier created by dense thickets of living shoots and standing *P. australis* litter, I did not see a significant difference in the number or richness of germinated seeds between low and high densities of *P. australis*. Finally, the composition of species in the seedbank varied greatly between samples, thus illustrating that large spatial heterogeneity in the seedbank composition exists, even at local (within 10 m) scales.

I have thus shown that the use of a glyphosate-based herbicide to control *P. australis* did not significantly affect the germination of resident species seeds in wetland seedbanks in treated areas of coastal marsh in the Long Point peninsula on Lake Erie. I attribute this to the tendency of glyphosate to tightly bind to soil particles and quickly be degraded by soil microorganisms (Dill et al. 2010; Franz et al. 1997). Furthermore, the herbicide was applied directly onto the canopy of

P. australis leaves and thus much of this chemical was likely intercepted by both living or standing dead biomass, and little actually reached the soil directly Therefore, in field conditions similar to this study, glyphosate will likely not affect the germination potential of dormant seeds.

The success of *P. australis* as an invasive species can largely be attributed to its ability to propagate vegetatively from tissue fragments (Bart and Hartman 2003; Haslam 1969a; b) as well as sexually through wind-dispersed seeds (Kettenring et al. 2016). Given the reproductive ability of *P. australis*, routine excavation work in infested areas or the mechanical control of living *P. australis* tissues requires careful BMPs for disposal. Recommended options for the safe disposal of *P. australis* tissues include composting, drying, or burying (California Invasive Plant Council 2012), however, composting has been cautioned against because the process may not destroy viable seeds (Michigan Department of Environmental Quality 2007; Ontario Ministry of Natural Resources and Forestry 2011), and drying may require transport and subsequent monitoring for up to one month (California Invasive Plant Council 2012; New Hampshire Department of Transportation 2008). The burial of *P. australis* tissues below 0.91 m of clean fill has been recommended by several North American agencies (California Invasive Plant Council 2012; New York State Department of Transportation 2004; New Hampshire Department of Transportation 2008; Ontario *Phragmites* Working Group 2015); however, no evidence could be found that justified the recommended burial depth. Given the potential of burial as a disposal technique, in Chapter 3 I investigated if burial is a reliable tissue disposal method across a range of burial depths using locally sourced sand fill. I expected that a negative relationship would exist between sand cap depth and the number of *P. australis* shoots that emerged, and anticipated that *P. australis* growth would cease at an overburden depth of roughly 1 m. I found that no *P. australis* shoots emerged from my capping treatments where the sand cap was 0.70 m or more. Thus, I concluded

that a safe recommendation for the disposal of living biomass would be burial under at least 1 m of clean fill that is free of *P. australis* seeds or vegetative propagules.

4.2 Implications and applications

4.2.1 The impact of glyphosate herbicide and Phragmites australis *density on the seedbank*

Because *P. australis* has a longer growing season and senesces later than most resident marsh vegetation (Farnsworth and Meyerson 2003), it is commonly recommended that glyphosate application take place in the fall (Hazelton et al. 2014; Ontario *Phragmites* Working Group 2015). This should protect non-target species, but still effectively control *P. australis*. Further, fall applications are justified by arguments that this is a time when *P. australis* is actively translocating resources down into the rhizomes for winter storage (Ontario Ministry of Natural Resources and Forestry 2011), and thus any herbicide applied should also be actively moved to the rhizosphere, where it should do the most damage to the invasive species (Duke and Powles 2008). However, the recommended practice of spraying glyphosate in the fall likely results in seeds reaching maturity before the plants are killed. At this point, each flowering stem may have already contributed 500-2,000 seeds to the local seedbank (Wijte and Gallagher 1996) and potentially have spread the invasion further by means of long distance transport (McCormick et al. 2016). Spraying earlier in the summer might also damage non-target species that have not yet senesced, but with the benefit of reducing the need for follow-up treatments by decreasing the amount of *P. australis* seeds entering the seedbank. I therefore recommend that, if non-target plants and interference with the habitat of breeding fauna can be avoided, managers consider treatment prior to seed set or at the onset of flowering to prevent the annual production *P. australis* seeds.

When conducting a restoration project, managers must plan for multiple years of monitoring. It is unclear how long *P. australis* seeds can remain viable in the seedbank (Baldwin et al. 2010); however, the removal of an established *P. australis* stand may create the conditions necessary for *P. australis* seedling germination and reestablishment in a remediated site. Given the demonstrated viability of *P. australis* seeds, follow-up monitoring in the years after control treatments have been applied is essential for early detection and eradication of remnant or recolonizing patches. Therefore, I recommend that managers involved in both wetland restoration and roadside management allot time and funds in the years following *P. australis* control for monitoring and spot treatment of *P. australis* seedlings. During this monitoring process, I would encourage managers to refer to Brisson et al. (2008) and Haslam (1971) for detailed information on the identification of *P. australis* seedlings, as these have different morphology than the rametes produced asexually by established rhizomes

Invasion by *P. australis* does not totally eliminate resident species from the seedbank, even at stem densities >60 live stems per m^2. This similarity between low and high density *P. australis* stands indicates that although the biomass of *P. australis* often excludes and out-competes resident plant species, a long lived and diverse seedbank can remain. Despite this, the presence of *P. australis* biomass will likely act as a competitive barrier to prevent ideal conditions for the germination and growth of resident species in the seedbank, therefore the mechanical removal of standing dead litter is just as important in the recovery of resident vegetation as the use of herbicide to kill living *P. australis* if the goal is to restore a wetland's vegetation community. I therefore recommend that any herbicide treatment of *P. australis* be combined with mowing or burning to clear the area for the germination and colonization by desirable species.

Little agreement was observed between what germinated in the greenhouse and what grew at the field sites where the seedbank samples were harvested from. This disparity shows that greenhouse trials may not serve to predict the future vegetation community in wetlands subject to annually variable water depths. Furthermore, large heterogeneity was observed within the seedbank of a small geographic area, thus extrapolation of these results to larger scales may be difficult. This heterogeneity reinforces the fact that a diverse seedbank is present to fill the resulting vacuum left after *P. australis* control, regardless of glyphosate application or the previous density of *P. australis*.

A germinability assay like I report on in Chapter 2 should be conducted to determine if other herbicide formulations used to control *P. australis* might affect the seedbank. Evidence that imazapyr herbicides may inhibit site recolonization and seed germination can be found in the literature (Mozdzer et al. 2008), thus I recommend further study into the impact of imazapyr and other herbicides on the seedbank and site recovery following application for *P. australis* control. Although this was not the focus of my study, additive surfactants may increase the overall toxicity of a herbicide, and the common Roundup surfactant POEA may reduce seed germination under the right conditions (Gomes et al. 2017). Therefore, any future studies that examine the impact of herbicide use on the seedbank should control for or consider the influence of additive surfactants.

4.2.2 Burial as a method to dispose of Phragmites australis *tissues*

Burial presents a simple, safe, and cost-effective disposal method that can avoid many logistical issues associated with competing options, such as composting and desiccation. Although 0.70 m was found to be the minimum depth to prevent regrowth, the invasive potential of *P.*

australis warrants a safety margin built into the recommended depth. To allow for a margin of error and variability, rounding to 1 m will essentially match the recommendation found in many BMP protocols and add an additional 0.30 m as a margin of safety. Thus, I recommend that managers seeking to bury *P. australis* biomass use a minimum burial depth of 1 m and adjust their BMPs accordingly. It must be stressed that that burial is only effective as a means to dispose of harvested *P. australis* biomass, and not as a means of *P. australis* control.

This research found that 0.70 m of sand prevented the growth of *P. australis*, but did not explore other capping materials or the influence of different moisture levels. Sand dredged from a deep source provided an excellent clean fill for this study; however, on-site soil material will likely differ drastically between project sites. Furthermore, flooding has been shown to decrease the survival of perennial rhizomes (Greet and Rees 2015). Future research related to the burial of *P. australis* tissues should therefore examine the impact of different overburden materials and flooded conditions on the regrowth of buried *P. australis* tissues.

My mesocosm study did not attempt to tease apart any effect of tissue fragment size or the relative survival of aboveground vs. belowground tissue types. The buried tissues were nearly all belowground in origin and were broken up into 0.15 to 0.30 m lengths and mixed before being distributed among mesocosms because this best mimicked the way that harvested tissues were being buried in at my study site. However, I recognize that larger fragments of tissue would possess greater energy stores may have a greater ability to produce shoots than the rhizome fragments used in this research. Future research into the efficacy of different burial depths on aboveground tissues (such as stem materials) would be beneficial to refine the burial BMPs further.

4.3 Conclusion

My research demonstrated that the fall application of glyphosate herbicide did not affect the richness or abundance of seeds germinating from wetland seedbanks, and neither did the stem density of *P. australis* from whence the seedbank was sampled. However, I found that the application of glyphosate between the period of flowering and seed set in *P. australis* (mid July in my study region) can reduce the amount of *P. australis* seed entering the seedbank; an action that may reduce the need for follow-up treatment of emerging *P. australis* seedlings in previously treated areas. In addition, I confirmed that burial of *P. australis* tissues in 1 m of sand fill can be an effective method for on-site disposal. These findings have expanded our understanding of the most effective means for reducing the spread of *P. australis* and restoring native plant communities. More work remains to be done, however. Although my research provided insight into the impact of glyphosate on the seedbank, a similar investigation would be useful if other herbicides (such as imazapyr) are used for *P. australis* control in the future. Finally, replication of my sand mesocosm study with *P. australis* shoot material, variable tissue fragment size fractions, different particle size fractions of fill and the incorporation of tissue flooding as additional experimental factors would provide valuable insight into the robustness of the burial disposal method across a variety of circumstances.

References

Ailstock MS, Norman CM, Bushmann PJ (2001) Common Reed *Phragmites australis*: control and effects upon biodiversity in freshwater nontidal wetlands. Restor Ecol 9:49–59.

Albert A, Brisson J, Belzile F, et al (2015) Strategies for a successful plant invasion: the reproduction of *Phragmites australis* in north-eastern North America. J Ecol 103:1529–1537.

Baldwin AH, Kettenring KM, Whigham DF (2010) Seed banks of *Phragmites australis*-dominated brackish wetlands: relationships to seed viability, inundation, and land cover. Aquat Bot 93:163–169.

Bart D, Hartman JM (2003) The role of large rhizome dispersal and low salinity windows in the establishment of Common Reed, *Phragmites australis*, in salt marshes: new links to human activities. Estuaries 26:436–443.

BASF Canada Inc. (2017) ARSENAL® PowerLine herbicide. BASF Canada Inc, Mississauga, ON

Belzile F, Labbé J, LeBlanc M-C, Lavoie C (2010) Seeds contribute strongly to the spread of the invasive genotype of the Common Reed (*Phragmites australis*). Biol Invasions 12:2243–2250.

Benbrook CM (2016) Trends in glyphosate herbicide use in the United States and globally. Environ Sci Eur 28:3.

Benoit LK, Askins RA (1999) Impact of the spread of *Phragmites* on the distribution of birds in Connecticut tidal marshes. Wetlands 19:194–208.

Bhattarai GP, Allen WJ, Cronin JT, et al (2016) Response to Blossey and Casagrande: ecological and evolutionary processes make host specificity at the subspecies level exceedingly unlikely. Biol Invasions 18:2757–2758.

Bhattarai GP, Meyerson LA, Cronin JT (2017) Geographic variation in apparent competition between native and invasive *Phragmites australis*. Ecol Soc Am n/a–n/a.

Blackburn LG, Boutin Cé (2003) Subtle effects of herbicide use in the context of genetically modified crops: a case study with glyphosate (Roundup®). Ecotoxicology 12:271–285.

Blossey B (1999) Before, during and after: the need for long-term monitoring in invasive plant species management. Biol Invasions 1:301–311.

Blossey B, Casagrande RA (2016) Biological control of invasive *Phragmites* may safeguard native *Phragmites* and increase wetland conservation values. Biol Invasions 18:2753–2755.

Blossey B, Schroeder D, Hight SD, Malecki RA (1994) Host specificity and environmental impact of the weevil *Hylobius transversovittatus*, a biological control agent of Purple Loosestrife (*Lythrum salicaria*). Weed Sci 42:128–133.

Bolton RM, Brooks RJ (2010) Impact of the seasonal invasion of *Phragmites australis* (Common Reed) on turtle reproductive success. Chelonian Conserv Biol 9:238–243.

Bonanno G, Giudice R Lo (2010) Heavy metal bioaccumulation by the organs of *Phragmites australis* (Common Reed) and their potential use as contamination indicators. Ecol Indic 10:639–645.

Braun HA, Kowalski KP, Hollins K (2016) Applying the collective impact approach to address non-native species: a case study of the Great Lakes *Phragmites* Collaborative. Biol Invasions 18:2729–2738.

Breen DB, Bailey SD, Violi HA (2014) Managing remnant and re-emerging Common Reed (*Phragmites australis*) infestations to improve treatment efficacy and mitigate damage to native plants. Invasive Plant Sci Manag 7:445–453.

Brisson J, de Blois S, Lavoie C (2010) Roadside as invasion pathway for Common Reed (*Phragmites australis*). Invasive Plant Sci Manag 3:506–514.

Brisson J, Paradis É, Bellavance M-È (2008) Evidence of sexual reproduction in the invasive Common Reed (*Phragmites australis* subsp. *australis*; Poaceae) in eastern Canada: a possible consequence of global warming. Rhodora 110:225–230.

Canadian Council of Ministers of the Environment (2012) CCME Summary Table (Glyphosate). Canadian Council of Ministers of the Environment

Canadian Council of Ministers of the Environment (1989) CCME Summary Table (Glyphosate). Canadian Council of Ministers of the Environment

California Invasive Plant Council (2012) Preventing the spread of invasive plants: best management practices for transportation and utility corridors. California Invasive Plant Council, California

Carlson ML, Kowalski KP, Wilcox DA (2009) Promoting species establishment in a *Phragmites*-dominated Great Lakes coastal wetland. Nat Areas J 29:263–280.

Catling PM (2005a) Effects of invasive alien plants on birds: some examples from North America. Biodiversity 6:30–39.

Catling PM (2005b) New "top of the list" invasive plants of natural habitats in Canada. In: Bot. Electron. News 345 1-7. http://www.ou.edu/cas/botany-micro/ben/ben345.html. Accessed 7 Nov 2016

Catling PM, Mitrow G (2011) The recent spread and potential distribution of *Phragmites australis* subsp. *australis* in Canada. Can Field-Nat 125:95–104.

Chambers RM, Meyerson LA, Saltonstall K (1999) Expansion of *Phragmites australis* into tidal wetlands of North America. Aquat Bot 64:261–273.

Cheshier JC, Madsen JD, Wersal RM, et al (2012) Evaluating the potential for differential susceptibility of Common Reed (*Phragmites australis*) haplotypes I and M to aquatic herbicides. Invasive Plant Sci Manag 5:101–105.

Clay K, Shearin ZRC, Bourke KA, et al (2016) Diversity of fungal endophytes in non-native *Phragmites australis* in the Great Lakes. Biol Invasions 18:2703–2716.

Clevering OA, Lissner J (1999) Taxonomy, chromosome numbers, clonal diversity and population dynamics of *Phragmites australis*. Aquat Bot 64:185–208.

Cronin JT, Kiviat E, Meyerson LA, et al (2016) Biological control of invasive *Phragmites australis* will be detrimental to native *P. australis*. Biol Invasions 18:2749–2752.

Derr JF (2008a) Common Reed (*Phragmites australis*) response to post-emergence herbicides. Invasive Plant Sci Manag 1:153–157.

Derr JF (2008b) Common Reed (*Phragmites australis*) response to mowing and herbicide application. Invasive Plant Sci Manag 1:12–16.

Dill GM, Sammons RD, Feng PCC, et al (2010) Glyphosate: discovery, development, applications, and properties. In: Nandula VK (ed) Glyphosate Resistance in Crops and Weeds. John Wiley & Sons, Inc., pp 1–33

Domenico PA, Schwartz FW (1990) Physical and chemical hydrogeology. Wiley

Duke SO, Powles SB (2008) Glyphosate: a once-in-a-century herbicide. Pest Manag Sci 64:319–325.

Eddelbuettel D, Francois R (2011) Rcpp: seamless R and C++ integration. Journal of Statistical Software 40(8), New York

Egley GH, Williams RD (1978) Glyphosate and paraquat effects on weed seed germination and seedling emergence. Weed Sci 26:249–251.

Ekstam B (1995) Ramet size equalisation in a clonal plant, *Phragmites australis*. Oecologia 104:440–446.

Ekstam B, Foreseby A (1999) Germination response of *Phragmites australis* and *Typha latifolia* to diurnal fluctuations in temperature. Seed Sci Res 9:157–163.

Fant JB, Price AL, Larkin DJ (2016) The influence of habitat disturbance on genetic structure and reproductive strategies within stands of native and non-native *Phragmites australis* (Common Reed). Divers Distrib 22:1301–1313.

Farnsworth EJ, Meyerson LA (2003) Comparative ecophysiology of four wetland plant species along a continuum of invasiveness. Wetlands 23:750–762.

Fell PE, Warren RS, Curtis AE, Steiner EM (2006) Short-term effects on macroinvertebrates and fishes of herbiciding and mowing *Phragmites australis*-dominated tidal marsh. Northeast Nat 13:191–212.

Fell P, Scott Warren R, Light J, et al (2003) Comparison of fish and macroinvertebrate use of *Typha angustifolia*, *Phragmites australis*, and treated *Phragmites* marshes along the lower Connecticut River. Estuaries 26:534–551.

Fér T, Hroudová Z (2009) Genetic diversity and dispersal of *Phragmites australis* in a small river system. Aquat Bot 90:165–171.

Fischer MS, Rodriguez RJ (2013) Fungal endophytes of invasive *Phragmites australis* populations vary in species composition and fungicide susceptibility. Symbiosis 61:55–62.

Franz JE, Mao MK, Sikorski JA (1997) Glyphosate: a unique global herbicide. American Chemical Society

Galinato MI, Van Der Valk AG (1986) Seed germination traits of annuals and emergents recruited during drawdowns in the Delta Marsh, Manitoba, Canada. Aquat Bot 26:89–102.

Goldenberg EM (2013) Innovative Control of *Phragmites* and Other Invasive Species: Specific Gene Silencing.

Gomes MP, da Silva Cruz FV, Bicalho EM, et al (2017) Effects of glyphosate acid and the glyphosate-commercial formulation (Roundup) on *Dimorphandra wilsonii* seed germination: Interference of seed respiratory metabolism. Environ Pollut 220, Part A:452–459.

Gotelli NJ, Ellison AM (2012) A Primer of Ecological Statistics, Second Edition, Second edition. Sinauer Associates, Inc., Sunderland, Massachusetts

Granéli W, Weisner SEB, Sytsma MD (1992) Rhizome dynamics and resource storage in *Phragmites australis*. Wetl Ecol Manag 1:239–247.

Greenberg DA, Green DM (2013) Effects of an invasive plant on population dynamics in toads. Conserv Biol 27:1049–1057.

Greet J, Rees P (2015) Slashing may have potential for controlling *Phragmites australis* in long-inundated parts of a Ramsar-listed wetland. Ecol Manag Restor 16:233–236.

Häfliger P, Schwarzländer M, Blossey B (2006a) Impact of *Archanara geminipuncta* (Lepidoptera: Noctuidae) on aboveground biomass production of *Phragmites australis*. Biol Control 38:413–421.

Häfliger P, Schwarzländer M, Blossey B (2006b) Comparison of biology and host plant use *of Archanara geminipuncta*, *Archanara dissoluta*, *Archanara neurica*, and *Arenostola phragmitidis* (Lepidoptera: Noctuidae), potential biological control agents of *Phragmites australis* (Arundineae: Poaceae). Ann Entomol Soc Am 99:683–696

Hallinger KD, Shisler JK (2009) Seed bank colonization in tidal wetlands following *Phragmites* control (New Jersey). Ecol Restor 27:16–18.

Halloran J, Anderson H, Tassie D (2013) Clean equipment protocol for industry. Peterborough Stewardship Council and Ontario Invasive Plant Council, Peterborough, Ontario

Haslam SM (1972) *Phragmites* Communis Trin. (*Arundo phragmites* L.,? *Phragmites australis* (Cav.) Trin. ex Steudel). J Ecol 60:585–610.

Haslam SM (1969a) The development and emergence of buds in *Phragmites communis* Trin. Ann Bot 33:289–301.

Haslam SM (1969b) Stem types of *Phragmites communis* Trin. Ann Bot 33:127–131.

Haslam SM (1970) The development of the annual population in *Phragmites communis* Trin. Ann Bot 34:571–591.

Haslam SM (1971) The development and establishment of young plants of *Phragmites communis* Trin. Ann Bot 35:1059–1072.

Havens KJ, Berquist H, Priest WI (2003) Common Reed grass, *Phragmites australis*, expansion into constructed wetlands: are we mortgaging our wetland future? Estuaries 26:417–422.

Hazelton ELG, Mozdzer TJ, Burdick DM, et al (2014) *Phragmites australis* management in the United States: 40 years of methods and outcomes. AoB PLANTS 6:plu001–plu001.

Hellings SE, Gallagher JL (1992) The effects of salinity and flooding on *Phragmites australis*. J Appl Ecol 29:41–49.

Henry CJ, Higgins KF, Buhl KJ (1994) Acute toxicity and hazard assessment of Rodeo®, X-77 Spreader®, and Chem-Trol® to aquatic invertebrates. Arch Environ Contam Toxicol 27:392–399.

Holdredge C, Bertness MD (2011) Litter legacy increases the competitive advantage of invasive *Phragmites australis* in New England wetlands. Biol Invasions 13:423–433.

Holm LRG, Plucknett DL, Pancho JV, Herberger JP (1977) The world's worst weeds distribution and biology. University Press of Hawaii, Honolulu

Honnay O, Bossuyt B (2005) Prolonged clonal growth: escape route or route to extinction? Oikos 108:427–432.

International Agency for Research on Cancer (2015) ARC Monographs Volume 112: evaluation of five organophosphate insecticides and herbicides. International Agency for Research on Cancer, Lyon, France

Jodoin Y, Lavoie C, Villeneuve P, et al (2008) Highways as corridors and habitats for the invasive Common Reed *Phragmites australis* in Quebec, Canada. J Appl Ecol 45:459–466.

Juneau KJ, Tarasoff CS (2013) The seasonality of survival and subsequent growth of Common Reed (*Phragmites australis*) rhizome fragments. Invasive Plant Sci Manag 6:79–86.

Keller BEM (2000) Plant diversity in *Lythrum*, *Phragmites*, and *Typha* marshes, Massachusetts, U.S.A. Wetl Ecol Manag 8:391–401.

Kettenring KM, McCormick MK, Baron HM, Whigham DF (2010) *Phragmites australis* (Common Reed) invasion in the Rhode River Subestuary of the Chesapeake Bay: disentangling the effects of foliar nutrients, genetic diversity, patch size, and seed viability. Estuaries Coasts 33:118–126.

Kettenring KM, McCormick MK, Baron HM, Whigham DF (2011) Mechanisms of *Phragmites australis* invasion: feedbacks among genetic diversity, nutrients, and sexual reproduction. J Appl Ecol 48:1305–1313.

Kettenring KM, Mock KE (2012) Genetic diversity, reproductive mode, and dispersal differ between the cryptic invader, *Phragmites australis*, and its native conspecific. Biol Invasions 14:2489–2504.

Kettenring KM, Mock KE, Zaman B, McKee M (2016) Life on the edge: reproductive mode and rate of invasive *Phragmites australis* patch expansion. Biol Invasions 18:2475–2495.

Kettenring KM, Whigham DF (2009) Seed viability and seed dormancy of non-native *Phragmites australis* in suburbanized and forested watersheds of the Chesapeake Bay, USA. Aquat Bot 91:199–204.

Kindt R, Coe R (2005) Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies. World Agroforestry Centre (ICRAF), Nairobi

Kirk H, Paul J, Straka J, Freeland JR (2011) Long-distance dispersal and high genetic diversity are implicated in the invasive spread of the Common Reed, *Phragmites australis* (Poaceae), in northeastern North America. Am J Bot 98:1180–1190.

Knezevic SZ, Rapp RE, Datta A, Irmak S (2013) Common Reed (*Phragmites australis*) control is influenced by the timing of herbicide application. Int J Pest Manag 59:224–228.

Koskinen WC, Marek LJ, Hall KE (2016) Analysis of glyphosate and aminomethylphosphonic acid in water, plant materials and soil. Pest Manag Sci 72:423–432.

Lane M, Lorenz N, Saxena J, et al (2012) The effect of glyphosate on soil microbial activity, microbial community structure, and soil potassium. Pedobiologia 55:335–342.

Lathrop RG, Windham L, Montesano P (2003) Does *Phragmites* expansion alter the structure and function of marsh landscapes? Patterns and processes revisited. Estuaries 26:423–435.

Lelong B, Lavoie C, Jodoin Y, Belzile F (2007) Expansion pathways of the exotic Common Reed (*Phragmites australis*): a historical and genetic analysis. Divers Distrib 13:430–437.

Lippe M Von der, Kowarik I (2007) Long-distance dispersal of plants by vehicles as a driver of plant invasions. Conserv Biol 21:986–996.

Lombard KB, Tomassi D, Ebersole J (2012) Long-term management of an invasive plant: lessons from seven years of *Phragmites australis* control. Northeast Nat 19:181–193.

Malecki RA, Blossey B, Hight SD, et al (1993) Biological control of Purple Loosestrife. BioScience 43:680–686.

Mal TK, Narine L (2004) The biology of Canadian weeds. 129. *Phragmites australis* (Cav.) Trin. ex Steud. Can J Plant Sci 84:365–396.

Maqueda C, Undabeytia T, Villaverde J, Morillo E (2017) Behaviour of glyphosate in a reservoir and the surrounding agricultural soils. Sci Total Environ 593–594:787–795.

Marks M, Lapin B, Randall J (1993) Element stewardship abstract for *Phragmites australis*.

Martin LJ, Blossey B (2013) The runaway weed: costs and failures of *Phragmites australis* management in the USA. Estuaries Coasts 36:626–632.

Mateos-Naranjo E, Perez-Martin A (2013) Effects of sub-lethal glyphosate concentrations on growth and photosynthetic performance of non-target species *Bolboschoenus maritimus*. Chemosphere 93:2631–2638.

McCormick MK, Brooks HEA, Whigham DF (2016) Microsatellite analysis to estimate realized dispersal distance in *Phragmites australis*. Biol Invasions 18:2497–2504.

McCune B, Grace J (2002) Analysis of ecological communities. Mjm Software Design, Gleneden Beach, Oregon

Melchior P, Weaver R (2016) Eurasian haplotype M *Phragmites australis* (Cav.) Trin. ex Steud., 1841 invasion in Minnesota, USA: a baseline for further monitoring in the upper Mississippi watershed. BioInvasions Rec 5:59–65.

Meyer D, Dimitriadou E, Hornik K, et al (2015) e1071: Misc functions of the Department of Statistics, Probability Theory Group (Formerly: E1071). TU Wien

Meyerson LA, Chambers RM, Vogt KA (1999) The effects of *Phragmites* removal on nutrient pools in a freshwater tidal marsh ecosystem. Biol Invasions 1:129–136.

Meyerson LA, Cronin JT, Pyšek P (2016) *Phragmites australis* as a model organism for studying plant invasions. Biol Invasions 18:2421–2431.

Meyerson LA, Saltonstall K, Windham L, et al (2000) A comparison of *Phragmites australis* in freshwater and brackish marsh environments in North America. Wetl Ecol Manag 8:89–103.

Meyerson LA, Viola DV, Brown RN (2010) Hybridization of invasive *Phragmites australis* with a native subspecies in North America. Biol Invasions 12:103–111.

Michigan Department of Environmental Quality (2007) A landowner's guide to *Phragmites* control. Michigan Department of Environmental Quality, Michigan

Minchinton TE, Bertness MD (2003) Disturbance-mediated competition and the spread of *Phragmites australis* in a coastal marsh. Ecol Appl 13:1400–1416.

Minchinton TE, Simpson JC, Bertness MD (2006) Mechanisms of exclusion of native coastal marsh plants by an invasive grass. J Ecol 94:342–354.

Mink PJ, Mandel JS, Sceurman BK, Lundin JI (2012) Epidemiologic studies of glyphosate and cancer: a review. Regul Toxicol Pharmacol 63:440–452.

Monsanto Canada Inc. (2016) Roundup WeatherMAX® With Transorb 2 Technology Liquid Herbicide. Monsanto Canada Inc., Winnipeg, MB

Monsanto Canada Inc. (2015) VisionMAX™ Silviculture Herbicide. Monsanto Canada Inc.

Moore GE, Burdick DM, Peter CR, Keirstead DR (2012) Belowground biomass of *Phragmites australis* in coastal marshes. Northeast Nat 19:611–626.

Mozdzer TJ, Hutto CJ, Clarke PA, Field DP (2008) Efficacy of imazapyr and glyphosate in the control of non-native *Phragmites australis*. Restor Ecol 16:221–224.

Nešković NK, Poleksić V, Elezović I, et al (1996) Biochemical and histopathological effects of glyphosate on Carp, Cyprinus carpio L. Bull Environ Contam Toxicol 56:295–302.

New Hampshire Department of Transportation (2008) Best management practices for roadside invasive plants. New Hampshire Department of Transportation, New Hampshire

New York State Department of Transportation (2004) Invasive species control methods for maintenance and construction. New York State Department of Transportation, New York State

Oksanen J, Blanchet FG, Friendly M, et al (2016) vegan: community ecology package.

Ontario Invasive Plant Council (2016) Clean equipment protocol. Ontario Invasive Plant Council

Ontario Ministry of Natural Resources and Forestry (2011) Invasive *Phragmites*-best management practices. Ontario Ministry of Natural Resources and Forestry, Peterborough, Ontario

Ontario Ministry of Natural Resources and Forestry (2014) Ontario Ministry of Natural Resources prescribed burn manual. Ontario Ministry of Natural Resources and Forestry

Ontario *Phragmites* Working Group (2015) Smart practices for the control of invasive *Phragmites* along Ontario's roads. Ontario *Phragmites* Working Group

Ostendorp W (1991) Damage by episodic flooding to *Phragmites* reeds in a pre-alpine lake: proposal of a model. Oecologia 86:119–124.

Peck JE (2010) Multivariate analysis for community ecologists: step-by-step using PC-ORD. MjM Software Design, Gleneden Beach, Or.

Piotrowicz-Cieslak AI, Adomas B, Michalczyk DJ (2010) Different glyphosate phytotoxicity of seeds and seedlings of selected plant species. Pol J Environ Stud 19:123–129.

Powles SB (2008) Evolved glyphosate-resistant weeds around the world: lessons to be learnt. Pest Manag Sci 64:360–365.

Rapp RE, Datta A, Irmak S, et al (2012) Integrated management of Common Reed (*Phragmites australis*) along the Platte River in Nebraska. Weed Technol 26:326–333.

Relyea RA, Jones DK (2009) The toxicity of Roundup Original Max® to 13 species of larval amphibians. Environ Toxicol Chem 28:2004–2008.

Reznicek A., Catling PM (1989) Flora of Long Point. Mich Bot 28:99–175.

Ridley H (1930) The Dispersal of Plants Throughout the World. L. Reeve and Co., LTD., Lloyds Bank Buildings, Ashford, Kent, pp 137–138

Rissoli RZ, Abdalla FC, Costa MJ, et al (2016) Effects of glyphosate and the glyphosate based herbicides Roundup Original® and Roundup Transorb® on respiratory morphophysiology of bullfrog tadpoles. Chemosphere 156:37–44.

Robichaud C, Rooney R (2017) Long-term effects of a *Phragmites australis* invasion on birds in a Lake Erie coastal marsh. J Gt Lakes Res.

Rolletschek H, Rolletschek A, Hartzendorf T, Kohl J-G (2000) Physiological consequences of mowing and burning of *Phragmites australis* stands for rhizome ventilation and amino acid metabolism. Wetl Ecol Manag 8:425–433.

RStudio Team (2015) RStudio: Integrated Development for R. RStudio, Inc., Boston, MA

Saltonstall K (2002) Cryptic invasion by a non-native genotype of the Common Reed, *Phragmites australis*, into North America. Proc Natl Acad Sci 99:2445–2449.

Saltonstall K (2016) The naming of *Phragmites* haplotypes. Biol Invasions 18:2433–2441.

Saltonstall K, Lambert A, Meyerson LA (2010) Genetics and reproduction of Common (*Phragmites australis*) and Giant Reed (*Arundo donax*). Invasive Plant Sci Manag 3:495–505.

Schummer ML, Palframan J, McNaughton E, et al (2012) Comparisons of bird, aquatic macroinvertebrate, and plant communities among dredged ponds and natural wetland habitats at Long Point, Lake Erie, Ontario. Wetlands 32:945–953.

Shearer JF, Harms NE (2012) Survey for pathogens of *Phragmites* in New York.

Short L (2015) Wymbolwood Beach *Phragmites* Removal Technique. Ontario *Phragmites* Working Group

Skinner LC, Rendall WJ, Fuge EL (1994) Minnesota's Purple Loosestrife program: history findings, and management recommendations.

Smith SM (2005) Manual control of *Phragmites australis* in freshwater ponds of Cape Cod National Seashore, Massachusetts, USA. J Aquat Plant Manag 43:50–53.

Swearingen J, Saltonstall K (2010) *Phragmites* field guide: distinguishing native and exotic forms of Common Reed (*Phragmites australis*) in the United States.

Ter Heerdt GNJ, Drost HJ (1994) Potential for the development of marsh vegetation from the seed bank after a drawdown. Biol Conserv 67:1–11.

Ter Heerdt GNJ, Schutter A, Bakker JP (1999) The effect of water supply on seed-bank analysis using the seedling-emergence method. Funct Ecol 13:428–430.

Ter Heerdt GNJ, Verweij GL, Bekker RM, Bakker JP (1996) An improved method for seed-bank analysis: seedling emergence after removing the soil by sieving. Funct Ecol 10:144–151.

Tewksbury L, Casagrande R, Blossey B, et al (2002) Potential for biological control of *Phragmites australis* in North America. Biol Control 23:191–212.

Thompson DJ, Shay JM (1985) The effects of fire on *Phragmites australis* in the Delta Marsh, Manitoba. Can J Bot 63:1864–1869.

Tozer DC (2016) Marsh bird occupancy dynamics, trends, and conservation in the southern Great Lakes basin: 1996 to 2013. J Gt Lakes Res 42:136–145.

Tsui MTK, Chu LM (2003) Aquatic toxicity of glyphosate-based formulations: comparison between different organisms and the effects of environmental factors. Chemosphere 52:1189–1197.

Tu M, Hurd C, Randall JM (2001) Weed control methods handbook: tools & techniques for use in natural areas. The Nature Conservancy

USGS Great Lakes Science Centre (2013) Innovative *Phragmites* control strategies for the Great Lakes restoration initiative.

Valkama E, Lyytinen S, Koricheva J (2008) The impact of reed management on wildlife: a meta-analytical review of European studies. Biol Conserv 141:364–374.

Voss EG, Reznicek AA (2012) Field Manual of Michigan Flora. University of Michigan Press

Weisner SEB, Strand JA (1996) Rhizome architecture in *Phragmites australis* in relation to water depth: implications for within-plant oxygen transport distances. Folia Geobot 31:91–97.

White JF, Kingsley KI, Kowalski KP, et al (2017) Disease protection and allelopathic interactions of seed-transmitted endophytic pseudomonads of invasive reed grass (*Phragmites australis*). Plant Soil 1–14.

Whyte RS, Trexel-Kroll D, Klarer DM, et al (2008) The invasion and spread of *Phragmites australis* during a period of low water in a Lake Erie coastal wetland. J Coast Res 111–120.

Wickham H (2009) ggplot2: Elegant graphics for data analysis. Springer-Verlag, New York

Wijte AHBM, Gallagher JL (1996) Effect of oxygen availability and salinity on early life history stages of salt marsh plants. I. different germination strategies of *Spartina alterniflora* and *Phragmites australis* (Poaceae). Am J Bot 83:1337–1342.

Wilcox KL, Petrie SA, Maynard LA, Meyer SW (2003) Historical distribution and abundance of *Phragmites australis* at Long Point, Lake Erie, Ontario. J Gt Lakes Res 29:664–680.

Williams GM, Kroes R, Munro IC (2000) Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. Regul Toxicol Pharmacol 31:117–165.

Windham L (2001) Comparison of biomass production and decomposition between *Phragmites australis* (Common Reed) and *Spartina patens* (Salt Hay Grass) in brackish tidal marshes of New Jersey, USA. Wetlands 21:179–188.

Zabaloy MC, Gómez E, Garland JL, Gómez MA (2012) Assessment of microbial community function and structure in soil microcosms exposed to glyphosate. Appl Soil Ecol 61:333–339.

Appendices

Appendix A: Map of study site properties in Long Point, Ontario.
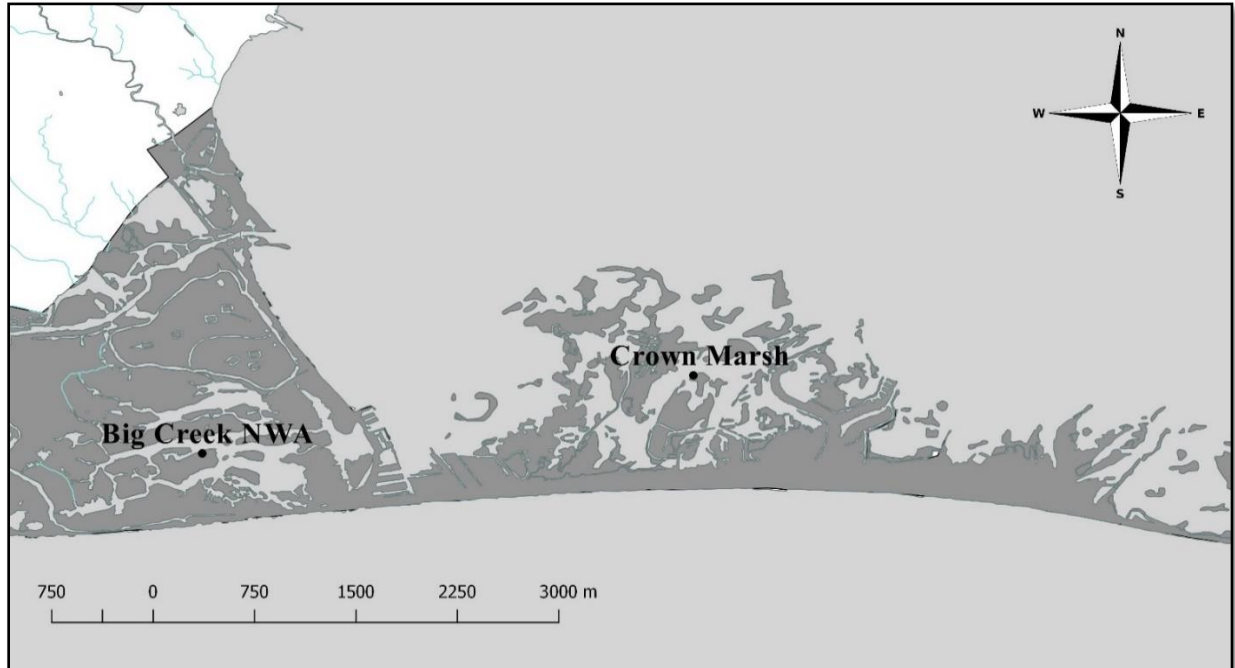
Appendix B: List of plant species observed in the Big Creek germinability assay, including 5 species that died before maturity and could not be identified. Identification was done using wetland field guides and confirmed using the Field Guide of Michigan Flora (Voss and Reznicek 2012). Several species could only be identified according to the genus, and were denoted by "spp." if it was likely that individuals from several species of a common genus were present but could not be differentiated or identified to species.

Botanical name	Common Name	Family
Verbena hastata	Blue vervain	Vervain
Alliaria petiolata	Garlic mustard	Brassicaceae
Barbarea vulgaris	Bittercress	Brassicaceae
Carex spp.	Sedge	Cyperaceae
Cirsium arvense	Canada thistle	Asteraceae
Eupatorium perfoliatum	Common boneset	Asteraceae
Juncacea spp.	N/A	Juncacea
Lycopus americanus	American horehound	Lamiaceae
P. australis	European Common Reed	Poaceae
Persicaria lapathifolia	Pale smartweed	Polygonaceae
Ranunculus sceleratus	Celery-leaved buttercup	Ranunculaceae
Scirpus pungens	Common threesquare	Cyperaceae
Solidago canadensis	Canada goldenrod	Asteraceae
Solanum ptycanthum	Eastern black nightshade	Solanaceae
Species #12	N/A	N/A
Species #17	N/A	N/A
Species #18	N/A	N/A
Species #19	N/A	N/A
Species #22	N/A	N/A
Typha spp.	Cattail	Typhaceae
Urtica dioica	Stinging nettle	Urticaceae

Appendix C: Scree plot of the final stress values from Dimensions 1-6 of the best NMDS solutions of Big Creek stem density. The values for Dimension 1 and Dimensions 2-6 were generated after 100 and 20 runs with real data, respectively, with a max of 100 iterations. These values were generated using R Studio.
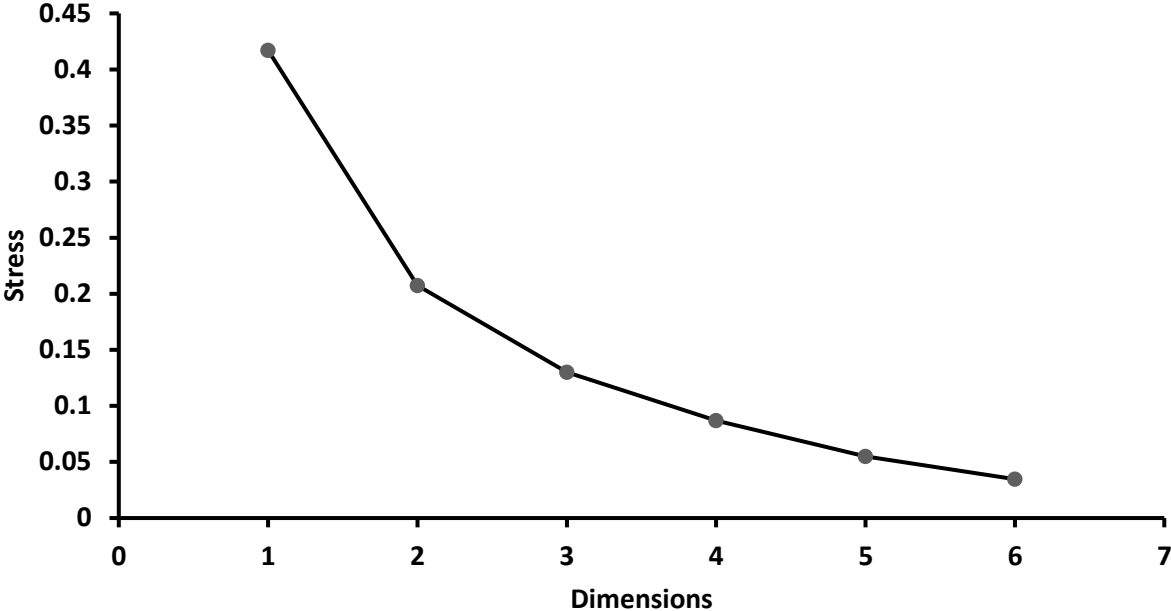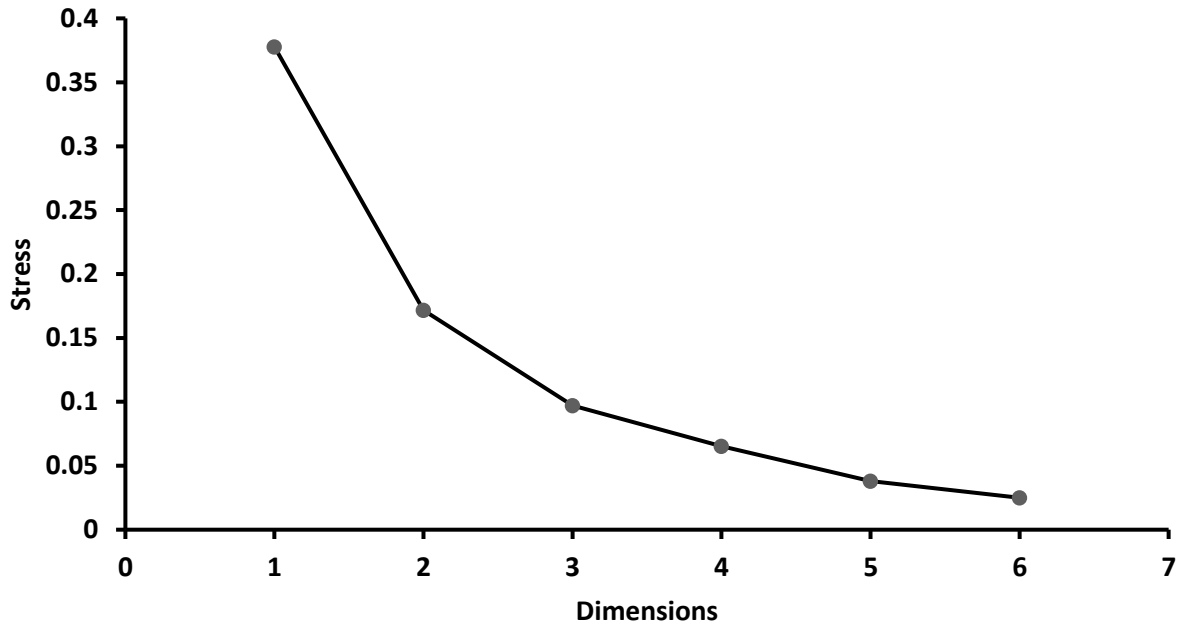
Appendix D: 3D NMDS ordination solution of a Bray-Curtis dissimilarity matrix using relativized and square root transformed stem density values of species that germinated during the Big Creek germinability assay (final stress of 0.13, Procrustes RMSE = 0.0002, max residuals = 0.0004) with plant species overlaid as vectors. Panel A shows the treatment factor with the control sites that were not sprayed (black circles) and sites sprayed with herbicide (grey triangles). Panel B displays *P. australis* density, with sites of high *P. australis* density (black circles) and low *P. australis* density (grey triangles) displayed.
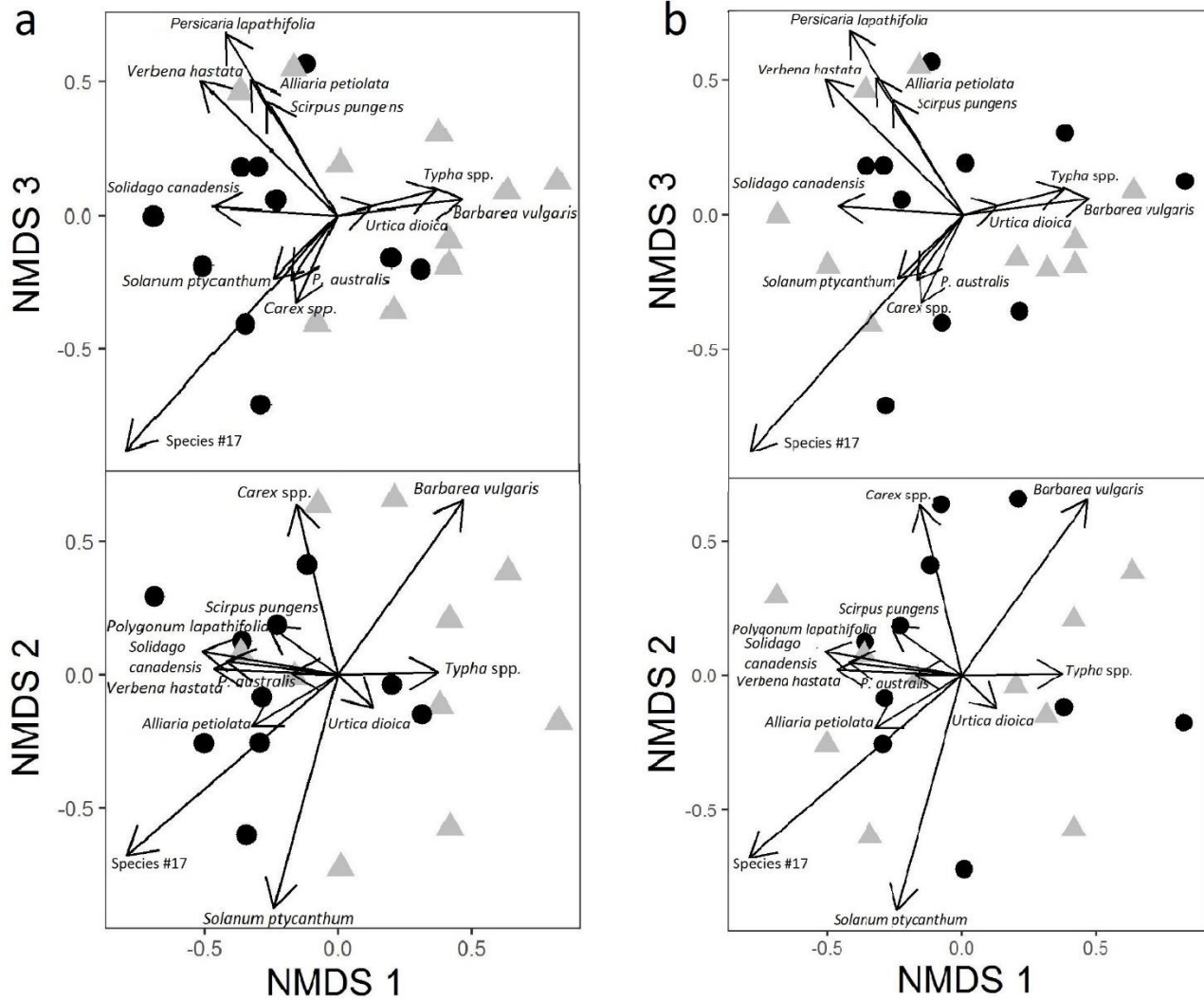
Appendix E: List of plant species observed in the Crown Marsh germinability assay. Three species were found in both the greenhouse germinability assay and surveys of seedbank sampling sites (signified by "Both") and three species either did not reach maturity and could not be identified. Identification of plants at both the sampling sites and greenhouse was done using wetland field guides and confirmed using the Field Guide of Michigan Flora (Voss and Reznicek 2012).   Several species could only be identified according to the genus, and were denoted by "spp." if it was likely that individuals from several species of a common genus were present but could not be differentiated or identified to species.

| Botanical name	Common Name	Family	Occurrence
Calamagrostis canadensis	Bluejoint	Poaceae	Sampling site
Campanula aparinoides	Marsh bellflower	Campanulaceae	Sampling site
Carex buxbaumii	Buxbaum's sedge	Cyperaceae	Sampling site
Carex spp.	Sedge	Cyperaceae	Sampling site
Carex lanuginosa	American woolyfruit sedge	Cyperaceae	Sampling site
Carex lasiocarpa	Woolyfruit sedge	Cyperaceae	Sampling site
Chara sp.	Muskgrass	Characeae	Sampling site
Cladium mariscoides	Smooth sawgrass	Cyperaceae	Sampling site
Eleocharis smallii	Common spikerush	Cyperaceae	Sampling site
Hypericum kalmianum	Kalm's St. Johnswort	Clusiaceae	Sampling site
Juncus brevicaudatus	Narrowpanicle rush	Juncaceae	Greenhouse
Lysimachia thyrsiflora	Tufted loosestrife	Primulaceae	Sampling site
Lythrum salicaria	Purple loosestrife	Lythraceae	Greenhouse
Mentha arvensis	Wild mint	Lamiaceae	Sampling site
Lamiaceae sp.	Mint	Lamiaceae	Greenhouse
Panicum sp.	Panicgrass	Poaceae	Greenhouse
P. australis	European Common Reed	Poaceae	Both
Scirpus pungens	Common threesquare	Cyperaceae	Both
Species #1	N/A	N/A	Sampling site
Species #6	N/A	N/A	Greenhouse
Species #8	N/A	N/A	Greenhouse
Solidago ohioensis	Ohio goldenrod	Asteraceae	Both
Stachys palustris	Marsh hedgenettle	Lamiaceae	Sampling site
Triadenum fraseri	Fraser's marsh St. Johnswort	Clusiaceae	Sampling site
Typha spp.	Cattail	Typhaceae	Greenhouse

Appendix F: Scree plot of the final stress values from Dimensions 1-6 of the best NMDS solutions of Crown Marsh stem density. The values for Dimension 1, Dimensions 2-5, and Dimension 6 were generated after 100, 20, and 45 runs with the data, respectively, with a max of 100 iterations. These values were generated using R Studio.

Appendix G: 3D NMDS ordination solution of a Bray-Curtis dissimilarity matrix using relativized species stem density values for species that germinated from the Crown Marsh germinability assay (final stress of 0.10, Procrustes RMSE = 0.0002, maximum residuals = 0.0003) with plant species overlaid as vectors. Control sites that were not sprayed (black circles) and sites sprayed with herbicide (grey triangles) according to the factor of treatment.
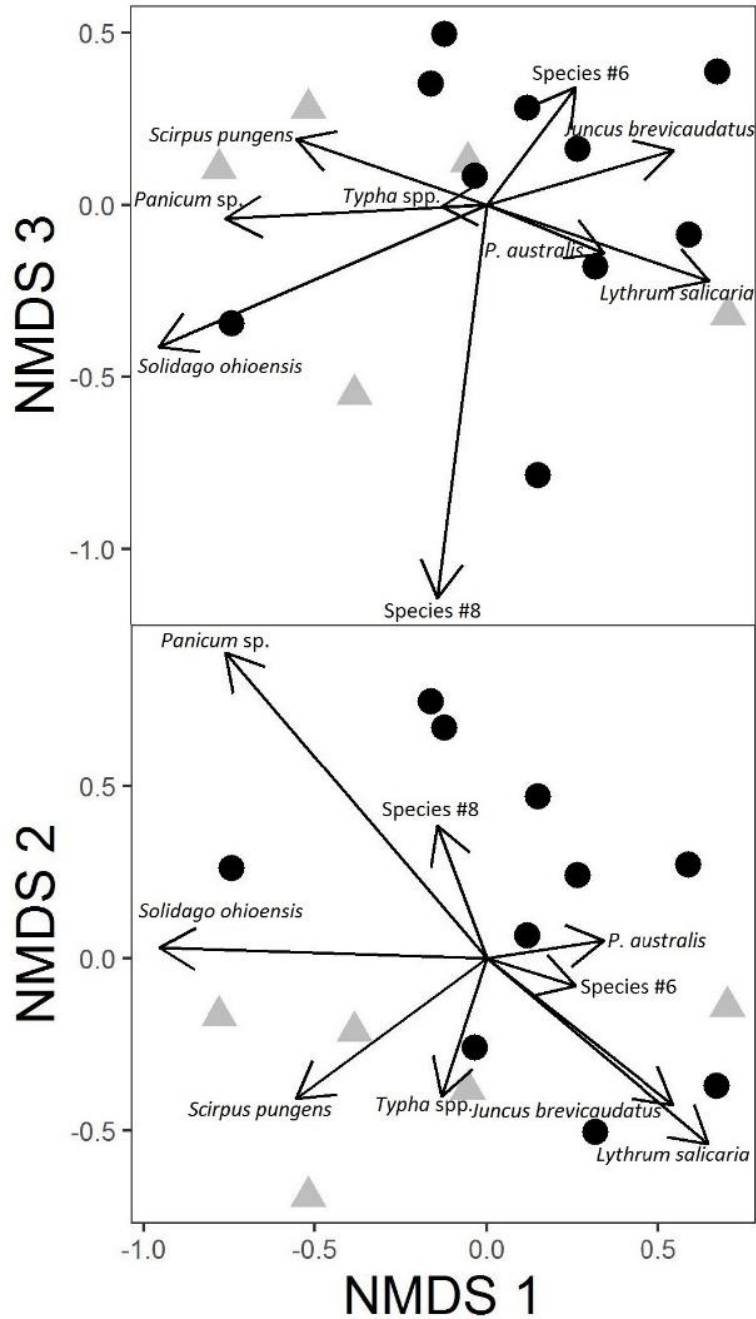
Appendix H: Map of control and treatment sites in Crown Marsh, Ontario. Five replicates are included in each site, and were the locations of seedbank sampling in 2015 and were surveyed in 2016.
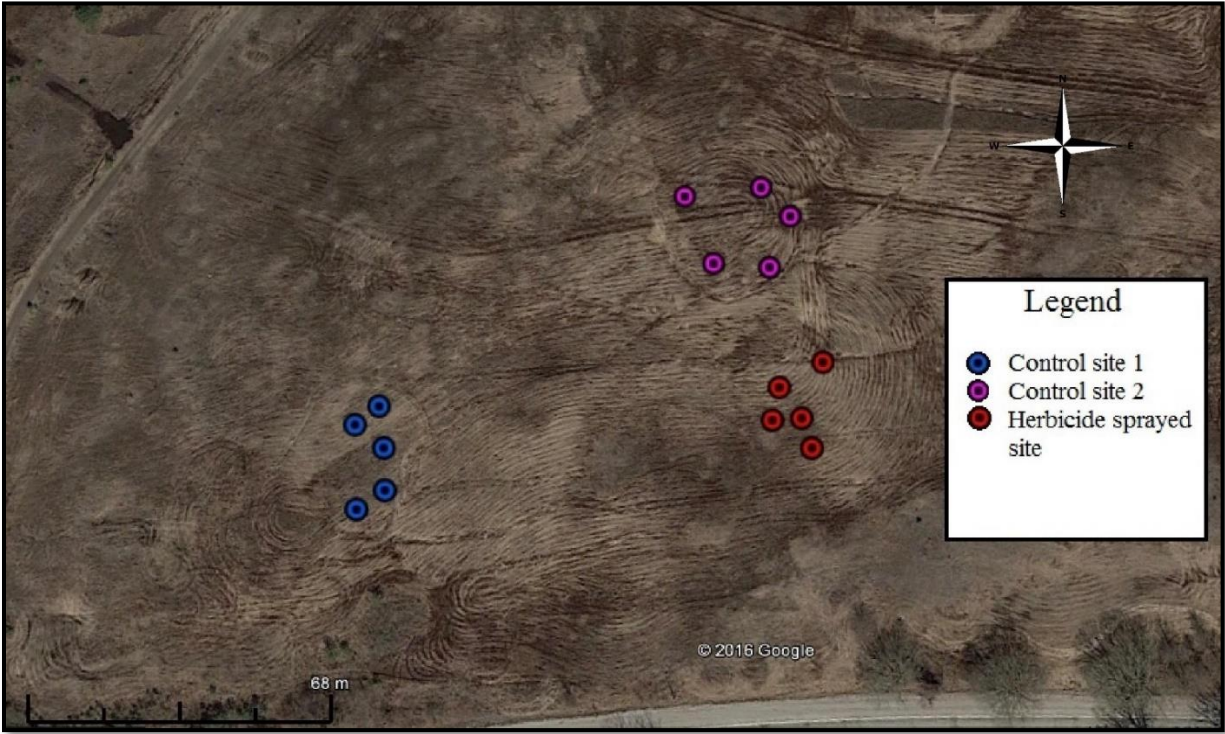
Appendix I: The process used to create Chapter 3 mesocosms. Mesocosms were created from plastic waste bins, then perforated with holes for drainage. A 20 cm mixture of peat and rhizomes was placed in the bottom of each mesocosm, and these were installed in Crown Marsh such that the top of each mesocosm was at the same elevation on the surface. These mesocosms were then monitored during 2016 for the presence of *P. australis* stems.