REVEGETATION WITH NATIVE PLANTS: A TEST OF BEST PRACTICES

REVEGETATION WITH NATIVE PLANTS: A TEST OF BEST PRACTICES

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Abstract

Restoration practitioners are tasked with recreating ecosystems using appropriate plant material that will provide ecological goods and services. However, best-practices for this type of intervention are not well developed for the southern Ontario landscape. Therefore, we evaluated approaches from four different aspects of seed-based restoration. First, we quantified the impact of seeding rate and application method on the success of grassland recreation. We also measured the impact of this restoration on the local bee community. Next, we compared a suite of native and nearly native wetland plants for their potential to prevent the establishment of invasive Phragmites australis. We measured the effect of competition on Phragmites across soil moisture and salinity gradients. Finally, we sought evidence for local specialization in a grassland forb, Monarda fistulosa, that would warrant policies to prevent the transfer of grassland seed for revegetation.

In re-creating grasslands from seed, we found an interaction between seeding rate and application method. At a high rate, both methods had the same outcome, but at a low rate, a two-phase application method produced better results than a single-phase method. However, we also found that a single-phase method produced target plant cover with a higher floristic quality index after three years. In one study region, restored sites supported a greater bee abundance than un-restored sites, but bee abundance did not change after restoration in all regions. Of all the native species tested, Phragmites was supressed most by Bidens frondosa, a fast growing annual. We also found evidence that Phragmites may be less competitive at low soil moisture, and more competitive at high soil salinity. Finally, we found no evidence of local adaptation in M. fistulosa at the watershed scale; instead, we see independent effects of site and seed origin. This implies that current site conditions may not be favorable to the offspring of relic populations, and that local genotypes may not always be the best choice for restoration.

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S.D.Weber, June 30, 2021

Contents

List of Figures & Tables

List of Abbreviations

i) MTO: Ontario Ministry of Transportation
ii) DOT: Department of Transportation (United States)
iii) FQI: Floristic Quality Index

Declaration of Academic Achievement

CHAPTER 1: General Introduction

CHAPTER 2: Informing Monarda fistulosa seed-transfer: are populations locally adapted?

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CHAPTER 3: Restoration at the roadside: does method matter and do bees benefit?

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CHAPTER 4: Native plants in competition with Phragmites: is resistance futile?

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CHAPTER 6: General conclusion

CHAPTER 1 Ecological Intervention, Revegetation & Reassembly

There is an increasing interest in the restoration of degraded ecosystems to provide greater ecological goods and services (Montoya, Rogers et al. 2012, Meli, Rey Benayas et al. 2014). Restoration provides an opportunity to test hypotheses about how communities assemble spontaneously, how practitioners can intervene in the process, and which steps in community assembly are not easily influenced through human intervention. Anthropogenic impacts such as global warming change the mechanisms through which communities have historically assembled and restoration practitioners may be motivated to focus on creating resilient, future-proof ecosystems rather than recreating lost assemblages (Miller and Hobbs 2007, Hobbs and Cramer 2008, Bullock, Aronson et al. 2011, Gutierrez 2018).

Terrestrial ecosystems can be classified based on climate, soils, as well as vegetation community (Lee, Bakowsky et al. 1998). As primary producers and habitat engineers, plants are the foundation of terrestrial ecosystems. Plants effects microclimate, they provide habitat and food either directly or indirectly for all life on land. It is no surprise then that restoration ecologists focus on revegetation, and on the addition and introduction of plants to re-create, restore or rehabilitate terrestrial habitats (Basey, Fant et al. 2015, Havens 2015, Olwell 2015, White, Fant et al. 2018).

Though sometimes used interchangeably, the terms restoration, recreations, rehabilitation, and revegetation can all take on different meanings in different contexts. Revegetation and rehabilitation may not aim to restore native species, for example, and otherwise may depend on exotic species to achieve a particular function. The term "restoration" can refer to the process of returning lost native species assemblages with reference to a nearby ecosystem or historical record. However, "restoration" is also used to describe efforts that focus on augmenting existing novel communities to create target levels of community diversity, drawing on novel, or nearby species assemblages to achieve that target. Some argue that extending the ranges of native plant communities, even at a small scale constitutes "re-creation", rather than "restoration", while others see it simply as assisted migration, the restoration of an ecological process, rather than an assemblage of particular species. The slight difference in terminology reflects, a larger philosophical debate as to whether the outcomes of human actions are inherently different to the outcomes of spontaneous ecosystems (Hobbs and Cramer 2008).

Though the appropriate term to describe restoration activities may be questioned, each is a form of human intervention into ecological processes. Arguably the value of the term "restoration" over "intervention" it is that it is equally general and all-encompassing. Arguably the term provides a positive filter for this work and helps appeal to public perception of investment in ecosystems (Hobbs and Cramer 2008). Here I use the terms "intervention" and "restoration" somewhat interchangeably. Intervention will be used to highlight the active human component, and restoration will be used in reference to popularly accepted terms such as 'restoration guidelines', and 'restoration practitioner'. For example, 'restoration ecology', is widely accepted to encompass the ecological study of rehabilitation, and recreation of habitats.

Re-Vegetation of the Herbaceous Layer in Southern Ontario

Southern Ontario is one of the most species-rich regions in Canada, but also the most densely population region. Because of this, intact ecosystems are rare, and the needs of wildlife are often at odds with the needs of humans (Kanter 2005). In the past decade, this region faced a net loss of natural areas. Rare species with legal protections have become even more rare, and not a single permit to affect these protected species was denied (OBC 2010, Saxe 2017).

In Southern Ontario high quality communities occur only as fragments in an otherwise developed and modified landscape. Restoration ecologists often aim to recreate communities with a similar species composition to some target community, typically thought of as an ecological reference (Balaguer, Escudero et al. 2014). They may also aim to augment particular ecosystem services, such as carbon sequestration, flood mitigation, or increasing pollination services. Though small and fragmented, these plant communities are conservation hubs, and sources of seed for habitat creation projects (Leimu, Vergeer et al. 2010). They also provide a glimpse of the ecosystem services provided by intact native plant communities.

There is particular interest in conserving bees and other pollinators in southern Ontario (Pindar, Mullen et al. 2017). Large-scale restoration in the region is also frequently targeted to support rare species of ground nesting birds, threatened trees, and migratory species such as the Monarch Butterfly. The region has a number of organizations dedicated to preserving grasslands and other unique habitats including wild-origin native plant producers. While restoration in southern Ontario has been researched from various perspectives, few primary empirical studies on herbaceous revegetation practices have been published based on work in this region and so not all local restoration policies and guidelines are equally informed by data collected in a local context with appropriate species.

Scientific perspectives in terrestrial revegetation

Both restored, and spontaneous communities result from a combination of niche-based interactions between species, and the niche-neutral, stochastic forces that shape communities over time (Funk, Cleland et al. 2008, Kembel 2009, Fischer, von der Lippe et al. 2013). When humans intervene in ecological systems, we manipulate both niche and niche-neutral interactions to meet preconceived restoration targets. When considering ecological intervention such as plant addition or seedbank augmentation, four main perspectives emerge that require evidence-based reasoning and therefore a solid scientific foundation.

First, locally sourced, and ecologically appropriate seed resources for use in restoration may be limiting in fragmented landscapes and can increase the costs of doing restoration. This means that restoration projects far from high quality natural areas must often transfer seeds from other regions, with unknown consequences to small, local populations, or to the restoration itself (Vanandel 1998, Hufford and Mazer 2003, St. Clair, Dunwiddie et al. 2020). In an effort to avoid unintended consequences, certain jurisdictions will follow a 'local-is-best' approach to restoration, though some ecologists question how universal this approach ought to be given future environmental change and our understanding of the adaptive process. Therefore, restoration practitioners must weigh the effects of local adaptation, and fragmentation of wild seed sources on the persistence of restored populations.

Next there are the technical aspects of human intervention, namely production, and installation of new restoration units. Standardized best-practices have been developed for a wide variety of grassland revegetation techniques in North America, particularly for creating grassland along roadways and other infrastructure corridors. These guidelines are developed to ensure restoration work is done efficiently and effectively, but also to meet particular standards or ecological goals. (Burton, Burton et al. 2006, Miller and Hobbs 2007, Haan 2010, Mola, Jimenez et al. 2011, Prevey, Knochel et al. 2014). Effort and resources are spent on preparing a site for revegetation and ensuring that plants establish successfully, but the potential costs of failed restorations are greater. In regions, like southern Ontario, where relatively few grasslands have been restored along roadways, practitioners are left to rely on anecdotal evidence or best practices developed in a different landscape, with different soils and species compositions.

Justifying this cost of restoration also requires long term monitoring to understand how restoration impacts the provisioning of ecosystem services, and if the expected ecological benefits were delivered. Therefore, measuring the success of a revegetation project ought to incorporate ecological benefits beyond the performance of the restored plant community. Understanding, for example, how a community of pollinators responds to the creation of a native wildflower meadow, is as important as understanding how well the restored plants performed (Dixon 2009, Noordijk, Delille et al. 2009, Baxter-Gilbert, Riley et al. 2015). The planting of 'pollinator strips' or 'wildflower corridors' is praised for supporting pollination services, but these aspects of restoration success or not often included in monitoring, likely due to the cost and effort required to meaningfully sample insect communities.

The biotic community itself is often the greatest barrier to restoration, particularly in degraded habitats, where invasive species dominate, and outcompete native species (Kennedy, Naeem et al. 2002, Von Holle, Delcourt et al. 2003, Byun, de Blois et al. 2013, Galatowitsch, Larson et al. 2016, Gallien and Carboni 2017). The final perspective considered here therefore, is the persistence of restored native plant communities in the face of invasion. Ontario is home to several invasive plant species, but one of the most disruptive in wetlands is the Common Reed (*Phragmites australis*, Phragmites hereafter). While controlling populations remains a priority, it remains unclear if restored landscapes will be able to repel future invasions from Phragmites. Some ecologists have suggested that native plant communities can be manipulated to be more resistant to invasion, though there is no scientific evidence of native plants being an effective biocontrol for Phragmites in Ontario.

Each perspective can provide scientific evidence to guide restoration policy and practice.

Research Questions

In order to provide experimental evidence from the perspectives above, I tested revegetation best-practices in Ontario that are currently supported by anecdotal evidence, or empirical studies in other contexts. I devised four sets of experiments, each asking a different question about seed-based restoration.

I began by asking whether local seeds perform better in restoration than seeds from regions farther away. I sought to measure the relative influence of seed provenance and site characteristics on resorted populations. Here, I worked with a model organism, *Monarda fistulosa*, to ask if seedlings perform best in their site of origin, or sites away from where they originate. An interaction between origin and site effects is evidence for local adaptation, displayed though a consistent homesite advantage. This study used a reciprocal transplant method with seedlings from four populations, representing 3 watersheds, and separated by approximately 40km. Transplants were monitored for two growing seasons, to test the following hypotheses:

1. Seedlings will accumulate more biomass and show higher reproductive fitness in their home site than in reciprocal(foreign) sites.
2. Seedlings in their home site will accumulate more biomass and show higher reproductive fitness than reciprocal(foreign) seedlings at the same site.

Next, I tested best practices in grassland establishment methods, specifically, those used to deliver seeds to prepared sites. I asked whether variation in establishment methods can significantly impact the composition or trajectory of a restored plant community over time. I installed experimental grassland strips along provincial highway corridor and studied their progression for three seasons after restoration. Randomized plots of native grassland species were established within each grassland strip using two different seeding rates, seeding methods, and mowing frequencies. I counted seedling density and measured aboveground biomass within each plot to test the following hypotheses:

1. An increased seeding rate (doubled) will result in a significantly higher proportion of target native species over time.
2. Hand-seeding through a two-phase method will result in a significantly higher proportion of target native species over time.
3. Annual mowing significantly increases target species cover over time.

The third experiment studied the response of native bee communities to local wildflower additions along the same roadsides restored in conducting the second experiment, described above. These experiments are presented together below in a single chapter. We asked if native plant additions will support a great abundance or diversity of bees, and tested a set of two related hypotheses:

1. Native wildflower addition along roadside verges will attract a greater abundance of bees to these locations.
2. Native wildflower addition along roadside verges will attract a greater diversity of bee genera to these locations.

Finally, the fourth experiment considers the longevity of restored communities, and will ask which plants show the greatest ability to resist invasion. Specifically, I will measure ability of wetland grasses and forbs, as well as simple combinations of these plants, to reduce the growth of the Common Reed, *Phragmites australis.* This experiment involved two different phytometer studies to score the level of competition between the invasive plant Phragmites australis (Haplotype M) and potential native, wild neighbors. The first manipulated salinity and moisture in a controlled greenhouse setting, and the second also tested the influence of moisture and salinity gradient in a field setting. We tested two sets of hypotheses:

1. Phragmites seedling can be supressed and killed by competing native species, and that diverse combinations have a stronger suppressive effect.
2. Moisture and salinity levels can influence the outcome of competition.

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CHAPTER 2 Informing *Monarda fistulosa* seed-transfer: are populations locally adapted?

Abstract

Local plants and seeds are usually encouraged in ecological restoration, but this is not best-practice for all species at all scales. We asked if populations of a grassland forb*, Monarda fistulosa*, are locally adapted at a watershed scale to inform seed-transfer policies in southern Ontario. A consistent homesite advantage, and significant origin by environment interaction signifies local adaptation.

We began by collecting seed from 4 wild populations of M. fistulosa, each form a different but adjoining watershed or sub-watershed. Seedlings were propagated at McMaster greenhouse and reciprocally transplanted back to the sites of all four source populations. A common garden was prepared within 2-6 m of the parent population at each site. Seedlings were planted systematically and monitored for two seasons. At the end of the study, all aboveground biomass was collected and separated into vegetative mass, floral mass, and seed mass. Viable seeds were separated, cleaned, counted, and weighted to estimate fitness. Vegetative biomass was also dried for fitness estimates.

We found a significant but independent effect of seedling origin and garden site on the fitness of M. fistulosa seedlings. Plants all origins performed best at one site, which may have also contributed the highest quality seedlings at each reciprocal site. Though this is not evidence for local adaptation at the watershed scale, it does imply that some source populations are better choices for restoration than others. Microclimate variation also appears to limit seedling success more than proximity between restoration site and source population.

These findings suggest that generalized seed-transfer regulations may have unintended negative impacts, and that seed sources might be evaluated based on seed provisioning in the wild, and informed by further reciprocal transplant studies at other scales.

Introduction

Ecosystem restoration aims to improve degraded landscapes, often by revegetating land with native plants (Miller and Hobbs 2007, Hobbs and Cramer 2008, Bichet, Tormo et al. 2010, Balaguer, Escudero et al. 2014, White, Fant et al. 2018). As we revegetate with native species, restoration ecologists move seeds within and beyond species ranges, usually with unknown consequences to resident, local populations.

Transferring plants between distinct populations carries a risk of genetic swamping and outbreeding depression through the disruption of co-adapted genes that provide local benefits (Hufford and Mazer 2003). Conservation officials may restrict seed transfer, and follow a 'local-is-best' approach to restoration, excluding non-local ecotypes to prevent outbreeding depression and the loss of unique genetic lineages (Broadhurst, Lowe et al. 2008). However, some ecologists have challenged the usefulness of a 'local-is-best' approach for plant re-introductions and suggest instead that the demonstrable loss of genetic diversity from fragmentation is much more concerning than the potential loss of unique lineages through outbreeding depression (Broadhurst, Lowe et al. 2008, Maschinski, Wright et al. 2013, Rehfeldt, Leites et al. 2018). The extent of local adaptation, and associated risks of outbreeding depression from mixing wild plant populations that are native to Ontario is almost entirely unknown.

While seed-transfer regulations for herbaceous species in Ontario are informal and vary across jurisdictions, tree seed-transfer is regulated formally by provincial policy, at least in projects managed by government. These policies allow for seed-transfer across watersheds, and between adjoining zones. Zones are meant to reflect potential differences in adaptive life history traits that have been observed to vary in some conifer species (OMNRF 2020). Though the zones are demarcated by climate and geological data, they are informed by relatively few peer-re viewed experimental studies, conducted only on a handful of tree species important to forestry (Beardmore and Winder 2011). Tree Seed Zones are sometimes used to guide the transfer of common herbaceous species as well, but locally rare species can be restricted at a smaller, watershed scale (CH 2014). As a result, these policies may restrict the majority of grassland species, since grassland habitats are rare on the landscape, but also an ideal reference community for early-successional restoration and revegetation of open, arid infrastructure corridors.

Local adaptation studies from southern Ontario are very few, though local adaptation was observed in the seed germination of Deer-berry (Vaccinium stamineum), a rare shrub at the northern extent of its range in southern Ontario (Yakimowski and Eckert 2007). Regional differences in seed performance are also being assessed for native prairie grasses such as *Andropogon gerardii* in southern Ontario. Early results suggest that populations at the northern edge of their range in the Rice Lake Plains are genetically indistinguishable from more southern populations from watersheds in the Norfolk Sand Plain (Deziel 2021). Local adaptation has been observed in Sitka Spruce at the periphery of its range, entirely outside of Ontario (Mimura and Aitken 2010). However it is unclear if all small, fragmented, or peripheral populations have the genetic diversity to allow for local adaptation, or have been isolated long enough to have become locally adapted (Aitken and Whitlock 2013).

The persistence of small populations depends on population genetics, demography, and site ecology (Keller and Waller 2002). Restored populations may be less fit compared to natural populations and re-introduced plants may not persist where they were historically present (Sluis 2002, Husband and Campbell 2004, Kindscher and Tieszen 2004, Godefroid, Piazza et al. 2011). Restored populations are influenced by several key factors, including, but not limited to the extent of local adaptation and associated risks of outbreeding depression. The success of restored populations also depends on the level of adaptive plasticity in source populations, maternal investment from source populations, genetic quality of the source populations, as well as year to year variation in growing conditions.

Plants can become locally adapted to variation in their environment, even at small scales (Aston and Bradshaw 1966), and may actually rely on inbreeding through increased self-fertility to achieve this (Antonovics 1968). Local adaptation to the same environmental gradient has even been observed in multiple taxa within a single community (Baughman, Agneray et al. 2019). Therefore, conservationists are often concerned about introducing non-local seeds through restoration that may threaten unique, locally adapted populations through outbreeding depression and the breakdown of co-adapted gene complexes (Lofflin and Kephart 2005, McKay 2005).

If plants are locally adapted, then we would expect them to perform better in their homesite than in foreign sites and expect they will also perform better than a foreign ecotype in their home site. In other words, we would expect to see an interaction between the site and origin effects on plant fitness. A meta-analysis of 1032 reciprocal transplants found that approximately 45% of transplant comparisons showed evidence for local adaptation in the strictest sense, but 71% documented at least a homesite advantage (Leimu and Fischer 2008). Though this study found local adaptation has been observed in species with various growth habits and life history strategies, it was more common in large populations (Leimu and Fischer 2008). A similar review found that the strength of homesite advantage increases with distance between populations, implying local adaptation, but phenotypic differences between populations were not correlated with the strength of a homesite fitness advantage or population size (Hereford 2009). However, plants from distinct lineages and multiple origins may not perform differently in a restoration setting at all (Baer, Gibson et al. 2014). Furthermore, habitat specialization is not always correlated with geographic distance, but rather with microsite variation (Bischoff, Cremieux et al. 2006), so a restoration approach that focuses only on local populations may actually miss populations best adapted to specific site conditions.

Though adjacent populations can be genetically differentiated across small-scale environmental gradients (Aston and Bradshaw 1966, Antonovics 1968) it is unclear how often mixing distinct populations has a negative outcome. While outbreeding depression has been documented in crosses from populations separated by as little as 100m, the same crosses made in different years did not show signs of outbreeding depression at all (Waser, Price et al. 2000). Although coadapted genes can breakdown when distant populations are mixed, the offspring from populations separated by intermediate distances can sometimes exhibit higher fitness than their parents, and an optimal crossing distance can be calculated (Lynch 1991). Also, Populations separated for more than 500 years may be more likely to show outbreeding depression when mixed (Frankham, Ballou et al. 2011). However, many populations in the Great Lakes basin have been isolated more recently through land-use practices and therefore may not be genetically distinct.

In addition to local adaptation by genetic differentiation, plants can also optimize their performance in heterogenous environments though adaptive phenotypic plasticity. Phenotypic plasticity is the ability to produce multiple phenotypes through environmentally cued gene expression (Thompson 1991). Plasticity itself is a heritable trait that evolves under natural under selection, and both local adaptation and plasticity can contribute to phenotypic variation (Via and Lande 1985). Some studies suggest that adaptive plasticity evolves in populations that experience environmental variation at scales less than one generation, but it can also evolve without selection favoring it, as a by-product of limited short-term selection on a specialized genotype (Rago, Kouvaris et al. 2019). Plasticity is also associated with increased genetic variability, and mutational variance, but not associated with a more rapid response to selection (Draghi and Whitlock 2012). While the relationship between plasticity and local adaptation is context specific, evidence suggests that plastic responses can predict the direction of adaptation by introducing developmental bias to the gene-pool under selection (Radersma, Noble et al. 2020). Despite its prevalence in plants globally (Stotz, Salgado-Luarte et al. 2021), phenotypic plasticity has rarely been incorporated into stewardship and restoration guidelines (Valladares, Matesanz et al. 2014).

Performance of a restored population is also influenced by propagule quality. Seed size, for example, may be a function of maternal investment, which reflects both environmental quality of the home site, and the genetic quality of parent plants (Stephenson 1984, Temme 1986). Given the pace of climate warming, local source populations may no longer be optimized to their environment (Rehfeldt, Leites et al. 2018). As a consequence, they may grow and flower, but provide fewer resources to seeds. Small source populations that suffer from inbreeding depression can contribute low quality propagules to restoration (Leimu, Vergeer et al. 2010). Generic, “local-is-best” seed-transfer policies could further restrict gene flow and actually reinforce the negative effects of habitat fragmentation in some species. Practitioners must consider the current risks of inbreeding depression as well as potential for outbreeding depression when conserving local lineages and transferring seed between populations (Basey, Fant et al. 2015, White, Fant et al. 2018).

Assisted migration, through informed seed transfer, may be necessary to maintain geneflow and adaptive potential in fragmented populations in order to cope with climate change (McLachlan, Hellmann et al. 2007, Havens 2015). Genetic rescue is the process of introducing non-local genotypes into inbred populations. Similarly, genetic admixture is the process of combining multiple seed sources into a restoration planting (Whiteley, Fitzpatrick et al. 2015, St. Clair, Dunwiddie et al. 2020). However, this is not always necessary. In some circumstances, small reintroductions from a limited gene pool are associated with heterozygote advantage, and not inbreeding (Pierson, Keiffer et al. 2007). Relying only on local sources may or may not lead to poor restoration outcomes (Maschinski, Wright et al. 2013). Given these complexities, the "local-is-best" approach to seed-sourcing for restoration may need to be re-evaluated (Havens 2015).

In order to test if seed-transfer restrictions at the watershed scale are warranted for grassland forbs, we asked if populations of *Monarda fistulosa* show evidence for local specialization within their respective, adjoining watersheds. Though this study follows a single species, *M. fistulosa* is a widespread forb, frequently used in habitat restoration across North America , and may be representative of early successional upland perennials with a similar range. This study may help to better inform seed-transfer policies for other wide-spread grassland forbs that share habitat with *M. fistulosa*.

In this study I I created a reciprocal transplant experiment to compare seedling fitness at multiple sites and from multiple origins. Seedlings grown from four wild populations of *M. fistulosa* were established into common gardens at each of the original source sites. Seedlings were monitored for 2 years, and their individual fitness was measured as aboveground biomass, seed number and individual seed mass. I asked whether populations of *M. fistulosa* from adjacent watersheds were locally adapted to their home site conditions. Specifically, I tested three predictions of local adaptation: 1) Is there a significant genotype-by-environment interaction for fitness? 2) Do local populations show a consistent homesite advantage, relative to non-local populations? 3) Do populations have their highest fitness in their home sites?

Methods

This study focused on a wide-ranging forb, native to east-central North America, Wild Bergamot, or *M. fistulosa.* Since local adaptation may be more prominent in large populations or species with large ranges (Leimu and Fischer 2008), we anticipated *M. fistulosa* would have had the opportunity to become locally adapted to different conditions across its wide range in southern Ontario. This species is also known to produce many seeds, with simple germination requirements, so relatively easy to collect, handle and propagate (Hamelin 2012). This species is also relatively fast growing, compared to similar perennial, meadow forbs. We studied four populations separated from each other by at least 30km (Table 1). The Kelso and Ancaster sites occur in topographically distinct, though adjoining, watersheds. The Cambridge and Oriskany populations are furthest from each other but occur in the same large watershed of the Grand River, with Cambridge to the North and Oriskany to the south.

Seed Collection

Seeds from each population were collected in September 2017. The dry, dehiscent fruit were collected from all capitula on 100 mature ramets. Ramets were haphazardly selected from a linear transect through the longest dimension of the population. Ramets were sampled at least 2m apart and contained at least three capitula. On average, we found capitula contain 50 florets, and therefore 50 potential fruits per capitula, and typically 2 seeds per fruit.
The capitula were allowed to dry at room temperature and ambient humidity for ten days. Seeds were cleaned from the fruit by rubbing over a wire mesh. 1/32-inch screen was used to separate seed from chaff. Fine dust and empty seeds were winnowed by hand using traditional methods. Finally, seeds were stored dry in sealed plastic bags at 5dC. Seeds were pooled by site and stored dry over winter until April of 2018. For more consistent germination, we treated all seeds to a four-week cold-moist stratification period.

Table 1: *Study sites consisting of wild populations of M. fistulosa sampled for seed used in the reciprocal transplant. Common gardens receiving transplants were established within 4m of the edge of the local wild population. Cambridge and Oriskany represent the upper and lower half of the largest single watershed, the Grand River Watershed.*

Site	*Description*	*Coordinates*
Ancaster	Clay, mid-Niagara Escarpment, meadow. Hamilton Region	43.247356, 79.950643
Cambridge	Sandy loam, railway prairie remnant, meadow. Upper Grand River	43.375632, 80.277810
Oriskany	Sandy loam, alvar thicket. Haldimand Co. Lower Grand River	42.947364, 79.945543
Kelso	Clay-loam, perched Niagara Escarpment meadow Halton Region	43.504856, 79.930844

Seed Germination

Seeds were planted in a greenhouse setting with ambient temperatures approximately 25dC. Seeds were planted in blocks of nine seedlings plugs, with two seeds sown per plug. Each block received seeds from the same population, and blocks were arranged systematically in a checkerboard pattern across the greenhouse benches to capture small scale variation in growing positions. Seedlings were numbered within their tray and randomly assigned to be transplanted at each study stie. Most seeds germinated within five days of being sown and were kept in the greenhouse until most seedlings had developed at least two nodes.

Transplants

Two sub plots were cleared to create common gardens at each site of our source populations. Plots were placed 2-6m apart and located within 2-6m of the local population. Ten seedlings from each population were transplanted into each population. Seedlings were planted systematically with five seedlings of each origin arranged in checkerboard pattern across the sub-plots. Seedlings were watered once every 3-5 days until the end of September in their first year and were not watered in the second year. No seedlings died in the first two weeks after they were planted. Several weeks later, two seedlings at the Oriskany site were upturned, likely by a browsing deer, and died0 from exposed roots next to the study plot. These seedlings were not replaced, and we kept these data points in our analysis. A few others did not overwinter well and died before the end of the study but are also included as valid data in our analysis.

Data Collection, Sampling & Analysis

At the end of September of the first growing season, all plants were measured for longest stem length(height) and recorded as dead or alive prior to fall senescence. By September of the second growing season, all plants were harvested. We measured the longest(tallest) vegetative shoots/stems (excluding flower heights) and counted the number of flower capitula/seed heads per individual plant. We then collected seeds and aboveground biomass from each individual plant. Final biomass was dried at 60dC for 48 hours and weighed. Seeds were removed from their capitula by rubbing over a wire mesh screen, and hand-winnowing chaff until this component contributed less that 1% to the total seed mass of 10 randomly selected capitula. Total seed mass was measured per individual plant, and individual seed mass was estimated by the average of ten individual seeds from each plant.

Eight seedlings died in total, and all of these are considered legitimate death; the observations are included in the data set with zero values for both biomass and seed production. Additionally, we removed two outliers from Kelso that produced extremely high biomass but did not produce seed. These plants were considered anomalies and are not considered in the data analysis.

I tested the effects of garden site, seed origin, and their interaction on fitness traits using analysis of variance. I used a log+0.01 transformation to correct for heteroscedasticity in observed values for seed number, individual seed mass, and aboveground biomass. Differences in fitness traits among origins within the same site were tested for significance using the back transformed Estimated Marginal Means. All statistical tests were performed on R (2021, version 4.0.3).

Results

By the end of the second year, the tallest plants at each site grew to between 95-118cm, though plant performance varied dramatically by site. Most plants were considerably smaller than this, with an average stem length of 34cm and an average biomass of only 2.5g. Overall, we had a 95% seedling survival rate. None of the study plants flowered in the first season, but all sites had flowering individuals in the second season. We did not detect a statistical interaction between site and origin effect that might suggest local adaptation, but rather independent site and origin effects on fitness, where all origins performed best at the same site.

Site Effects

We found a significant effect of study site on *M. fistulosa* biomass, resulting from greater performance at the Kelso site compared to the other sites (Table 2). After two years growth, plants from all origins were up to five times larger at the Kelso site than other study sites. Plants derived from all origins also produced more seeds at the Kelso site (Table 3, Figure 1b), approximately ten times more than at Oriskany, the other site where plants from all origins produced seed. Nearly all plants at Kelso flowered and produced seed, whereas fewer than 25% of plants flowered at Oriskany, resulting in fewer seeds produced at this site.
We also observed a site effect on seed size, but only between the two sites where plants of all origins produced seed, Kelso and Oriskany (Figure 3, Table 4).

The relationship between seed production and individual seed mass also differs between sites (Figure 4b). At Ancaster, a negative correlation between seed size and mass suggests a trade off in seed number and seed size perhaps due to stress, whereas a positive correlation in Cambridge suggests plants are prioritizing limited resources to reproductive fitness: both high seed number and large seed size.

Origin Effects

Plant biomass was significantly predicted by seedling origin (Table 2), driven by the high performance of seedlings derived from the Kelso population compared to the Oriskany population (Table 2; Figure 1a). Though plants from Ancaster produced the most seed at their home site, seedling origin did not predict seed production overall at all four sites (Figure 1, Figure 2, Table 3). Overall, we found no difference in individual seed size among plants of different origins (Figure 3, Table 4).
Plants of different origins share similar, weak-positive correlations between seed size and seed number (Figure 4a). We see no relationship between these traits in plants from the most productive sites, Kelso and Oriskany, implying no trade-off between investing in large seeds or many seeds.

Though overall, seedling origin explains less variation than site factors, origin did explain some variation in plant biomass where plants derived from Oriskany were consistently smaller than plants derived from Kelso populations across all sites (Figure 1), though these differences were not always statistically significant. We also observed that plants from Kelso were larger and produced more seed than plants from Oriskany and Cambridge, respectively, at their home site in Kelso (Figure 2). Plants from Cambridge produced slightly more seeds in their home site than plants from other origins, and plants grown from Ancaster seed grew slightly larger in their home site than plants at away sites, though these differences were not statistically significant (Figure 1).

Table 2: *ANOVA of second year biomass of reciprocally transplanted M. fistulosa plants, testing the effect of seed origin, and garden site. All origins are equally represented at all garden sites.*

predictor	*sum of squares*	*d.f.*	*mean squares*	*F*	*p*
origin	0.48	3	0.16	3.52	***0.02***
site	7.69	3	2.56	56.95	***<0.05***
origin x site	0.43	9	0.05	1.06	0.39
error	6.48	144	0.05		

Table 3: *ANOVA of second year seed production of reciprocally transplanted M. fistulosa plants, testing the effect of seed origin, and garden site. All origins are equally represented at all garden sites.*

predictor	*sum of squares*	*d.f.*	*mean squares*	*F*	*p*
origin	2.86	3	0.95	1.447	0.232
site	109.00	3	36.33	55.254	**<0.050**
origin x site	7.62	9	0.85	1.287	0.249
error	94.69	144	0.66		

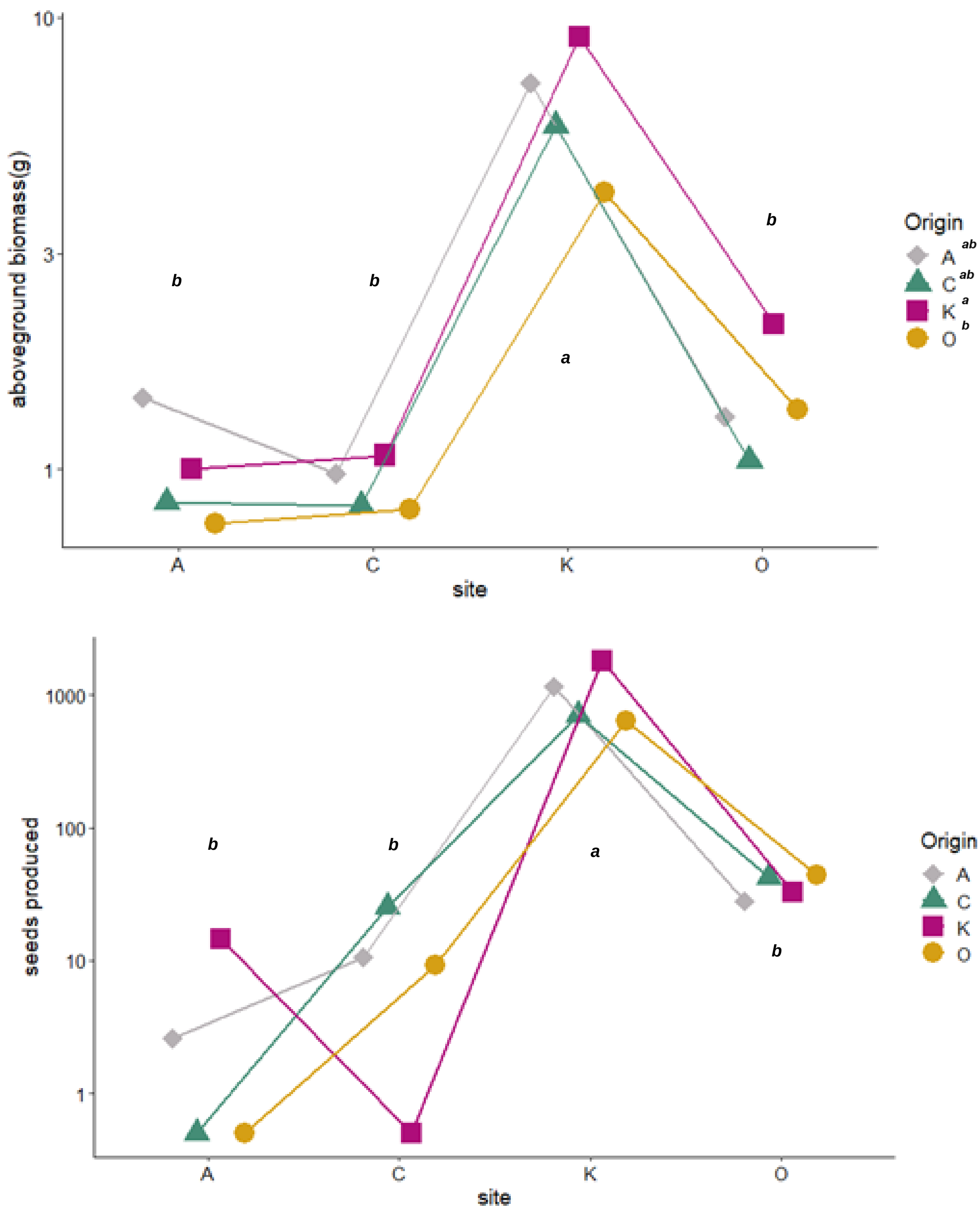

Figure 1: (a; top) second year biomass and (b; bottom) second year seed production of reciprocally transplanted *M. fistulosa* seedlings. Sites (labels on figures) and seed origins (labels on legend) that did not differ are labeled with the same lowercase letter (Tukey HSD, p<.05).

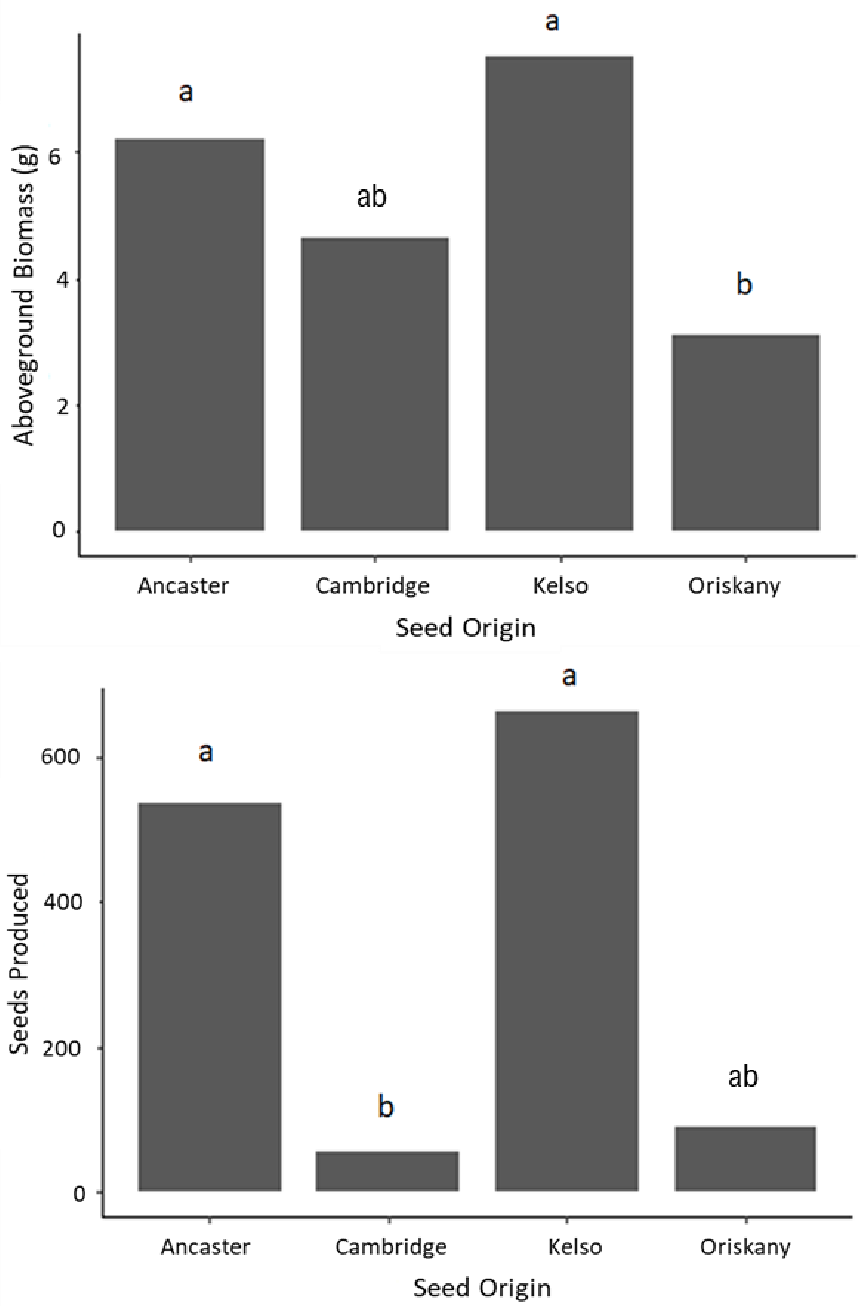

Figure 2. *Differences in mean seed production and aboveground biomass of M. fistulosa, grown from seed collected at four sites and transplanted into the Kelso site. Means are backtransformed from log values. Significance was determined using estimated marginal means and significant differences are indicated by different letters. Pairwise differences in seed production and biomass were not significant within any other sites.*

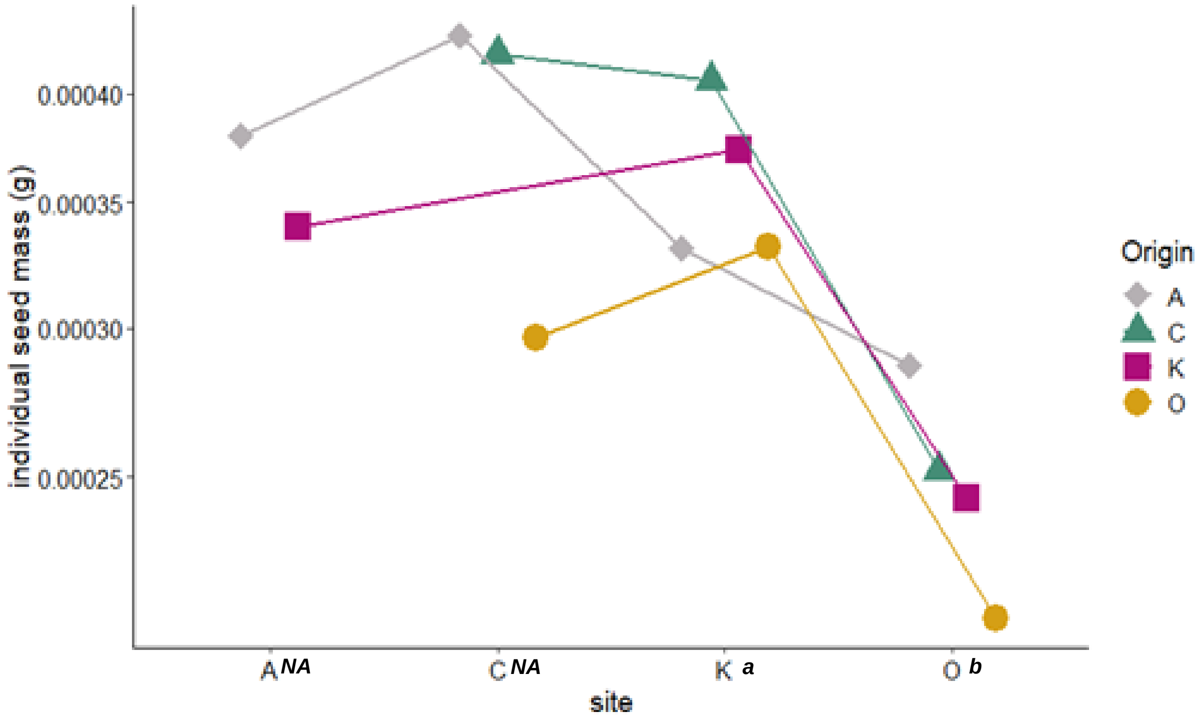

Figure 3. *Individual seed mass across sites. Contrasts could only be made between two sites since not all origins produced seed at Ancaster and Cambridge. Kelso and Oriskany did produce different sized seeds (p=0.001, contrast of estimated marginal means)*

Table 4: ANOVA of individual seed mass of reciprocally transplanted *M. fistulosa* plants, testing the effect of seed origin, and garden site. All origins are equally represented at all garden sites.

predictor	*sum of squares*	*d.f.*	*mean squares*	*F*	*p*
origin	2.240e-09	3	7.450e-10	0.535	0.661
site	2.909e-08	3	9.695e-09	6.965	**0.001**
origin x site	5.390e-08	6	8.980e-10	0.645	0.694
error	5.708e-08	41	1.392e-09		

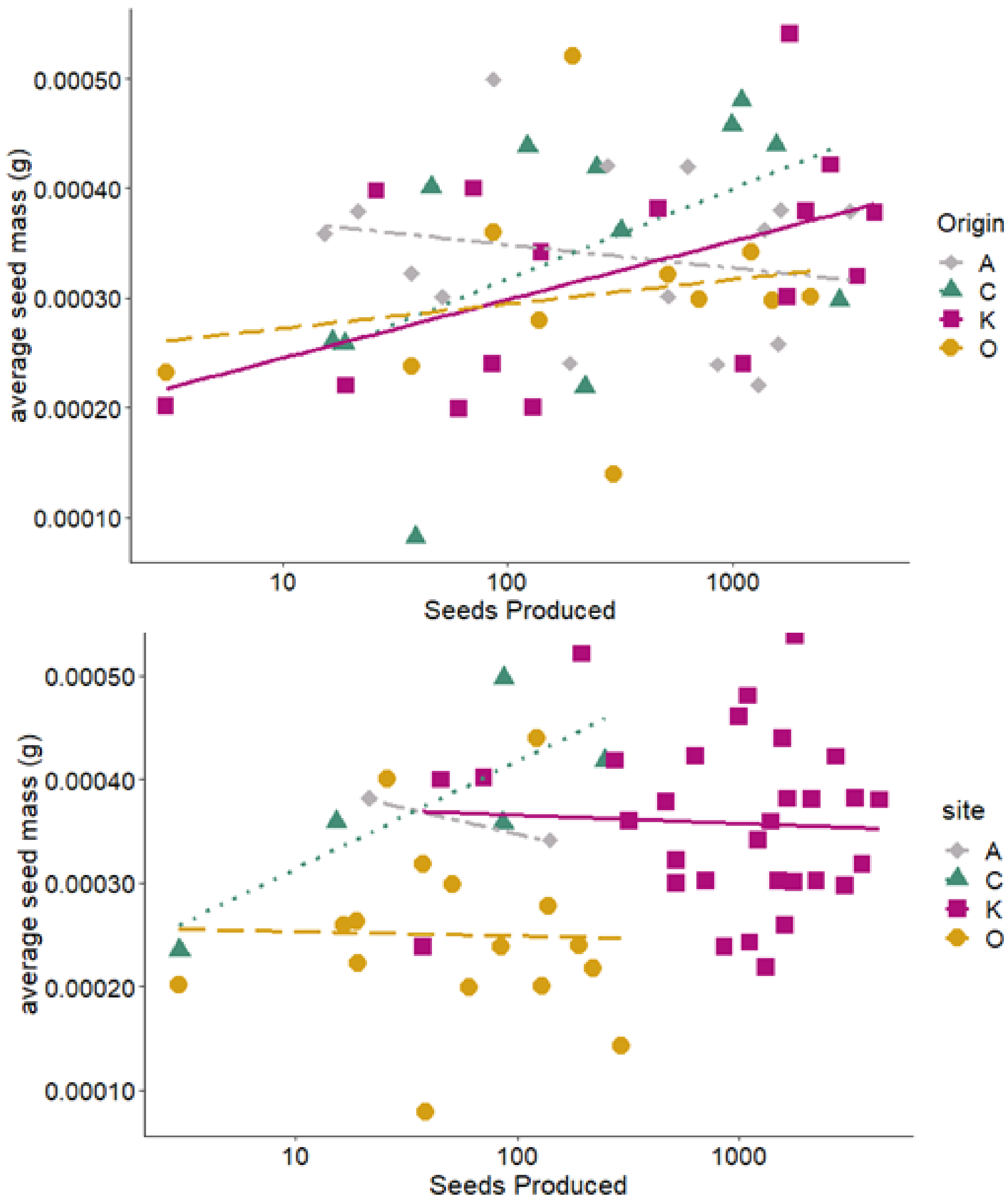

Figure 4 *The influence of origin (a; top) and site (b; bottom) on the number and individual seed mass. We found only a significant effect of site on seed number and mass Table 2, Table 3).*

Discussion

We performed a reciprocal transplant study to test if populations of *M. fistulosa* are locally adapted to the watersheds they occur in. We transplanted seedlings into common gardens established within several metres of one of four source populations. Plants were harvested at the end of two growing seasons for fitness estimates, to judge whether variation in fitness is driven by an interaction between site effects and origin effects, and whether seedlings consistently perform best when transplanted back into their homesite, the site of origin. Surprisingly, a majority of seedlings flowered at only one of four sites, though in all sites the gardens were established within 2-6 metres of where mature *M. fistulosa* plants occur. This implies that some sites were more favorable for seedling establishment than others, at least in the years studied. Though we did not detect convincing evidence for local adaptation, we did detect independent effects of site and origin on fitness.

Based on our observation of fitness, we reject the hypotheses that populations of *M. fistulosa* are locally adapted to their home site at the watershed scale. Instead, we found evidence that site and seed origin influence success independently of each other. We also observed that local-scale site differences drive regional translocation outcomes in *M. fistulosa*, and that some populations contribute higher quality propagules to translocation than others.

Site Effects

Because we did not detect an overall genotype-by-environment interaction, we can conclude that the fitness of *M. fistulosa* was driven independently by variation in environmental conditions, as well as seed quality (Via and Lande 1985). Furthermore, the strong site effect affirms that differences in conditions between sites in different watersheds significantly impacted the fitness of *M. fistulosa* but did so independently of where the seeds come from. Some of our study sites were simply more favorable to establishing *M. fistulosa* seedlings than others, regardless of seedling origin. Although it is often recommended that restoration materials be sourced from wild populations as close to the restoration site as possible (McKay 2005), these results show that local *M. fistulosa* seedlings do not perform any better than seedlings from other watersheds, particularly at stressful sites.

Variation in seedling performance can be driven by differences in site soils and climates (Liu, Wang et al. 2020). Though we did not measure soil characteristics during the study, descriptive records of site soils suggest that Kelso may have had the most intermediate soil texture, and perhaps the least disturbed soil flora. Sites may also experience favorable climate conditions for establishment in different years (Vanandel 1998). Different aspects of fitness may also be favored by temporally variable resources. For example, sites favorable for pollination and seed set may not be favorable for seed provisioning later in the season. This was observed at Oriskany, where all plants flowered, and produced roughly 100 seeds each, yet individual seeds had a low mass (Figure 3).

Though common garden plots at each site were located within four metres from the source populations used in this experiment, seedlings may not have been transplanted into a favorable microsite. *M. fistulosa* seeds are relatively thinned shelled and desiccate after long dry periods. Therefore, seedlings may be able to thrive under only a narrow range of ideal conditions, and those conditions may not actually be found within 2-6m of the parent plants at most populations studied. The performance of *M. fistulosa* is apparently impacted more by fine-scale variation in growing conditions than climate or ecosystem features that vary at the watershed scale. Even where local adaptation has been detected in grassland forbs, populations were differentiated among local microsites rather than between broader geographic regions (Bischoff, Cremieux et al. 2006). The results are also consistent with annual variation in favorable establishment conditions (Waser, Price et al. 2000), and we might observe a different pattern if seedlings were established in a more favorable year

Some plants may become adapted to establishing in specific microsites created by pioneer species and show poor establishment away from such “nurse” species (O'Brien, Carbonell et al. 2020). *M. fistulosa* may be locally adapted at a fine scale to different nurse environments that help seedlings cope with stress. Given the small, thin-walled seeds of *M. fistulosa*, this species may relay on establishing in biocrusts or in the shade of other plants that moderate the microclimate (Havrilla, Leslie et al. 2019). Perhaps in the preparation of the common-garden beds, the nurse environment was removed, which impacted growing conditions at three of the four sites studied.

At all sites but Kelso, optimal establishment conditions may no longer occur. These site differences could reflect a change in the biotic community since the parent population established, potentially associated with a change in climatic conditions, or the introduction of invasive competitors, herbivores, or disease. Highly fragmented and disturbed communities often have extinction debt, evidenced by low genetic diversity or fitness in some populations of adults that are otherwise able to persist (Helm, Oja et al. 2009). Indeed, reproductive traits of plants in some communities reflect historical landscape structure, suggesting that extinction debt is driven by a mismatch between functional traits and current conditions of altered habitats (Lecoq, Ernoult et al. 2021). Therefore, we might conclude that the three populations of *M. fistulosa* occurring at sites where seedlings of all origins performed poorly are a kind of ‘ghost’ of past ecosystems and associated growing conditions that no longer exist (J.S. Harding, E.F. Benfield et al. 1998). Present diversity can reflect past land management practices

(Mennicken, Kondratow et al. 2020), and so populations with low recruitment may no longer be growing in conditions favorable to seed germination or seedling establishment.

Origin Effects

M. fistulosa does not appear to be locally adapted at the watershed scale, given the lack of genotype-by-environment interaction affecting fitness. Overall, the fitness of *M. fistulosa* seedlings was significantly influenced by their origin, independent of where they were planted. This implies that some source populations may produce seeds that are larger and healthier than others. Seed quality may be influenced by the identity of parents and their own genetic quality, as well as increased maternal investment from source plants growing in ideal conditions (Stephenson 1984, Temme 1986, Mojzes, Kalapos et al. 2021).

Populations in ideal conditions may pass along a fitness benefit to their offspring by producing higher quality seeds. Larger seeds produce better restoration outcomes in other grassland plants (Jakobsson and Eriksson 2000), so maternal investment might explain seedling performance in our study. Although maternal stress can increase offspring performance in some early successional plants (Mojzes, Kalapos et al. 2021), we did not find that the sites where seedling struggled most also contributed the best performing seedlings overall. Based on differences in second year biomass and seed production between genotypes across all sites, we might conclude that the parent population at Kelso is the heathiest, or the least stressed, and that both Cambridge and Ancaster may be relatively unhealthy and more highly stressed. We also see that where significant differences between origins do occur, it is plants that originate from Kelso that perform best, potentially due to increased seed provisioning at their maternal site, though this was not measured. A homesite-advantage may only be evident in well provisioned seedlings, or in ideally situated populations. Seedlings grown in less ideal sites may simply be less capable of displaying ranges in fitness that might signify a local homesite advantage.

Though seeds were collected from the same number of individuals from each original population, all populations are nested within broader, regional metapopulations, each with a potentially unique level of genetic diversity. Seeds collected from genetically diverse populations typically generate better restoration outcomes (Reynolds, McGlathery et al. 2012), and so Kelso may be the most genetically diverse population, while the three other sources may suffer from inbreeding (Leimu, Vergeer et al. 2010).

Conclusion

These results echo another study of *M. fistulosa* restored in Ontario that found significant variation in the fitness of seedlings from different sources but found no difference between those that were collected from natural populations and those collected from restored, and arguably less diverse populations. Instead, they concluded that wild populations had larger maladaptation differentials and gradients than hypothesized, because the *M. fistulosa* habitats are frequently degraded (Hamelin 2012).

Our study demonstrates that the "local-is-best" approach to restoration may miss opportunities to improve the success of plant reintroduction. It may be worthwhile to compare the performance of different potential source populations, or incorporate multiple sources to ensure restoration genotypes are well matched to the site characteristics (Havens 2015, St. Clair, Dunwiddie et al. 2020). Optimal source populations could be identified by weighing seeds to infer quality where few seeds are produced, or through reciprocal transplants. In perennial forbs like *M. fistulosa,* this test could be accomplished within a few growing seasons, and in the end, save on long-term project costs. Furthermore, these findings imply that soil characteristics, rather than the resident local plant community would best inform species selection for restoration.

Conservation policy increasingly relies on population genetics, and the science of local adaptation to frame assisted migration and climate adaptation strategies. Our work shows that generalizing seed-transfer protocols may prove difficult, and that local seed sources may not always be the best for restoration (OMNRF 2020). Local specialization is an interpretation of individual comparisons between populations of the same species; therefore, the ecological meaning of that specialization differs between comparisons, and varies across scales of space and time. These complexities mean that all seed-transfer should be approached on a case-by-case basis.

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CHAPTER 3 Revegetation at the roadside: does method matter and do bees benefit?

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Preface

Restoring roadsides with native grassland plants may provide several benefits, including reduced long-term maintenance costs, increased ecosystem services and habitat for wildlife. However, the use of native seed to revegetate roadsides is not a common practice in Ontario. This may be in part due to a lack of scientific evidence to inform seed installation and to clarify the expected benefits. In this report, we present the results of our work studying the use and ecological benefit of native grassland plants for revegetating roadsides in southern Ontario. In experimental native seedings along Ontario roadsides, we tested the efficacy of different seeding methods in achieving target native plant cover, and in increasing local bee abundance and diversity.

We found that the density of target native plant species in the first three years was increased by doubling the seeding rate from 15kg to 30kg, or by using a two-stage seeding method (broadcast seeding followed by hydro mulching) at a low rate. The one-stage method underperformed at a low seeding rate, likely because the one-stage hydroseeding method allows more seed to be lost in the equipment and reduces seed-to-soil contact, decreasing germination and seedling survival. We therefore recommend that seeding rates be doubled or a two-stage method be used when hydro-seeding with native plant seeds.

In addition to increasing native plant diversity, we found that native plantings increased bee abundance in some locations. Therefore, successful native plantings may increase pollinator habitat, which in turn could benefit nearby agricultural areas. However, bees did not respond positively to restoration at all locations, and average bee abundance also varied significantly between different geographic areas. This variation among locations suggests that landscape factors, such as habitat connectivity, natural cover and land use, can have a significant impact on bee communities. Our findings can help to inform best practices for native plantings along Ontario roadsides. We have also included in the appendix a jurisdictional scan detailing the restoration approaches of other North American jurisdictions. Altogether, we present evidence that revegetating roadsides with native grassland plants can have positive impacts on both threatened plant communities and our native bee species.

Introduction

Roadsides are often degraded environments and may be challenging to revegetate with ecologically appropriate plants, especially on erodible slopes, on contaminated ground, or in the presence of invasive species (Forman and Alexander 1998, Staab, Yannelli et al. 2015). Despite the challenges of harsh growing conditions, roadsides can also be ecologically valuable habitats (Phillips, Bullock et al. 2020). Often these landscapes are targeted for restoration, especially in areas fragmented by agriculture, urbanization and forestry (Jacobson, Fukamachi et al. 2014, Lázaro-Lobo and Ervin 2019). Though already common practice in some jurisdictions across North America, interest in using native plants to revegetate roadsides in Ontario has grown slowly (Appendix 1, Ontario Ministry of Transportation jurisdictional scan).

In many cases, the use of native seed in restoration is more effective at meeting project goals than traditional cover crops (HarperLore and Wilson 1990, Arthur and Gartshore 2004, Tinsley 2007, Brandt, Henderson et al. 2011, Grant, Nelson et al. 2011). Roadsides planted with native species may reduce the long-term cost of revegetation. Since many native grassland plants are adapted to drought-prone soils, they may require less water to establish than traditional exotic cover crops. Tall prairie grasses may help act as natural snow fencing and reduce plowing costs (Johnson 2008). Grasslands have also been known to sequester carbon, improve water quality, and mitigate flooding (Forman and Alexander 1998, Ruiz-Jean and Aide 2005, Kucharik, Fayram et al. 2006, Hansen and Gibson 2013). Road verges may also act as habitat for pollinating insects, allowing them to migrate, and colonize new habitats (Hopwood 2008). Ecosystems that are ecologically connected through mobile organisms like pollinators have increased levels of other ecosystem services that plant communities provide (Bullock, Aronson et al. 2011, Phillips, Bullock et al. 2020).

Roadside Restoration Best Practices

The demand for roadside restoration has generated numerous guiding documents that offer best-practices for creating native grasslands along roadsides (Swan, Cripps et al. 1993, Armstrong, Roberts et al. 2001, Robson and Kingery 2006, Neufeld 2008, IDT 2009, Brandt, Henderson et al. 2011, FHWA 2017, Harper-Lore, Johnson et al. 2017, WDT 2017). However, recommendations in these documents are rarely supported by experimental evidence or peer-reviewed literature. While they are helpful in documenting both successful and unsuccessful restorations, they serve as anecdotal evidence for best practices rather than a scientific test of how variation in these practices impacts restoration outcomes. Native seed addition will typically result in increased plant diversity, at least in the short term (Grant, Nelson et al. 2011), yet few peer-reviewed, and manipulative experiments have compared seed installation methods, and none have been conducted in Ontario.

Best practices for roadside restoration have been studied, but few experiments have tested how variation within a particular seed-installation methods on establishment of target plants. Experiments on US highways have found that tilling the site prior to seeding increases target plant cover, that the presence of invasive species reduces target cover and that fertilization has no effect on target cover (Skousen and Venable 2008). Studies have also shown that conservative grassland species can perform as well as generalist plants, though target cover of grassland species increases on sandy or coarse soils (Haan 2010). On roadsides in Newfoundland, restored community composition was a function of roadside microsite variation, sorting species based on their auto-ecological traits. A related study demonstrated that installing seeds under a layer of mulch can be more successful than installing the seeds over top of the mulch (Karim and Mallik 2008, Mallik and Karim 2008). While these examples clarify patterns of plant establishment in roadside environments, the plants and communities studied are not representative of open habitats in southern Ontario. These experiments also do not test how variation in grassland seed installation methods impact roadside restoration outcomes.

In practice, the method of seed installation may vary considerably by site, target species, and project goals (Williams 2013, Harper-Lore, Johnson et al. 2017). Hydroseeding is a popular method that involves spraying a slurry of seed and water onto the site. This slurry may also include bulking agents and tackifiers which help seed stick to the site. This slurry is typically mixed continually in a mobile tank to ensure the seed is evenly suspended, and then forced through a valve-controlled hose to apply the slurry across the site. Hydroseeding is often viewed as the most efficient way to seed verges around infrastructure, but this method can also promote weeds, and lead to poor restoration outcomes compared to broadcast or mechanical drill seeding (Faucette, Risse et al. 2006, Matesanz, Valladres et al. 2008, Mola, Jimenez et al. 2011). Factors such as slope, aspect, and the type and number of hydro-seed stages used can all influence the restoration outcome (Gonzalez-Alday, Marrs et al. 2008). Determining best practices for hydroseeding cannot be done by comparing different restorations, as species selection, site preparation, timing of seeding, and variation in equipment use between operators may all overshadow any meaningful effects of seed installation method on restoration performance. A planned experiment is the best way to assess best practice in hydroseeding.

Roadside Restoration Performance

The success of roadside restoration can be scored based on the performance of target vegetation. Performance may be evaluated based on growth or visual cover, measures of above ground biomass, or measures of floristic quality and diversity (Chiarucci, Wilson et al. 1999, Jog, Kindscher et al. 2006). Some restoration targets evaluate the fidelity of a restoration to either a local reference community, or to the seed mix used. It can be difficult to compare results of different restoration studies because of the variation in metrics reported. Furthermore, there may be good reason to evaluate other ecological indicators to understand how restoration affects specific ecological processes. However, only about 38% of studies evaluate or monitor more than two ecological indicators (Ruiz-Jean and Aide 2005).

Since the purpose of roadside restoration is often to support wildlife, measuring the response of other communities to native plant addition is an obvious performance indicator. Though roadsides present a unique restoration opportunity, it remains unclear if increasing native plant cover or diversity near roads supports greater insect populations. Some restored roadsides can attract a greater diversity and abundance of pollinators (Hopwood 2008), but paradoxically roads are responsible for a large number of insect deaths (Baxter-Gilbert, Riley et al. 2015). Roads bordered by meadows are responsible for greater insect mortality than roadsides bordered by lawn grass and may therefore be poor choices for restoration (Keilsohn, Narango et al. 2018). However, pollinator road-mortality increases when pollinator populations increase, so population dynamics cannot be judged on death rates alone. The extent to which roadsides benefit populations may be landscape and species dependent (Munguira and Thomas 1992). Thus, further investigation into the effects of roadside restoration on pollinator populations is required.

Questions & Hypotheses

With growing interest in pollinator health, land managers are seeking ways to create and improve existing habitat for bees and butterflies (Pindar, Mullen et al. 2017). At the onset of this research, the Ontario Ministry of Agriculture, Food and Rural Affairs had drafted legislation to support a *Pollinator Health Action Plan*, which called for private and public land managers to restore and conserve crucial bee habitat. In response, The Ontario Ministry of Transportation has identified over 160,000km of roadside habitat that could be restored or managed to increase pollinator habitat (Weber, Baker et al. 2020). Our research objectives were developed in collaboration with MTO staff, and the project was supported through the *Highway Infrastructure Innovation Funding Program*. We began by summarizing the grassland restoration methods used in other regions of North America. We have also summarized the political and institutional support that has allowed the rapid and wide-ranging use of native grassland species within infrastructure corridors including railways, hydro corridors, green roofs, and roadsides (Appendix 1). We then designed two separate approaches to research and evaluate seed-based ecological restoration at the roadside.

We designed a field experiment to test how variation in seeding practices impacts the success of restored roadside grasslands. In a factorial design, we manipulated seeding rate and hydro-seed application methods randomly across plots within a series of roadside restorations in 2016. A third of our plots were mowed in both years following restoration, a third were mowed only in the first year and a third did not receive a mowing treatment. We also established control plots with a non-native seed mix to compare establishment rates with the native species seed mix. We measured several metrics of restoration success density, including cover, plant diversity, floristic quality bee diversity. We ask the following questions:

i) How did the seed installation methods affect restoration success?
ii) Did mowing frequency impact the restoration success?
iii) Do roadsides that have recently been restored with native grassland plants support a greater abundance or diversity of bees than nearby unrestored roadsides?

Methods

Roadside Study Sites

We selected 2 highways in southwestern Ontario to conduct our manipulative experiment. One is located on Ontario Highway 7, south of the town of St. Mary's (SM), in a region dominated by clay loam soils. The second is located on Ontario Highway 3, south of the town of Tillsonburg (TB) in a region dominated by sandy soils, with a more recent history of intact prairie-grassland vegetation communities. We chose roadsides that were greater than 6m wide and were dominated by exotic pasture or sod grasses. Sites were adjacent to agriculture, residential and commercial lawn space, and contained a variety of mostly exotic species, though native *Solidago* and *Symphyotrichum* species were present at all experimental sites prior to seed augmentation.

All sites were selected on the north side of provincial highways in sections oriented east –west. At each location, we created two 200x5m rectangular experimental sites in 2016, running roughly parallel to the road edge, by clearing the existing vegetation. At TB, this was accomplished by applying a .01% glyphosate solution to the area, followed two weeks later by rototilling. At SM, because the existing pasture grasses had formed dense hummocks, with more accumulated biomass, we used a strip-dozer to remove 6-8 inches of topsoil, along with the sod.

Experimental sites were divided into a linear sequence of 5x5m plots. A native seed mix was installed in November, though the installation method varied between each plot. Plots received either a high or low rate of seed (30kg/ha or 15kg/ha respectively), which was either installed using a one-stage hydro-seed application, or hand-broadcast first and hydro-mulched after using a two-stage installation method. These treatment combinations were randomly applied to subplots, along with four control plots at each site.

The seed mixture was almost identical in both locations, though three and four species were included in the mix at only one site (Table 1). Species ratios are based on the size of individual seeds, with small, fine seeds included at a lower level than large, coarse seeds (grasses). Some larger seeded forbs were included at twice the rate of forbs with smaller seeds. Switchgrass, unlike other grasses sown has a small, fine seed and was included at a lower level.

We also applied a mowing treatment randomly to plots to study the impact of repeated annual mowing. Plots were either mowed each year, mowed once after the first year, or were not mowed. We compared mowing frequency with target cover in the third year, as well as invasive cover in the third year. To represent invasive cover, a subset of the exotic plant community was classified as invasive based on ability to form monocultures in the local landscape.

Vegetation Measurements

A summary of our plot treatments and measurements is included in Table 2. At the end of the first growing season, we estimated the proportion of individual native seedlings in a 3/4m2 subplot of each plot. Subplots were located haphazardly, but roughly in the centre of each plot and at least 1m from the edge of the plot in any direction. Rarely, vegetative shoots from pre-existing vegetation persisted by the end of the first year. For our estimates, we considered perennial ramets as equivalent to individual one-year old seedlings.

In August of the second growing season, we estimated the proportion of target native plant cover using the biomass of plants growing within a 3/4m^2 sub-plot for every plot. The top five most visually abundant plants were clipped to the ground, their biomass collected, and dried for 24hours before being weighed. Any other species that were present in lower abundance were noted, but not collected. Biomass collection was followed by a second round of mowing for plots that received annual mowing. At the end of the third growing season, biomass was collected as above to estimate the proportion of target species in each plot. Data were analyzed using ANOVA, following a transformation of log+0.01.

We also calculated the Floristic Quality Index (FQI) of the restored communities after three growing seasons. We calculated FQI using an equation for grassland monitoring that relies on presence/absence of species in each plot (Jog, Kindscher et al. 2006) and not their relative biomass within plots. Coefficients of Conservatism used in the FQI calculations are derived from species occurrence information in Ontario (Oldham, Bakowsky et al. 1995).
Since each site received a different species of *Verbena* and *Penstemon,* occurrences of these taxa are reported only to genus level in our results. We also used the average Coefficient of Conservatism of *P. hirsutus* and *P. digitalis*, as well as *V. hastata* and *V. stricta,* to calculate FQI scores.

Bee Community Measurements

We documented the relative abundance and diversity of bees at the restored sites, compared to paired, un-restored, control sites. These control plots were located roughly 2km from their paired experimental restoration site, on the same side of the road.

The bee survey protocol followed standard bee cup trapline sampling protocols (Roulston, Smith et al. 2007). The local insect community was sampled by using pan-traps (small, brightly coloured cups filled with slightly soapy water). We began sampling in July of the summer following seed installation. Each year, we sampled every two weeks until the end of September, but in the second year we began sampling in June, and in the third year we began in May. Sampling days only occurred on calm, dry days with temperatures over 16 degrees.
Sites were sampled by placing 20 traps, roughly in a line, 5m apart, but avoiding tall, dense, or woody patches of vegetation. Traps were placed across each 5x300m restored roadside site, and across an unrestored control site with similar dimensions. Traps were left out for 8-hour periods, after which all arthropod samples were retrieved and placed in ethanol. All bee and wasp specimens were isolated, and bee species were identified to genus level.

We used ANOVA to test for differences in abundance between sites in each year. To represent changes in species richness, we used the iNEXT program in R to create saturation curves of species richness as a function of sampling effort. This provides an extrapolation of our observations to help judge if our sampling method was adequate to capture the true diversity present. While this is not a statistical test, we can conclude that sites are different in their diversity if their confidence intervals do not overlap.

Table 1 *Species included installed in the seed mix at both sites unless indicated. Low Rate is shown at 7kg/ha; the amounts are doubled for the High Rate at 14kg/ha*

Species	kg	%	FQI
Forbs			
Aquilegia canadensis	0.1	1.43	5
Asclepias syriaca	0.2	2.86	0
Desmodium canadense	0.1	1.43	5
Drymocallis arguta	0.1	1.43	7
Heliopsis helianthoides	0.2	2.86	3
Lespedeza capitata	0.1	1.43	7
Monarda fistulosa	0.1	1.43	6
Oenothera biennis	0.1	1.43	6
Rudbeckia hirta	0.1	1.43	0
Rudbeckia laciniata	0.2	2.86	7
Solidago ptarmicoides	0.1	1.43	9
Symphyotrichum novae-angliae	0.1	1.43	2
Grasses			
Elymus canadensis	1	14.29	8
Elymus riparius	1	14.29	7
Elymus villosus	1	14.29	7
Elymus virginicus	1	14.29	5
Panicum virgatum	0.1	1.43	6
Sorghastrum nutans	1	14.29	8
Tillsonburg Only			
Lupinus perennis	0.1	1.43	10
Penstemon hirsutus	0.1	1.43	7
Sporobolus cryptandrus	0.1	1.43	2
Verbena stricta	0.1	1.43	7
St. Mary's Only			
Juncus tenuis	0.2	2.86	0
Penstemon digitalis	0.1	1.43	6
Verbena hastata	0.1	1.43	4

Table 2 *Summary of plot treatments, and measurements.*

Treatments	***Description Summary***	***Date***
Rate	low(15kg) or high 30(kg)	Nov. 1, 2016
Method	1-stage (hydro-seed) or 2-stage (hand-seed and hydro-mulch)	Nov. 1, 2016
Mow 1	2/3s of plots were mowed to 20-50cm	Sept 1, 2017
Mow 2	1/3 of previously mowed plots are mowed again to 20-50cm	Sept 1, 2018

Measurements	***Description***	***Date***
Seedling Number	number of all seedlings within m^2 (number of ramets or culms of existing vegetation not destroyed in site preparation)	July 15-30, 2017
*Aboveground Biomass 1**	within m2 including floral and vegetative mass (g)	Aug 1-15 2018
*Aboveground Biomass 2**	within m^2 including floral and vegetative mass (g)	Aug 1-15 2019
Floristic Quality Index	Rank based on species conservatism, three years after restoration	Aug 15 2020

All plant species were collected and later sorted into groups of native, native-target and non-native species for analysis.

Results

Vegetation Cover & Community

There was an effect of site on initial target seedling density (Table 3; Figure 1), with differences observed between adjacent sites in SM and between SM West and TB West. A two-stage method produced more target seedlings overall. As anticipated, initial target seedling density was higher in plots seeded at a high rate (Table 3; Figure 1). Doubling the seeding rate resulted in nearly three time the target seedling number using a one-stage method. However, doubling the seeding rate only doubled the target seedling density using a two-stage method. This difference is largely explained by an interaction between seeding rate and seeding method, such that a double-stage method improved the outcome of a low seeding rate more than the outcome of a high rate (Table 3; Figure 1). The interaction is pronouncing an otherwise significant independent effect of seeding method (Table 3), with a two-stage method producing a greater number of target seedling on average. We also detected an interaction between site and seeding rate, shown as a greater increase in target seedlings, form the low rate to the high rate, at the TB sites than at the SM sites. (Figure 2, Table 3).

We also compared plots established with a two-stage method to control plots seeding with a "Pipeline Blaze" mix at the producer's rate and using the recommended two-stage method. Significantly more target native seedlings were found in the plots seeded with the native mix, than target cover crop seedlings were found in the plots seeded with the Blaze mix (p=0.019, Type III Anova, df=1, F=5.666. SS=21943; Figure 2).

Table 3 *ANOVA Table of results for initial target seedling cover*

source	sum of squares	d.f.	F	sig.
site	32626	3	4.17	***0.008***
rate	111725	1	42.9	***<0.01***
method	15632	1	5.99	***0.017***
site x rate	34472	3	4.41	***0.006***
site x method	3924	3	0.50	0.682
rate x method	19409	1	7.44	***0.007***
site x rate x method	44545	3	0.58	0.629
residual error	208491	80		

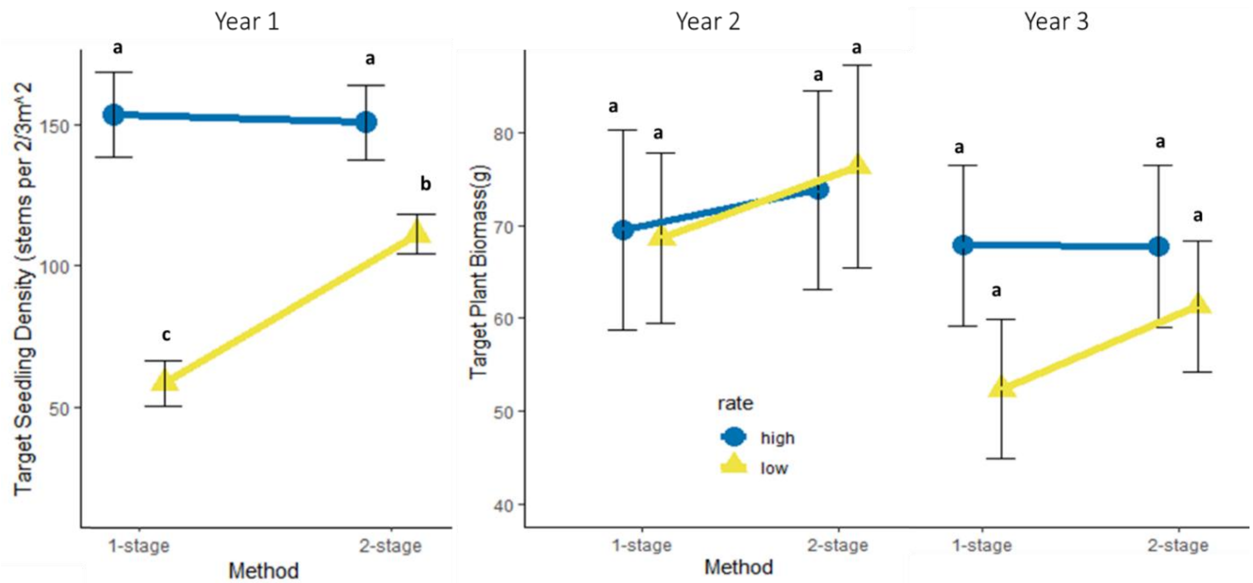

Figure 1 *Initial seedling density, and subsequent target native plant biomass for three years following roadside restoration. Significantly different outcomes are assigned different letters.*

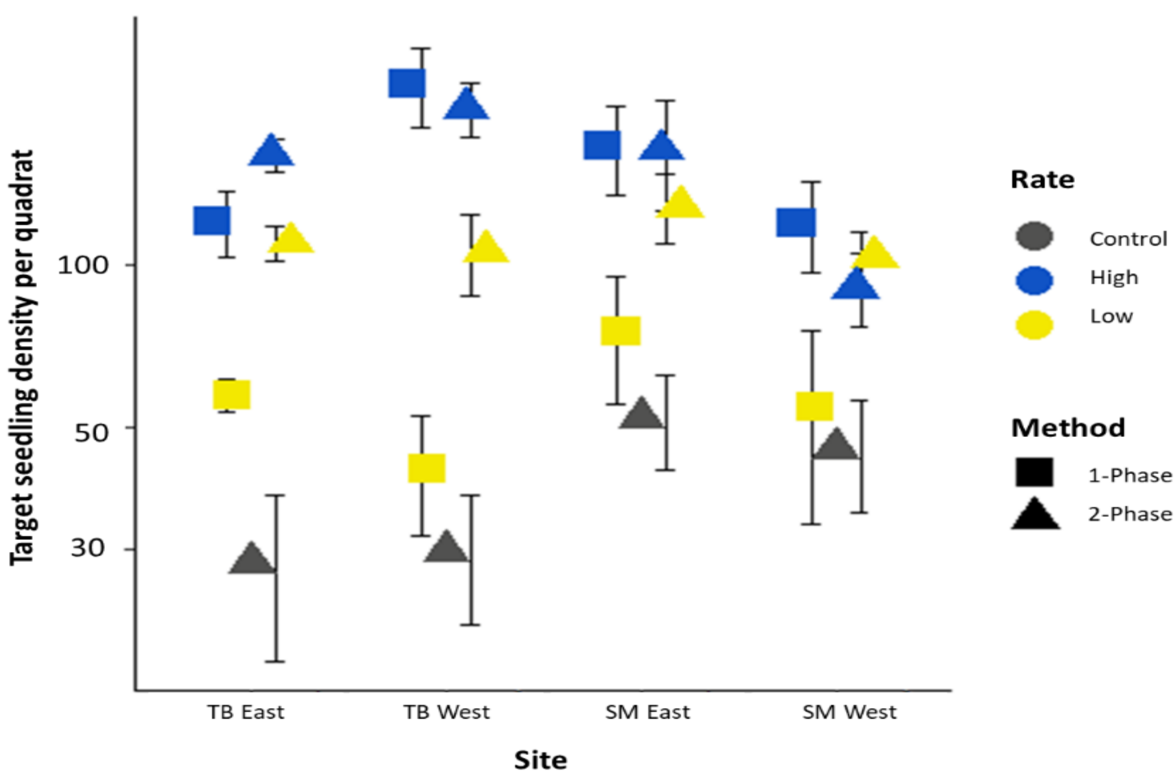

Figure 2 *Effect of site, method, and rate on initial target native seedling density, compared with control seeding of traditional cover crop.*

There was a nearly significant effect of site on target plant biomass in the second growing season (2018, Type III Anova, $p=0.068$, $df=3$, $F=2.473$, $SS=14739$). This effect is driven by the Tillsonburg East site which produced less target biomass compared with all other sites ($p<0.05$, contrasts of estimated marginal means). We did not detect any effect of site, method or rate on target plant biomass by the end of the third season.

We also did not detect any relationship between target biomass and mowing regime by the end of our study. Though mowing has been associated with restoration outcomes in other studies, we did not detect a relationship between mowing frequency and non-native plant biomass by the end of the third growing season ($p=0.463$, Type III Anova, $df= 1$, $F=0.544$, $SS=2500$). Target species cover by the end of the third year was also not associated with mowing frequency ($p = 0.236$, Type II Anova, $df=1$, $F=1.429$, $SS=2116$).

We observed the roadside plant community composition change over the course of our experiment. Species that established quickly in the first year and dominated in the second year like *Oenothera biennis*, were largely replaced by a more diverse assemblage of target native plants (Figure 3). After three years, the Floristic Quality Index was higher at both TB sites than SM sites (Table 4, Figure 4a). However, one of the most abundant target species in the third year*, Rudbeckia hirta*, has a Coefficient of Conservatism of 0, which means it contributes little to FQI scores (Figure 3, Table 1).

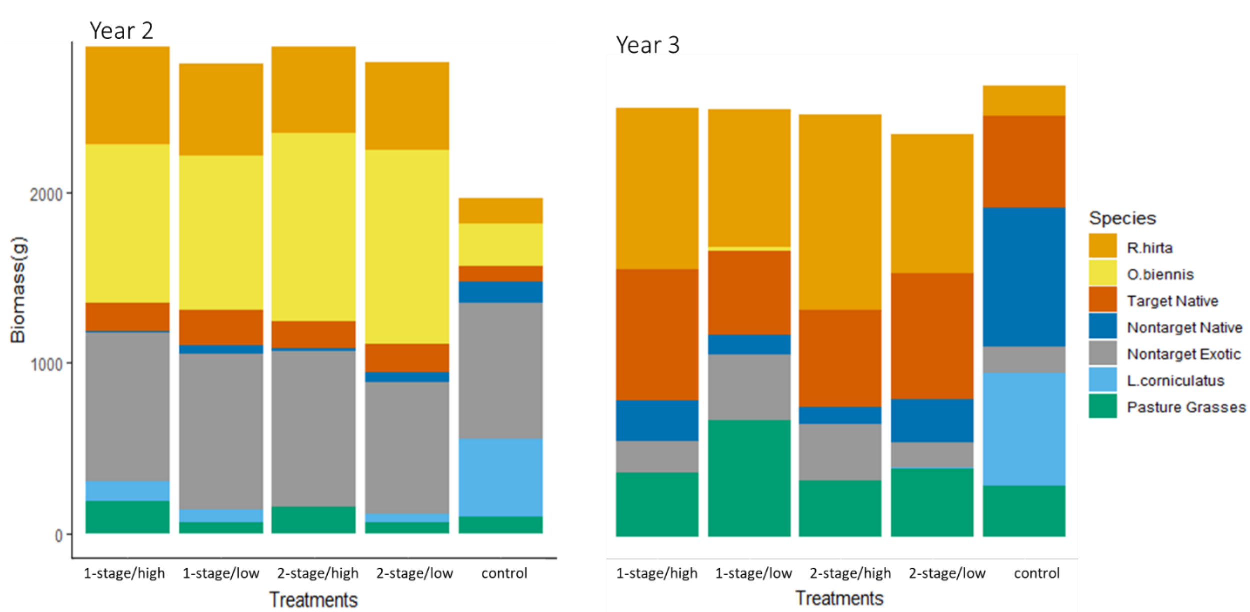

Figure 3 *Changes in dominant vegetation biomass across treatment types from second to third growing season*

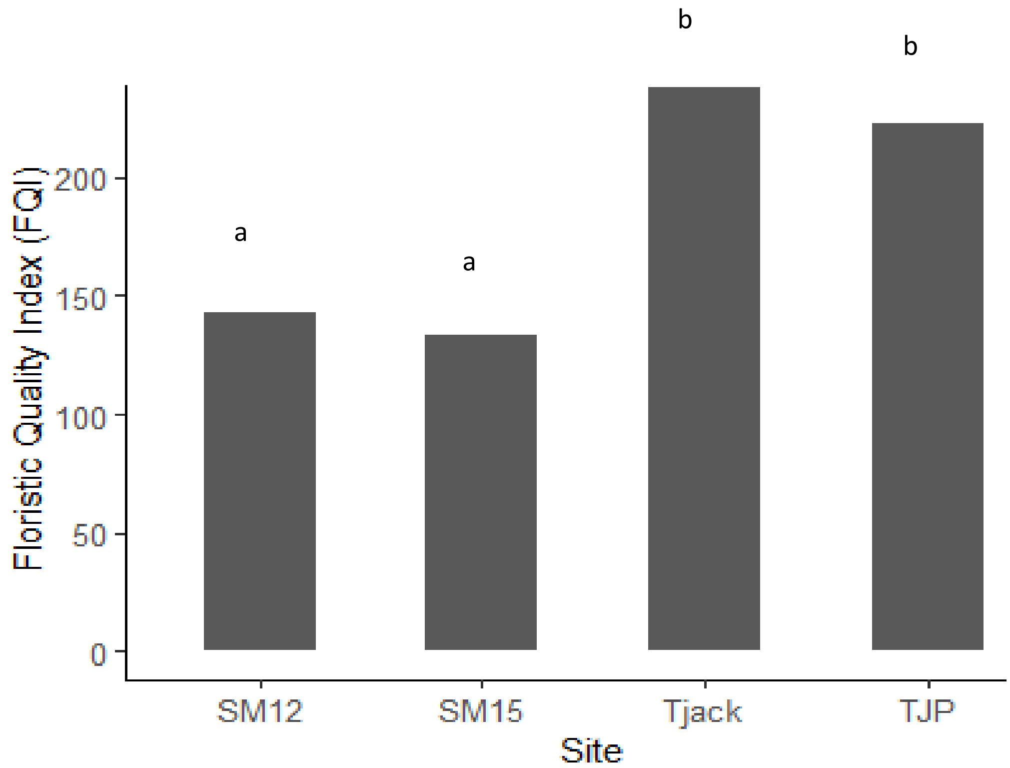

Figure 4 *Floristic quality of restored roadisde sites*

Table 4 *Type III ANOVA results showing impact of site and seeding approach on floristic quality.*

source	**sum of squares**	**d.f.**	**F**	**sig.**
site	99.22	3	6.643	***0.001***
rate	0.450	1	0.090	0.765
method	0.020	1	0.004	0.951
site x rate	21.22	3	1.421	0.242
site x method	15.62	3	1.046	0.376
rate x method	0.060	1	0.013	0.909
site x rate x method	13.77	3	0.922	0.434
residual error	398.3	80		

Bee Abundance & Diversity

At our experimental roadside restoration sites, we collected 797 individual bees across three years from 21 different genera (Figure 5). As expected, the trapping method favored small bees. As a result, few honeybees or bumble bees were caught, though they were commonly observed on the landscape visiting plants at the restored sites. Over three years, the abundance of bees increased in restored sites compared to their paired unrestored sites, but only at sites in Tillsonburg. This increase in bee abundance was not observed at SM sites, although target plant cover was similar in both locations. Landscape level differences were not quantified as part of the present study, but may include differences in agricultural intensity, forest cover, and connectivity.

We did not detect a notable difference in bee diversity between restored and unrestored sites (Figure 6). Diversity increased slightly over three years at all sites, though the weak relationship between sample size and diversity means that our estimates are likely inadequate to draw conclusions. This result highlights the tremendous amount of effort required to gather meaningful data with regards to community response to restoration over time.

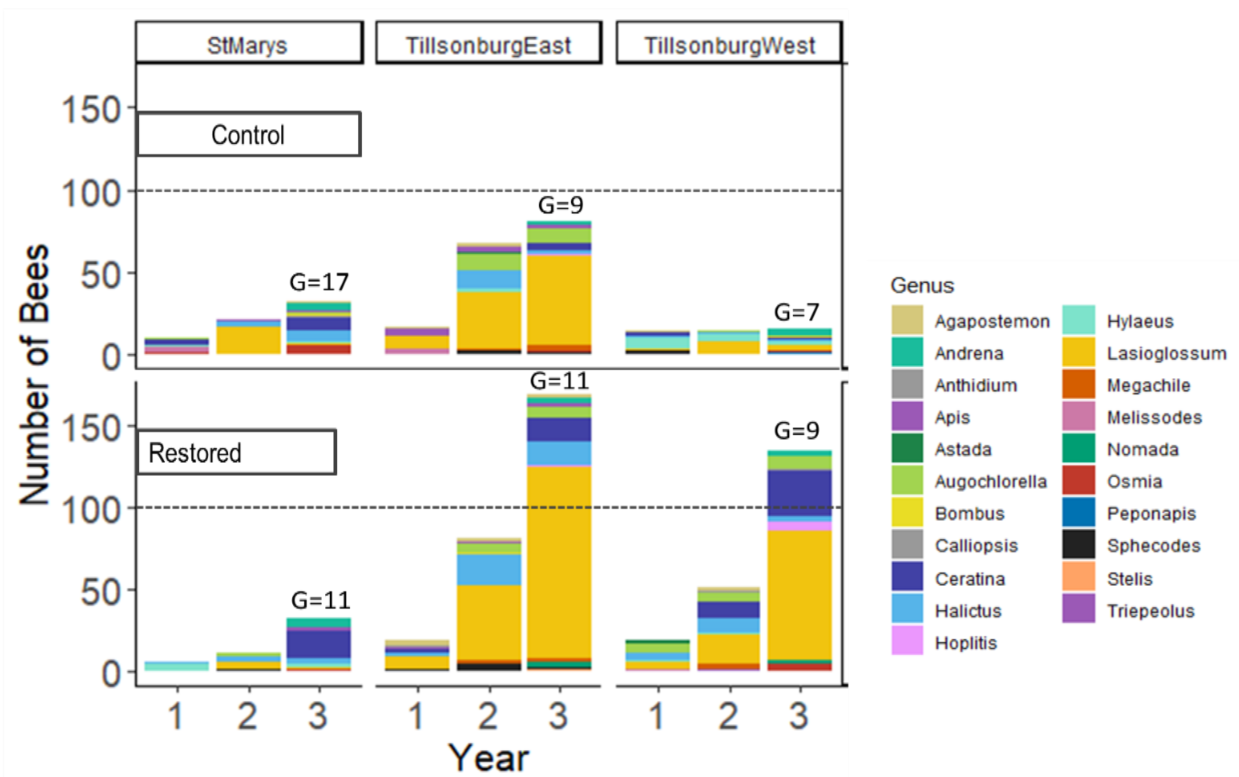

Figure 5 *Changes in bee abundance in the years following native seed addition, comparing restored roadsides to paired, unrestored sites. G=Genus Richness*

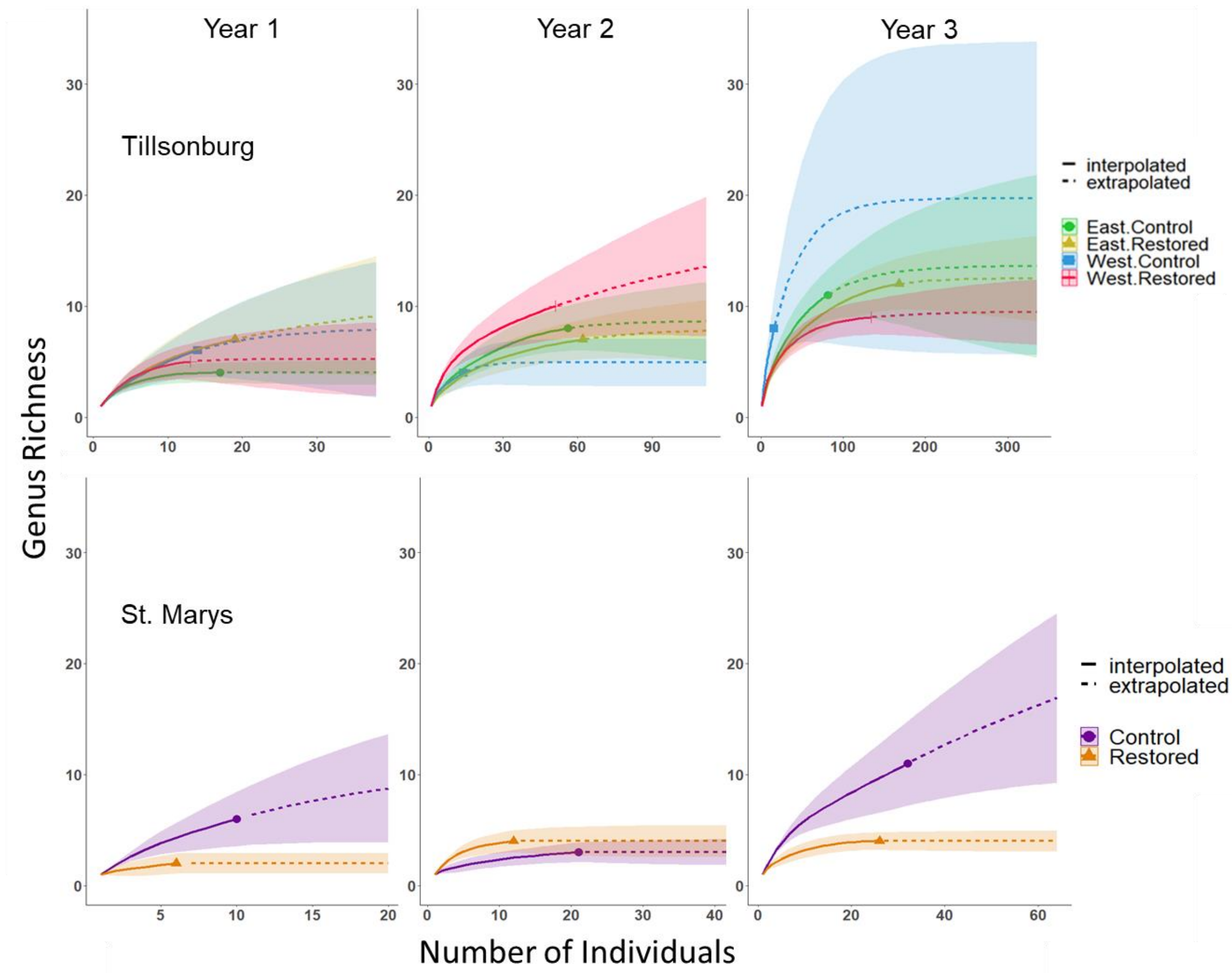

Figure 6 *Change in bee genus richness on restored roadsides compared to unrestored roadsides in the three years following native seed addition. Two sets of sites were used in Tillsonburg, only one set of sites was used in St. Mary's.*

Discussion

Our research shows that revegetating roadsides in Ontario with native seeds can be successful, and that variation in seeding methods may influence success, though project timing, site preparation, species selection and maintenance may also play a role in the success of restorations. Hydro-seeding in a single step will require approximately twice the number of seeds as broadcast seeding and applying a mulch on top of the seed. While the two-stage broadcast method does help to achieve green and sable conditions faster with less seed, eventually all sites show a similar amount of target native plant cover and so this method may only be necessary where erosion is a problem.

The interaction between seeding rate and the number of stages used to install seed may help explain why the success of different installation methods varies so much (Williams 2013). In some instances, hydro-seeded sites can take longer to mature, and the relative success of any one method appears to be case specific (Matesanz, Valladres et al. 2008, Brandt, Henderson et al. 2011, Williams 2013). Additionally, hydroseeding can favor small-seeded species (Montalvo et al 2002), so variation in seed mix composition may also explain the case-specific success of one installation method over another.

Poor performance of the one-stage method at a low seeding rate may be driven by mechanistic differences between installation methods, and the amount of seed-to-soil contact that each method provides. Applying the seed hydraulically in a mulch slurry forces the seeds through a system of pipes~~,~~ and hoses. This process introduces many opportunities for seeds to get lodged in seams, and other crevices in the equipment (Harper-Lore, Johnson et al. 2017). There were fewer opportunities in the two-stage method for seed to be lost in handling.

Applying seed that is suspended within a slurry of paper mulch may also prevent some seeds from settling into the soil at an ideal depth for over-wintering or germination (Arthur and Gartshore 2004). Our results suggest that increasing the volume of seed used can make up for inefficiencies that the hydroseed method introduces and therefore support prior recommendations for higher application rates for hydroseeding (Cain, 2003).

Similar to other studies (Hopwood 2008, Blaauw and Isaacs 2014), we found that bee abundance did increase at some restored sites over time. This suggests that the use of native seed along roadsides may help support pollinator populations. However, average bee abundance and the response of bee abundance to restoration varied by location. Thus, the benefits of local-scale habitat creation on the bee community may be modulated by landscape level characteristics, such as ecological connectivity or the availability of nesting sites (Cranmer, McCollin et al. 2012, Bennett and Isaacs 2014, Connelly, Poveda et al. 2015).

Though we did observe a greater abundance of bees at some restored sites over time, we did not detect a significant change in the level of diversity within the bee communities sampled. This may be due to our relatively low sampling effort causing a wide margin of error for each site. It is also possible that a long-term relationship between restoration and bee diversity is obscured by annual variation in population sizes caused by fluctuations in climate or other stochastic events. However, in extant plantings, we did find a positive relationship between bee genus richness and native plant diversity. Thus, though bee diversity does not appear as sensitive to restoration as bee abundance, there is some evidence that restoration of native plant communities can increase bee diversity.

Recommendations

- Based on our research, restoration experience, and the jurisdiction literature review we offer the following recommendations for creating pollinator habitat and increasing ecosystem functioning along road corridors:
- Species mixes should be tailored to soil texture and moisture availability.
- Seed mixes should contain species that are locally native to the regions they are used and be applied during a winter dormant period as would happen in nature.
- Native seed mixes should be targeted to sites that are not at high risk for erosion and be seeded along with a nurse crop to avoid the need for overseeding with non-native species, which can cause restoration failure.
- Seeding rates should be doubled or a two-stage method should be used when hydro-seeding with native plant seeds.
- Native seed mixes take 3 or more years to become established, and performance evaluations cannot be started until at least August, following a winter seeding.
- Restoration sites will likely be dominated by non-target weeds in the first year, and species composition should be expected to change annually for at least the first three years.
- Long-term management, such as mowing or burning, may be required to maintain native grassland plant diversity in restored sites.
- Abundance-weighted Floristic Quality Index was the most sensitive indicator of native plant diversity and vegetation quality in extant plantings and may therefore provide to be a useful indicator of success in future monitoring.
- Oversight during planning and implementation should be provided by an expert in native seed addition, with a commitment to the restoration outcome.

- If the restoration of specific ecosystem functions is a goal of native roadside plantings, then monitoring should include indicators of ecosystem function as the response of other ecosystem components may differ from the response of vegetation communities.
- Bees may be supported by roadside restoration in some regions, though site selection should prioritize those that help to form linkages with existing habitat.
- Restoration plans should consider the impact of the soil microbial community on vegetation establishment.

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CHAPTER 4: Native plants in competition with Phragmites: is resistance futile?

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Preface

Phragmites australis (Common Reed, hereafter *Phragmite*s) is one of the most invasive plants in Ontario. Preventing further spread is as important as controlling extant populations. Phragmites spreads along road corridors, being favored over native vegetation by frequent disturbance, and road salt which creates saline ditches that Phragmites can dominate in. Furthermore, Phragmites has the potential to produce a large number of seed propagules. Newly disturbed soil provides an opportunity for these seed propagules to establish in the absence of competition form existing vegetation, facilitating invasion. Roadwork can create these opportunities, unfortunately, and so restoration practitioners are tasked with revegetating landscapes that do not become dominated by invasive plants, like Phragmites. There is evidence that diverse, native plant communities can resist invasion through competitive exclusion of species with similar niches, or through more efficient and complete use of local resources. Some practitioners suggest that native plants can exclude noxious weeds, though there is little empirical evidence to support using native species seed as Phragmites biocontrol. Our research tested the hypothesis that the seedlings of native plants that occupy a similar niche may suppress Phragmites seedlings when planted together. We also tested the hypothesis that roadside invasions are driven by seed dispersal, rather than transfer of vegetative root fragments between roadwork sites.

We conducted three related experiments. First, in a greenhouse setting we grew Phragmites seedlings as a phytometer in competition with several native plant species. We grew them in competition with salt-tolerant plants, as well as simple combinations of multiple species to understand how the competitive effect on Phragmites may differ with increased diversity and density. We exposed seedlings to different levels of soil moisture and soil salinity to replicate variation of roadside soil conditions. The second experiment repeated the phytometer design of the first, growing Phragmites seedling in competition with a native plant seedling, one-on-

one and in simple communities. However, this study was conducted outside in a series of cleared areas along a vegetated parking-lot buffer that receives salt run-off from the surrounding urban area. Finally, we tested roadside populations for genetic relatedness in order to gauge the relative contribution of short-distance seed rains and long-distance vegetative transfer to new invasions.

We found further evidence that Phragmites performs better in high salinity and can sometimes overcome competition more easily at higher soil moisture and salinity, giving it an advantage over native plants in salty roadside conditions. Of all the species and communities of species tested, *Bidens frondosa* had the greatest negative impact on Phragmites biomass. Few of the other species were able to significantly reduce Phragmites seedling growth in either the greenhouse or the field. However, multi-species environments were more competitive than single species environments.

We also found that there is no association between the relatedness of populations and their proximity to one another, but we do see an association between specific genotypes and specific roads. Furthermore, we found that there is considerable variation within genetic clusters on the landscape, suggesting that most dispersal is road-facilitated spread of seeds, and not root fragments. targeted seed addition.

Introduction

Study 1 & 2: Suppression of Phragmites by native plants

Phragmites australis (Cav.) Trin. Ex Steudel (Common Reed; hereafter Phragmites) is a cosmopolitan grass, with historical lineages native to the Great Lakes region, and throughout North American. However, hybridization between American and Eurasian subspecies of Phragmites has formed an invasive haplotype with enhanced competitive traits called Haplotype 'M' (Saltonstall 2002, Meyerson, Viola et al. 2009, Kettenring and Mock 2012). These traits allow Phragmites to quickly colonize drainage ditches, or other disturbed, salty or moist environments such as roadsides(Lelong, Lavoie et al. 2007). Most research has focused on controlling existing invasions, and the role of human activity in facilitating the spread of Phragmites; however, the extent to which native plant communities may act as barriers to new invasions has not been well studied (Hazelton, Mozdzer et al. 2014).

Phragmites thrives in moist areas but will also grow in a range of seasonally wet or mesic environments, and has even formed upland ecotypes (Zheng, Chen et al. 2002). Salt tolerance has enabled the M haplotype to invade coastal marshes as well (Vasquez, Glenn et al. 2005). Phragmites achieves its highest stem-density under saline conditions and can survive in salinity as high as 400 mM NaCl (Vasquez, Glenn et al. 2005, Meyerson, Viola et al. 2009). Photosynthetic capacity in haplotype M is higher than native genotypes; they may require less nitrogen as well (Mozdzer and Zieman 2010). Phragmites is highly competitive underground, rooting deeper than most native wetland species to access nitrogen-rich pore water, facilitating the decomposition of soil organic matter (Mozdzer, Langley et al. 2016). Though clonality may drive competition with established plant communities, high seed production and dispersal allow Common Reed to spread to new habitats quickly. However, patches must be genetically diverse, as fertilization requires outcrossing (Kettenring and Mock 2012)

Newly disturbed sites, such as recently renovated road edges and ditches provide the perfect opportunity for wind-dispersed weeds to invade. Restoration practitioners are therefore tasked with revegetating these landscapes in such a way that excludes noxious weeds, such as Phragmites (Maheu-Giroux and de Blois 2006, Lombard, Tomassi et al. 2011, Rohal, Cranney et al. 2019). In Ontario, drainage ditches are most often revegetated with Eurasian agronomic cover crops like clover and red fescue, though a variety of wildflower and native grassland seed may be used as well (Appendix 1). Some practitioners have questioned the ability of monospecific cover crops to adequately repel invasion by noxious weeds including Phragmites. This has led to the hypothesis that certain communities of native plants may be more likely to resist invasion (D'Antonio, Levine et al. 2001, Peter and Burdick 2010, Byun, de Blois et al.

2013). Though Phragmites can spread into high quality, species-rich habitats, it is unclear how the seed bank diversity in a newly disturbed site impacts invasion success.

Communities with high species diversity may have increased resistance to invasion through niche overlap (Peterson, Allen et al. 1998, Kennedy, Naeem et al. 2002). Invading species may take advantage of empty niches in communities with reduced diversity, and often favor disturbance prone areas. On the other hand, diverse plant communities exhibit vegetational inertia, maintaining their own optimal microhabitat, even in the face of invading species. Therefore, sites with intact native communities may be able to resist invasion for longer periods (Von Holle, Delcourt et al. 2003). Intact and diverse native communities overshadow, and crowd out invading seedlings though niche complementarity (Naeem, Knops et al. 2000). Communities with empty niches can be invaded more easily, since invaders are more likely to succeed when they are functionally and phylogenetically distant from resident species (Peterson, Allen et al. 1998, Davis, Grime et al. 2000, Gallien and Carboni 2017).

There is limited, but compelling, evidence that some native plant communities or particular species may be able to resistant to invasion by Phragmites. In this study, Phragmites clones (root cuttings) were grown with a series of individual native freshwater marsh species, and small communities made of these species. The native species were categorized into functional groups: annuals, non-rhizomatous perennials, short, rhizomatous perennials, and tall, rhizomatous perennials. Notably, Phragmites performed worse with annuals, and with mixtures that contained all four functional groups (Peter and Burdick 2010, Byun, de Blois et al. 2013)

Using a similar phytometer approach, my research aims to build on the findings of the two studies above by focusing on Phragmites seedlings, rather than vegetative clones, and testing the competitive effect of native freshwater species that may be considered for roadside seeding in southern Ontario. I tested two hypotheses, first that native species, or combinations of these species can significantly reduce the establishment of new Phragmites seedlings. The second hypothesis tested was that soil moisture and salinity facilitate the spread of Phragmites by mediating the outcome of competition. Through experimental plantings, using Phragmites as a phytometer, we attempted to answer the following questions:

1. Can the seedlings of native species suppress Phragmites seedlings?
2. How does competitor diversity change the outcome of competition between Phragmites and native species seedlings?
3. Which native species can lethally outcompete Phragmites most often?
4. Are salt-tolerant halophytic plants, of regional native origin more effective at suppressing Phragmites than locally native wet-meadow (wetland edge) species?
5. Does soil moisture and soil; salinity influence the outcome of competition between native species and Phragmites?

Study 3: Genetic structure of Phragmites along regional highways

In order to better understand the extent to which populations of Phragmites spread by local seed rain, as opposed to long-distance transfer of vegetative fragments, we sought to understand genetic variability of Phragmites populations on our regional roads.

While collecting seed for our first two studies, we observed a pattern of decreasing seed production with increasing distance from the north shore of Lake Erie. This observation led us to the hypothesis that some populations may contribute more to spread than others, and that most populations across the region would relate to highly fecund populations such as those near Long Point (Lake Erie).

To test this hypothesis, we collected DNA samples from roadside populations of Phragmites and tested them for evidence of clustering of genotypes, or overdominance of genotypes on the landscape. This research was conducted in parallel with our first two studies. Understanding the relative role of short-distance seed dispersal in the successful spread of Phragmites is crucial to identifying the most effective method to resist invasion.

Methods

We designed two phytometer experiments to compare the growth of Phragmites seedlings in competition with different native wetland species, and communities of these species. In the first experiment, we manipulated the identify of competing species as well as moisture and salinity within a greenhouse environment. In the second experiment, we grew Phragmites outside in field conditions along a vegetated parking lot buffer. In this field study, Phragmites seedlings were grown with the best competitors from the greenhouse. In the field study, we took advantage of a natural moisture and salinity gradient to test the role of these soil properties on the outcomes of competition. Genetic testing of roadside populations was conducted in parallel with the two experimental studies and is outlined in Study 3 following the methods of the phytomer experiments.

Seed Sources and Phragmites *Germination Trial*

All seedlings used in this experiment were grown at the McMaster Biology Greenhouse. Phragmites seedlings were propagated from mixed-origin seed, collected from ten populations located up to 150km from McMaster University. Phragmites seed was collected from 10 inflorescences from each population. Only culms over 2m tall and located in saturated soil or soft mud were sampled. We extracted viable seed from each inflorescence using a Shop Vac. The extracted material was screened through a series of soil grading sieves, a fine wire kitchen sieve and finally winnowed clean of dust and any remaining inert material. Seeds were pooled per population, weighed, and the average seed number per inflorescence was estimated using the weight of 50 seeds from each population, where possible. Prior to our competition studies, we tested Phragmites seed germination alone to verify the assumption that *Phragmite*s is capable of producing a large number of viable seeds per ramet. These results are presented at the top of the Results section to follow.

Table 1 *Competition treatments for potted* Phragmites *seedlings under experimental greenhouse conditions.*

Competitive environment	*Competitor*	*Seedlings per pot*
Control	none	1
solo	*Panicum virgatum (L.)*	2
solo	*Elymus riparius (Weig.)*	2
solo	*Elymus virginicus (L.)*	2
solo	*Oenothera biennis (L.)*	2
solo	*Bidens frondosa (L.)*	2
solo	*Monarda fistulosa (L.)*	2
solo	*Verbena hastata (L.)*	2
solo	*Rudbeckia laciniata (L.)*	2
solo	*Solidago sempervirens (L.)*	2
solo	*Spergularia maritima (L.) Chiov*	2
multi(halophyte)	*S. sempervirens, R. laciniata*	3
multi(forb)	*O. biennis, R. laciniata*	3
multi(grass)	*E. virginicus, P. virgatum*	3
multi (grass & forb)	*O. biennis, P. virgatum*	3

Competitor seedlings were propagated from wild-origin (local provenance) seeds; most were purchased from a local source-identified commercial producer of native seed (St. Williams Nursery & Ecology Centre, 2016 catalogue). Seeds from the adventive halophyte species *Solidago sempervirens* and *Solidago maritima* were collected from spontaneous populations within 20km of McMaster University. This specie's has spread island from its historical coastal range through salty road ditches. Seeds of all species were cold-moist stratified for 30 days. Rather than optimizing the stratification for each species individually based on their minimum requirements, we chose equal stratification periods for all species to simulate a shared seasonal change.

Study 1: Greenhouse Phytometer Design

Competitive Environment

Phragmites was grown alone as an experimental control and was also potted together in competition with other plants. We created 32 replicates of 14 different competitive environments, using native species, halophyte species and simple combinations or communities of these species, summarized in Table 1. Seeds of the same species were first germinated together in trays and transplanted at the 1-2 leaf stage into pots either alone or together in a competitive environment. One pot of each competitive environment type, including one control pot, were randomly assigned to one of 32 trays, split between two greenhouse benches. The bench that each tray was assigned to is not used as a blocking factor since both benches received similar light exposure, and all trays were otherwise treated equally.

Moisture & Salinity

In order to mimic variation in roadside environments, we also applied a watering treatment. After a two-week staging period in which all trays were watered as needed with fresh water, we began applying the moisture and salinity treatments. Each tray received one of four moisture treatments, moist-fresh, moist-saline, dry-fresh or dry-saline. Treatments were applied systematically to trays in a checkerboard fashion so that there were equal numbers of each treatment combinations on each bench, and they were spread out evenly across any variations in light environment.

All trays received roughly 500ml of water once a week on the same day, regardless of treatment. High moisture treatments were created by limiting drainage in a tray to a single opening (2-3mm). Low moisture treatments were created by letting moisture drain freely from the trays through a series of several larger holes (8-10mm)

For the salinity treatments we applied saltwater solution once a week, beginning with a 50mol/ppm solution, and increasing concentration by 50mol/ppm each following week to a maximum of 200mol/ppm. We provided an equal amount of additional fresh water to trays receiving the freshwater treatment on the same day that the salinity treatment was applied.

Sampling & Analysis

Plants were monitored for 2 months for death or shedding of biomass (leaves). Where possible, biomass and plants that died before the end of the experiment were collected. In very few cases, leaves were shed onto the ground, and could not be assigned to an individual pot. At the end of the experiment, aboveground biomass from each individual plant was clipped, and dried before being weighed. We used ANOVA to test for differences in the total biomass of Phragmites from different competitive environments, and the effect of moisture and salinity on biomass and the outcome of competition. We also used Estimated Marginal Means for pairwise comparisons of competitive environment, and Contrast Statements to compare the effect of groups of competitive environments. All analyses were done in R v4.0.3

Study 2: Field Phytometer Design

Competitive Environment

In the year following the greenhouse competition experiment, we constructed a similar series of competitive environments for Phragmites seedlings but situated outside in a local restoration site (McMaster University West Campus). Competitors were chosen from those that performed best in the prior greenhouse experiment across moisture and salinity treatments. We also grew Phragmites groupings alone as a control for comparison.

We constructed competitive environments within a series of 12 plots, placed roughly 5-10m from the edge of the vegetated buffer, running parallel to the edge. We avoided areas with dense *S. canadensis* cover due to its rapid clonal expansion and those areas that were flooded in spring. Each plot was segmented into six 25cm × 25 cm subplots, arranged in a 2x3 grid. Existing vegetation was cleared for plug planting, and roots were removed as much as possible, up to 10cm deep. Each subplot had six seedlings total; three Phragmites and three competitors (Table 2), except for the Phragmites-only treatment with only three Phragmites and no competitors. In each subplot, we planted one replicate of each competitive environment treatment (Table 2). Each plot contained a replicate of the single-species competitive environments, assigned randomly to a subplot. Half the plots were assigned a *S. sempervirens*

Community competitive environment, while the other half received a *B. frondosa* Community competitive environment. One sub-plot at each plot was also planted with Phragmites only, as a control.

Seeds were sourced from the same supplier and populations as in the greenhouse experiment, and also stratified in the same way. Seedling were started in May at the McMaster Biology Greenhouse in ambient natural lighting and begin germinating at roughly 20 degrees. Seedlings were grown in small plug trays (72) and transplanted between the 2 to 3 leaf stage (leaf pairs in *B. frondosa*). Seedlings were watered well when planted. A total of seven seedlings died in the first week and were replaced. All of the replacements survived the first week of planting, and so observations began approximately 2 weeks after all seedlings were established.

Moisture & Salt Gradient

The site chosen to create the competitive environments in Table 2 is a recently restored buffer zone between a parking lot at McMaster University (Lot M) and the adjacent channelized creek. The site has a strong natural salinity gradient because of parking lot and road maintenance. We took advantage of these gradients to provide a moisture and salinity treatment similar to the prior greenhouse experiment. The north end of the site receives runoff from the university campus, and busy local roads, all of which are salted in the winter. Snow plowed from the parking lot is also piled primarily on the north end of the vegetated buffer, bringing salt with it. We know from previous work that this site exhibits a patchy but otherwise directional moisture gradient.

The highest moisture and salinity occur at the north end which is dominated by halophyte plants, such as *Symphyotrichum subulatum (Michx.) G.L. Nesom,* and *S.maritima* as well as a number of common annual weeds. Adventive populations of *S. sempervirens* are infrequent, though this species has been expanding its range in Ontario primarily via road corridors from the gulf of the St. Lawrence southwest.

Toward the southern end of the vegetated buffer where soils are less saline, and slightly drier, *Solidago canadensis (L.)* dominates away from the parking lot edge, while native sedges and rushes can be found in small depressions and slowly draining pools where the parking lot meets the buffer. Phragmites has also colonized some of these patches and appears to be spreading.

Sampling & Analysis

We took three soil moisture and salinity readings within every subplot using a moisture probe (WET-Sensor, WET-UM-1.4, Delta T Devices Ltd.), roughly six weeks apart, withing 12-24 hours after rainfall. Moisture and salinity values were averaged across the season for each subplot. We also measured the change in longest leaf length from the beginning of our study to the end, at which point all aboveground biomass was collected, dried, and weighed. The results of this paper will focus on observed differences in Phragmites biomass alone to judge the competitive effect of other species on this invasive plant.

We used Type III Anova to test for differences in Phragmites biomass between competitive environments, including the control environment that were planted without competitors. We also Type III Anova to tests for effects of soil moisture and salinity on Phragmites biomass directly, as well as to test for interactions that might suggest moisture and/or salinity mediated the outcome of competition. We used Contrast Statements (estimated marginal means) to determine the difference between competitors and groups of competitors in their effect on Phragmites. All analyses were done in R v4.0.3. A log +.1 transformation was applied to Phragmites biomass prior to analysis to ensure homoscedasticity and normality of data, meeting the assumptions of our linear model.

Table 2 *Competitive environments of potted* Phragmites *seedlings in outdoor field soil conditions. *S. sempervirens is a halophyte.*

competitive environment	*competitors*	*Seedlings/subplot*	*Subplots*
control	none	3	12
monoculture	*E. riparius*	6	12
monoculture	*R. laciniata*	6	12
monoculture	*B. frondosa*	6	12
monoculture	*S. sempervirens**	6	12
S. sempervirens Community	*S. sempervirens, E. riparius, R. laciniata*	6	5
B. frondosa Community	*B. frondosa, E. riparius, R. laciniata*	6	7

Study 3: DNA Sampling & Sequencing

We began by collecting 51 Phragmites leaf samples from 39 stands growing along roadsides between Long Point and Hamilton, Ontario. A single sample was taken for the first 33 Phragmites stands, and 3 samples were taken from the other 6 stands. Leaf samples were preserved in a silica-gel desiccant.

DNA was extracted from 20mg of leaf sample, using the Qiagen *DNEasy Plant Mini Kit*, following adjustments to the manufacturer's protocol made by others (Lambertini, Frydenberg et al. 2008). We used microsatellite analysis on eight loci to determine genetic variation, and used primers developed by others (Saltonstall 2002). Final sequencing was conducted at the National Resources DNA Profiling and Forensics Lab at Trent University, using a 48-capillary ABI 3730 DNA analyser. Genotyping was achieved through Genemarker 1.91 software, and genetic distance was calculated as a Bruvo Genetic Distance using GenAIEx 6.503 software. We used R v3.6.1 for clustering analysis through the factoExtra package. Both hierarchical and k-means clustering approaches were used. In addition to DNA samples, we also measured soil conductivity at each of the sampled Phragmites stands.

Results

Phragmites Seed Germination

Prior to growing Phragmites seedlings for our experiments, we compared seed set per inflorescence and germination rates among ten roadside populations in the region between Hamilton and Long Point. This was done to justify pooling of populations for greater genetic diversity and a greater volume of material to handle. We detected considerable variation in seed-set per inflorescence in the populations of Phragmites that were used as seed sources for our study. Figure 1 shows that germination rate increases with increasing number of seeds per inflorescence. While this correlation is not significant (Pearson Correlation, $r=0.535$, $p=0.137$), we do see a quasi-binomial distribution where most populations sampled are producing over 10 seeds per inflorescence, and that most of these seeds are capable of germinating at a rate of about 45% or higher, with two populations, Hagersville Wetland, and the Desjardin Canal, that produced very few seeds, and very poor-quality seeds. The Long Point and Port Rowan populations produced the most seeds per inflorescence with the highest germination rate. Highway 24 produced similarly high-quality seeds, but fewer of them.

Study 1: Greenhouse Phytometer

Overall, the presence of a competitor significantly reduced the biomass of Phragmites. By the end of our study, only 14 Phragmites seedlings died (3%). Seedling death occurred in all soil conditions, though no seedlings died in the control environment. Twice as many seedlings died in single species competitive environments than multi species competitive environments. The largest, and fastest growing competitor was *B. frondosa*. We did not detect a significant effect of salinity, but did find an effect of moisture, as well as an interaction between competitor and moisture that effected Phragmites biomass (Table 3, Figure 2).

Competitive Environment

All competitive environments significantly reduced Phragmites biomass compared with the control of no competition, except for the environment with *P. virgatum.* While Phragmites growing with *M. fistulosa* were significantly smaller than plants growing without competition, the were not significantly smaller than those growing with *P. virgatum*. (Table 5, Figure 2). Phragmites biomass was most severely reduced by growing with the annual native *B. frondosa*, though to a similar degree as *V. hastata,* the Forb Community, and the Salt Community (Table 5, Figure 1,). Two seedlings died in competition with each *B. frondosa* and *V. hastata*, as well as *S. maritima* and the Salt Community. Competitive environments of multiple species, the Communities, were generally more competitive than the competitive environments of single species, though the Mixed Community (grass and forb) was significantly less competitive than the Salt or Forb Communities, but similar to the Grass Community (Figure 1, Table 4, Table 5)

Moisture & Salinity

Overall moisture had a significant effect on the outcome of competition. In most competitive environments, Phragmites biomass is greater in high moisture treatments than in low moisture treatments (Table 3, Figure 1). However, we also detected an interaction between competitive environment and moisture level; Phragmites was less impacted by moisture when growing with *B. frondosa* and *S. maritima*.

We did not detect an influence of soil salinity driving the outcome of competition, though presumably the salt-tolerance of Phragmites does confer some competitive advantage in certain situations. Though we successfully increased soil salinity through the course of the season, we did not observe Phragmites seedlings responding differently to competition under high salinity levels.

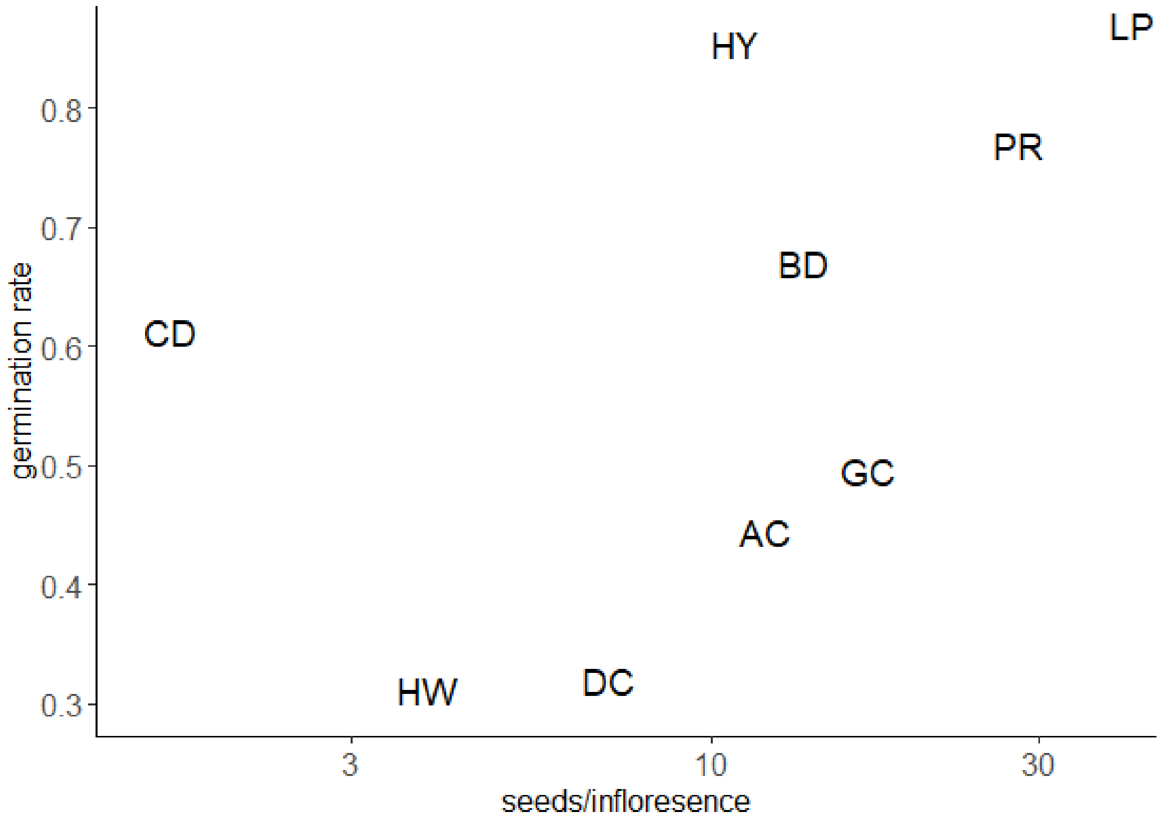

Figure 1 *Phragmites seed germination rate as a function seed number per inflorescence. Populations are from the same study region as those considered in Study 3. AC=Ancaster, BD=Brantford, CD=Cambridge Ditch, DC=Desjardin Canal (Dundas), GC= Gateway Church (Hamilton), HY=Highway 24 (north of Delhi), HW= Hagersville Wetland, LP= Long Point Marsh, PR=Port Rowan (inland Long Point)*

Table 3 *Results of ANOVA for the greenhouse phytometer study, showing a significant effect of competitive environment, a significant effect of moisture, and a significant interaction between competitor and moisture effecting* Phragmites *seedling biomass.*

predictor	*sum of squares*	*d.f*	*F*	*p*
competitor	*60.11*	14	27.497	**<0.001**
moisture	*14.88*	1	97.274	**<0.001**
salinity	*0.45*	1	2.883	0.090
competitor x moisture	3.95	14	1.807	**0.035**
competitor x salinity	1.02	14	0.467	0.950
moisture x salinity	0.17	1	1.083	0.299
competitor x moisture x salinity	1.21	14	0.554	0.900
residual error	65.586	420		

Table 4 *Group comparison of treatment effects on* Phragmites *biomass under competition from contrast statements. Significant contrasts are in bold font. The most competitive environment is highlighted in bold font where the contrast is significant.*

contrast	*df*	*difference*	*SE*	*t ratio*	*p*
*single species vs c**ommunity***	*420*	*2.050*	*0.413*	*4.956*	***<.001***
halophytes vs local species	420	-0.072	0.442	-0.162	0.871
***forbs** vs grasses*	420	-2.21	0.337	-6.551	**<.001**

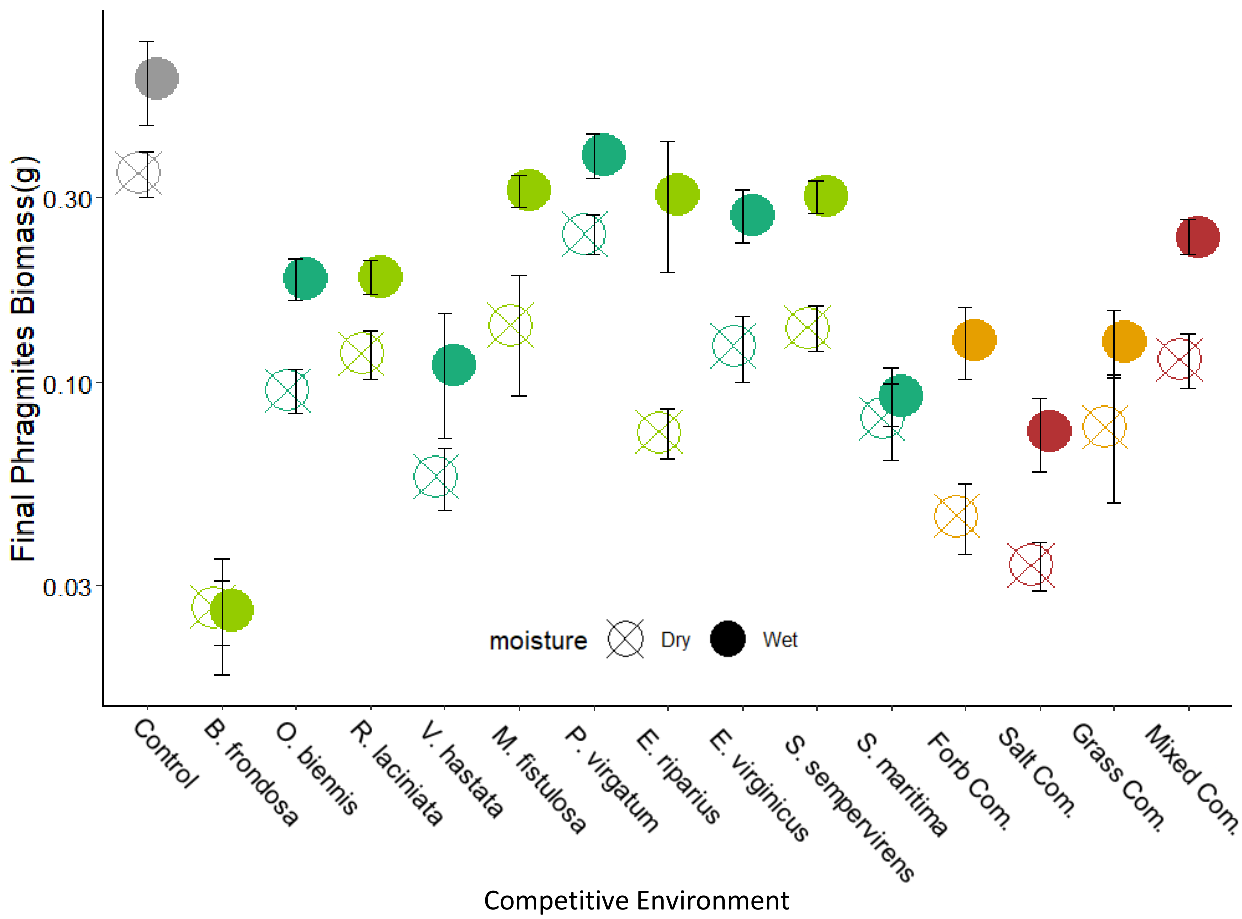

Figure 2 *Phragmites seedling biomass across competitive environments in the greenhouse phytometer study.*

All competitive environments significantly reduced Phragmites *biomass compared with the control, except for P. virgatum.* Phragmites *grown in high moisture were bigger than those in dry environments. Though this difference was not statistically significant at the p=0.05 level, it may be biologically meaningful. Table 5 is a summary of pairwise differences between competitive environments using estimated marginal means.*

Table 5 *Matrix of pairwise contrasts showing ratios of* Phragmites *biomass in competitive environment A, divided by* Phragmites *biomass in competitive environment B. Significant contrasts are in bold font where p<0.05.*

Blue shading illustrates the magnitude of competitive effect of each environment compared to the control. Green shading indicates where environment A reduced Phragmites *biomass more than B. Brown shading indicates where B reduced* Phragmites *biomass more than A. Competitive Environments: C=Control; no competitor, BF=B. frondosa, CF=Forb Community, CG=Grass Community, CM=Mixed Community, CS=Salt Community, ER=E. riparius, EV=E. virginicus, MF=M. fistulosa, OB= O. biennis, PV=P. virgatum, RL=R. laciniata, SM=Spergularia maritima, SS=S. sempervirens, VH= V. hastata*

		B														
	CE	*C*	*BF*	*CF*	*CG*	*CM*	*CS*	*ER*	*EV*	*MF*	*OB*	*PV*	*RL*	*SM*	*SS*	*VH*
	C		**4.00**	**3.34**	**2.73**	**1.93**	**2.88**	**2.18**	**1.87**	**1.77**	**2.19**	1.31	**2.05**	**1.66**	**2.82**	**3.01**
	BF	**0.25**		0.84	**0.68**	**0.48**	0.72	**0.55**	**0.47**	**0.44**	**0.58**	**0.33**	**0.51**	**0.42**	**0.71**	0.75
	CF	**0.30**	1.20		0.82	**0.58**	0.86	**0.65**	**0.56**	**0.53**	**0.66**	**0.39**	**0.61**	**0.50**	0.84	0.90
	CG	**0.37**	**1.46**	1.22		0.71	1.06	0.80	**0.68**	**0.65**	0.80	**0.48**	0.75	**0.61**	1.03	1.10
	CM	**0.52**	**2.07**	**1.73**	1.41		**1.49**	1.13	0.97	0.92	1.13	**0.67**	1.06	0.86	**1.46**	**1.56**
A	*CS*	**0.35**	1.39	1.16	0.95	**0.67**		0.76	**0.65**	**0.61**	0.76	**0.45**	**0.71**	**0.58**	0.98	1.05
	ER	**0.46**	**1.83**	**1.53**	1.25	0.89	1.32		0.86	0.81	1.00	**0.60**	0.94	0.76	1.29	1.38
	EV	**0.54**	**2.14**	**1.79**	**1.46**	1.03	**1.54**	1.17		0.95	1.17	**0.70**	1.10	0.89	**1.51**	**1.61**
	MF	**0.57**	**2.26**	**1.89**	**1.54**	1.09	**1.63**	1.23	1.06		1.24	0.74	1.16	0.94	**1.59**	**1.70**
	OB	**0.46**	**1.71**	**1.53**	1.25	0.88	1.32	1.00	0.85	0.81		**0.60**	0.94	0.76	1.29	1.38
	PV	0.76	**3.06**	**2.56**	**2.09**	**1.50**	**2.20**	**1.67**	**1.43**	1.35	**1.67**		**1.57**	1.27	**2.15**	**2.30**
	RL	**0.49**	**1.95**	**1.63**	1.33	0.94	**1.41**	1.06	0.91	0.86	1.07	**0.64**		0.81	1.37	**1.47**
	SM	**0.60**	**2.41**	**2.02**	**1.65**	1.17	**1.74**	1.32	1.13	1.07	1.32	0.79	1.24		**1.70**	**1.82**
	SS	**0.36**	**1.42**	1.19	0.97	0.69	1.02	0.77	**0.66**	**0.63**	0.78	**0.46**	0.73	**0.59**		1.07
	VH	**0.33**	1.33	1.11	0.91	**0.64**	0.96	0.72	**0.62**	**0.59**	0.73	**0.43**	**0.68**	**0.55**	0.93	

Study 2: Field Phytometer

In the field study, a greater proportion of Phragmites died than in the greenhouse study (30%), including four individuals that died in the absence of competition, likely due to stressful growing conditions. However, these control sites ranged across moisture levels, and salinity levels (Figure 3, Figure 4). The influence of competitive environment alone was slightly significant, though we also found a significant three-way interaction between our soil factors and the competitive environment (Table 6). While soil moisture and salinity did not influence Phragmites biomass alone, we did detect an interaction that influenced the outcome of competition, between both factors as well as the competitive environment.

Competitive Environment

Overall, final Phragmites biomass was influenced by the type of competitive environment it was growing in. Similar to the greenhouse study, we observed the greatest reduction in Phragmites biomass when growing with *B. frondosa*. Unlike the greenhouse study, we detected a significant effect of competitive environment on average Phragmites biomass in the field (Table 6), but no single competitive environment type reduced Phragmites biomass compared to the control environment (Table 7). *B. frondosa* and *S. sempervirens* environments were each more competitive than *R. laciniata* environments, but alone have a similar effect to all other competitive environments (Table 7, Figure 3, Figure 4).

However, we did find that competitive environments comprised of multiple species (Communities) were able to reduce Phragmites biomass more than competitive environments comprised of a single species, planted at the same density. We did not detect a difference between competitive environments that contain salt-marsh halophytes, and environments with species of local freshwater wetlands (Table 8), which is surprising because we observed an interaction between competitive environment and salinity, as well as moisture (Table 6).

Moisture & Salinity

We found that soil moisture and salinity interact to effect Phragmites seedling biomass such that the effect of one salinity depends on the magnitude of effect from soil moisture, and vice versa. Furthermore, we detected a significant three-way interaction between these two soil traits and the competitive environment (Table 6). This means that the response of Phragmites to its competitive environment depends on how each species responds to changes in both soil traits, while the effect of one soil trait depends on the level of the other soil trait, and the effect of both traits depend on the species growing in the soil (Figure 5). Due to this complex response, the results of a three-way interaction can be difficult to interpret.

Final Phragmites biomass was not influence directly by either soil moisture or salinity alone, but by an interaction between these soil conditions and also by an interaction between competitive environment and the soil conditions (Table 6). We can see that Phragmites responds to variation in soil salinity differently across competitive environments. For example, we see that the community environments are more sensitive to increases in salinity than single species environments and are therefore less able to reduce the growth of Phragmites. This effect is seen in the difference among slopes of their respective lines in Figure 3. Surprisingly, we also see that Phragmites actually grows larger with *R. laciniata* than without it, across all salinity levels, implying a facilitative rather than competitive effect of *R. laciniata* on Phragmites (Figure 3).

Phragmites seedlings also responded to variation in soil moisture differently across competitive environments. When growing with strong competitors, Phragmites seedlings were more drastically reduced under dry conditions. However, the response of Phragmites to competition with *R. laciniata* and *E. riparius* is more consistent over the range of moisture levels. Again, we see that both of these species appear to facilitate Phragmites, at least at low moisture levels (Figure 4).

Comparing Phragmites' response to competition across all competitive environments and both soil factors is less straightforward, but some patterns emerge. First, it appears that *S. sempervirens* may be a better competitor at high salinity levels when moisture is low, illustrated by the cluster of smaller yellow circles in Figure 5. In the same figure we see the competitive effect of the *B. frondosa* Community is sensitive to increasing salinity, but this is slightly more pronounced at higher moisture levels, indicated by the cluster of small red squares.
Overall, the competitive environment including *B. frondosa* alone was able to reduce Phragmites seedling biomass most consistently across soil conditions. Although the competitive effect of community environments was greater at low salinity (Figure 3, Figure 4, Figure 5).

Table 6 *Summary of Type III ANOVA comparing the effects of competitor, soil moisture and soil salinity on log (Phragmites biomass+.1*).

predictor	*sum of squares*	*d.f.*	*F*	*p*
Competitive environment	4.877	1	2.161	**0.049**
moisture	0.144	6	0.383	0.537
salinity	0.079	1	0.476	0.491
competitor x moisture	5.608	1	2.485	**0.024**
competitor x salinity	5.230	6	2.317	**0.035**
moisture x salinity	0.347	6	0.922	0.338
competitor moisture x salinity	5.613	1	2.487	**0.024**
residual error	70.710	188		

Table 7 *Matrix of pairwise contrasts showing ratios of* Phragmites *biomass in competitive environment A divided by* Phragmites *biomass in competitive environment B. Significant contrasts are in bold font where p<0.05.*
Blue shading illustrates the magnitude of competitive effect from each environment, Control/Competitive Environment. Green shading indicates where A reduced Phragmites *biomass more than B. Brown shading indicates where B reduced* Phragmites *biomass more than A. C=Control. Competitive Environments: BC= B. frondosa Community, BF=B. frondosa, ER=E. riparius, RL=R. laciniata, SC= S. sempervirens Community SS=S. sempervirens*

		B						
	CE	*C*	*BC*	*BF*	*ER*	*RL*	*SC*	*SS*
	C		1.228	1.527	0.962	0.741	1.523	1.243
	BC	0.814		1.243	0.783	0.603	1.240	0.857
A	*BF*	0.655	0.805		0.630	**0.486**	0.998	0.689
	ER	1.040	1.277	1.587		0.771	1.584	1.094
	RL	1.350	1.658	**2.058**	1.297		**2.055**	1.420
	SC	0.657	0.806	1.002	0.631	**0.487**		0.691
	SS	0.805	1.167	1.451	0.914	0.704	1.447	

Table 8 Preplanned pairwise comparisons of estimated marginal mean biomass of Phragmites in different competitive environments. Significant contrasts are shown in bold font.

contrast	*d.f.*	*difference*	*SE*	*t ratio*	*p*
single species vs ***community***	188	1.970	0.163	3.937	**<.001**
halophytes vs local species	188	-0.430	0.246	2.997	0.082
R. laciniata alone vs ***in community***	188	0.721	0.163	4.416	**<.001**
E. riparius alone vs ***in community***	188	0.461	0.015	2.997	**<.050**

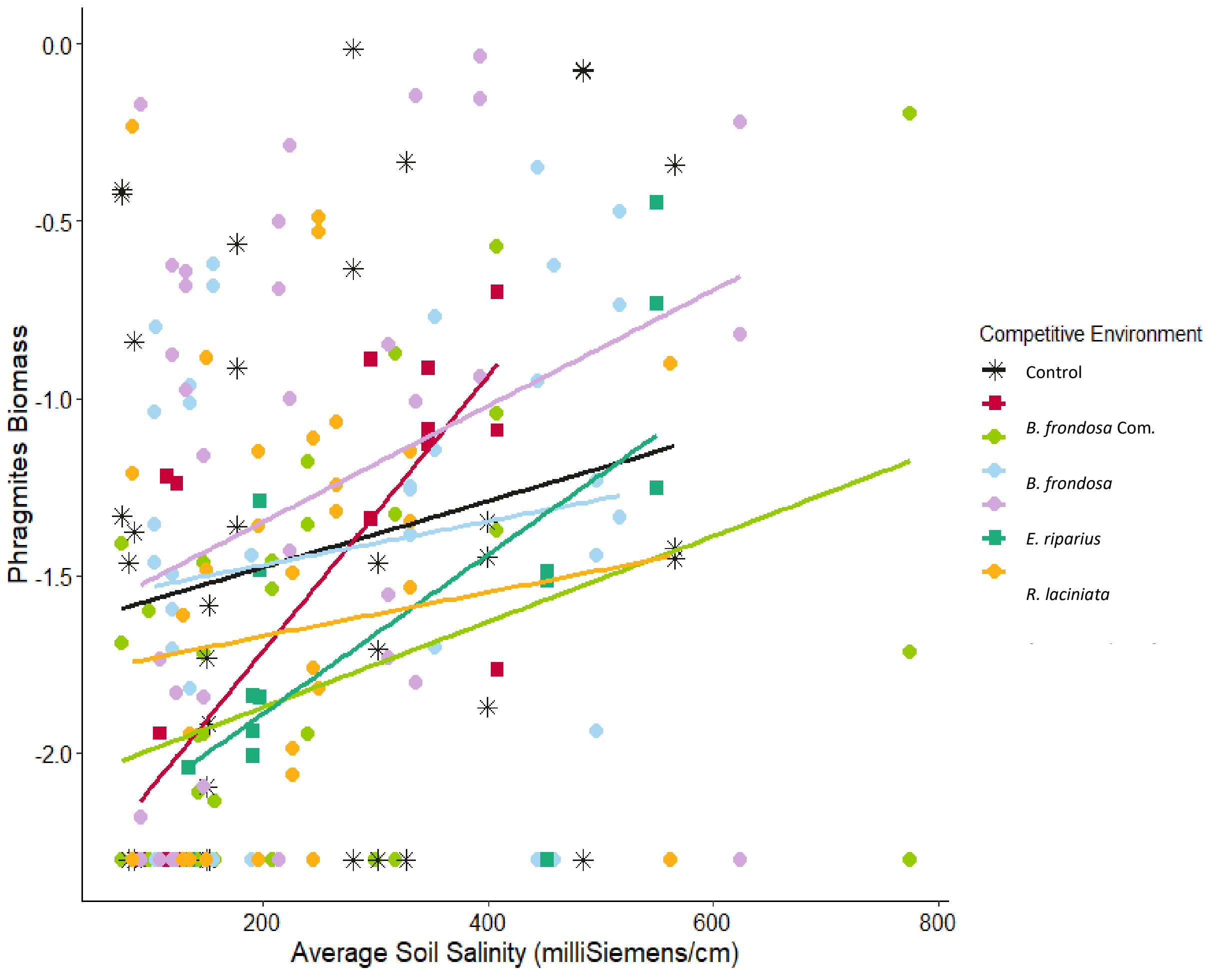

Figure 3 Influence of Salinity on the competitive effect of native plants on Phragmites seedlings.

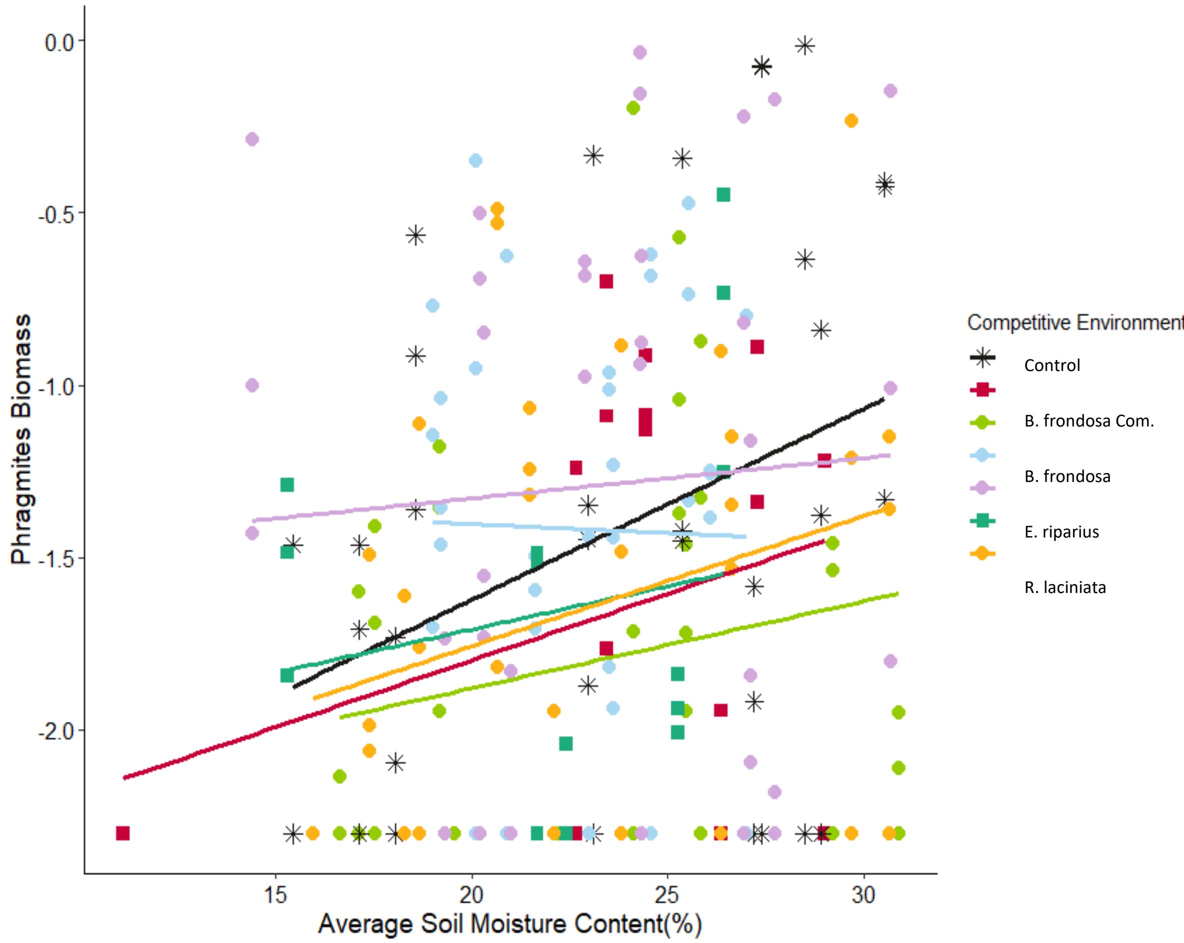

Figure 4 Influence of Moisture on the competitive effect of native plants on Phragmites seedlings.

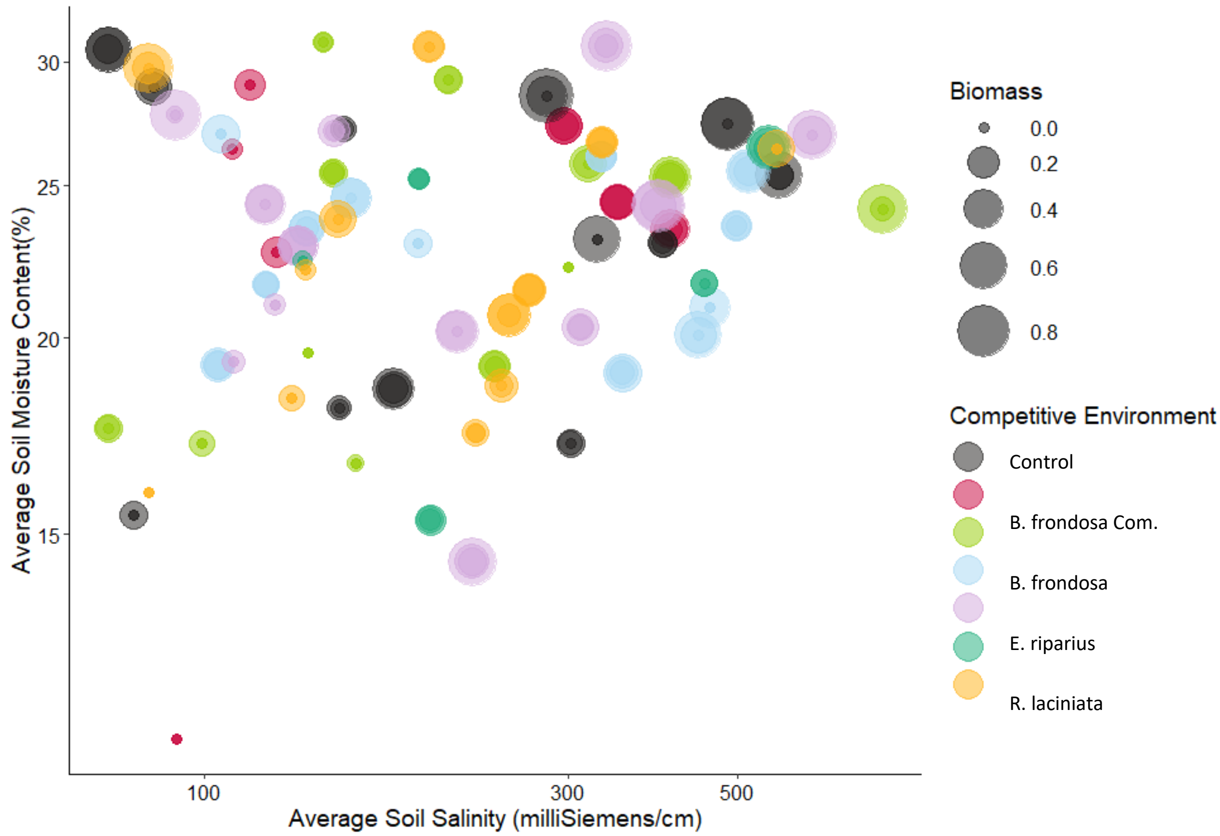

Figure 5 Phragmites *biomass across competitive environments (Com=Community), soil salinity and moisture.*
The amount of biomass is represented by the size of the circle for each seedling. Each cluster of overlapping circles represents the three individual Phragmites *seedlings planted per subplot that share the same soil moisture and salinity. Log scales are used on both axes.*

Study 3: DNA Sampling & Sequencing

The results of our initial seed germination tests further support the hypothesis that seed dispersal and therefore seedling establishment may be driving invasion of Phragmites. To test this hypothesis, we collected genetic and geographic data from populations across the landscape to gauge how much genetic variation exists between populations, within populations, and if related populated are close together on the landscape.

Genetic Clustering

Both methods, hierarchical and k-means clustering, identified six distinct genetic clusters among populations of Phragmites in our study area (Figure 6). However, genetic distance was not correlated with geographic distance (Figure 7). While some related populations are clustered geographically, others are spread out across the entire study area suggesting long-range dispersal patterns (Figure 5). However, most of the genetic clusters seen across the study area are represented in Long Point. This may suggest that Long Point is acting as a dominant source population or simply that existing natural variation in the region is more likely to be found in a large population such as at Long Pont. While we don't see an association between relatedness and distance on the landscape, we do see that certain genotypes (genetic clusters) are associated with particular roads (Figure 8).
Furthermore, we see that there is considerable variation within distinct genotypes (Figure 6), which would imply that sexual reproduction, via seed dispersal is contributing to the spread of Phragmites in the region more that the movement of clonal root fragments. Overall, out study provides further evidence that highways are acting as vectors for the spread of Phragmites seeds.

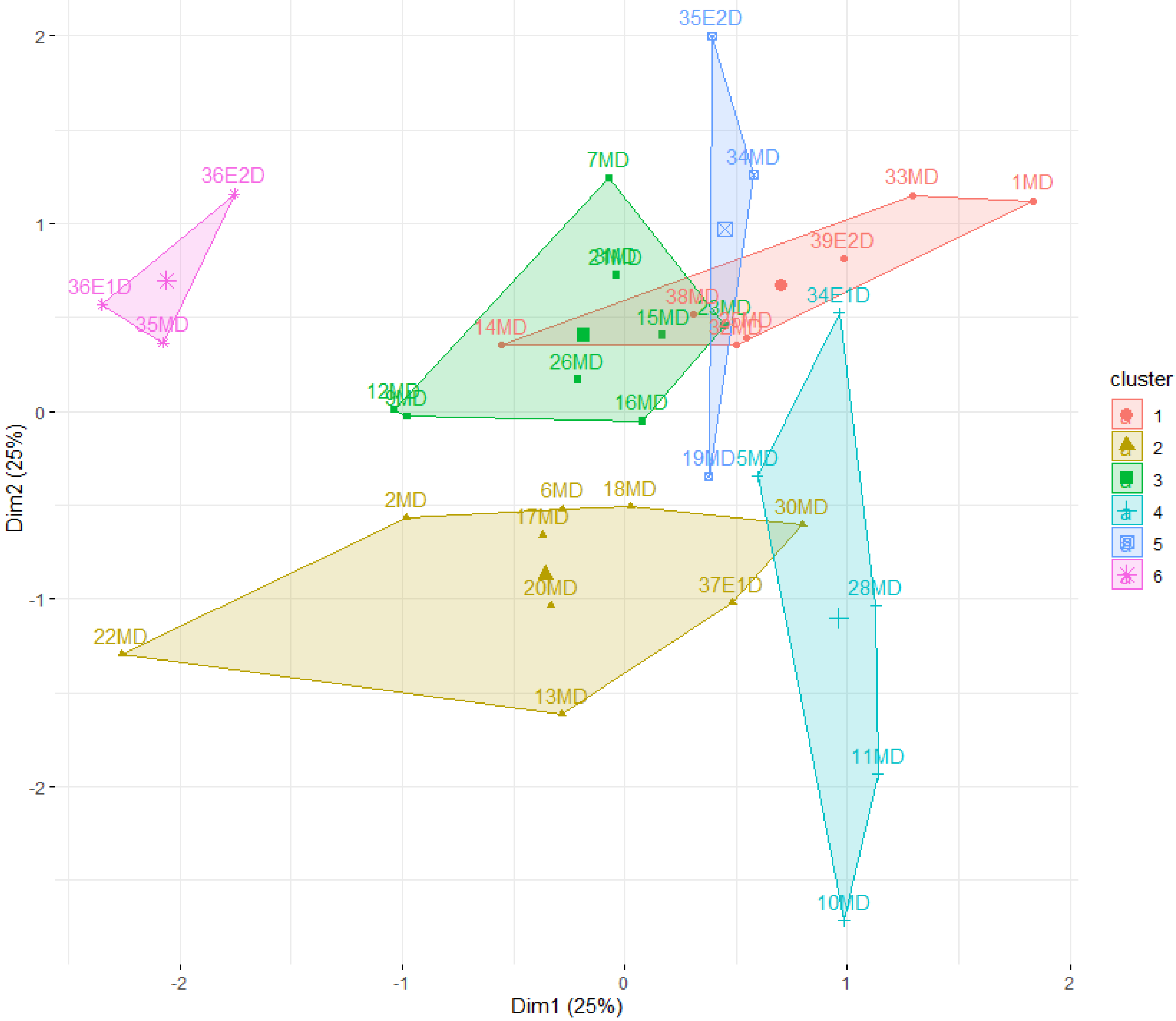

Figure 6 *Genetic clusters among* Phragmites *populations in the study region, identified using hierarchical clustering.*

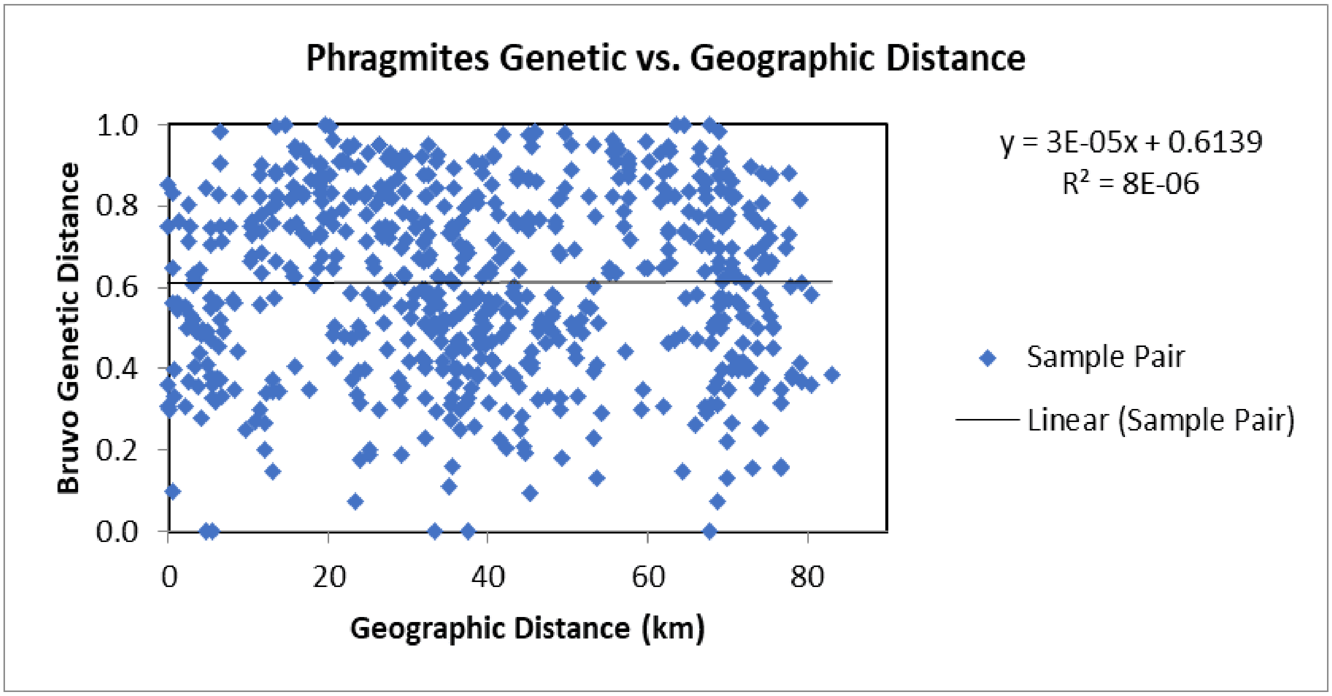

Figure 7 *Pairwise comparison of* Phragmites *samples shows no relationship between genetic distance and geographic distance*.

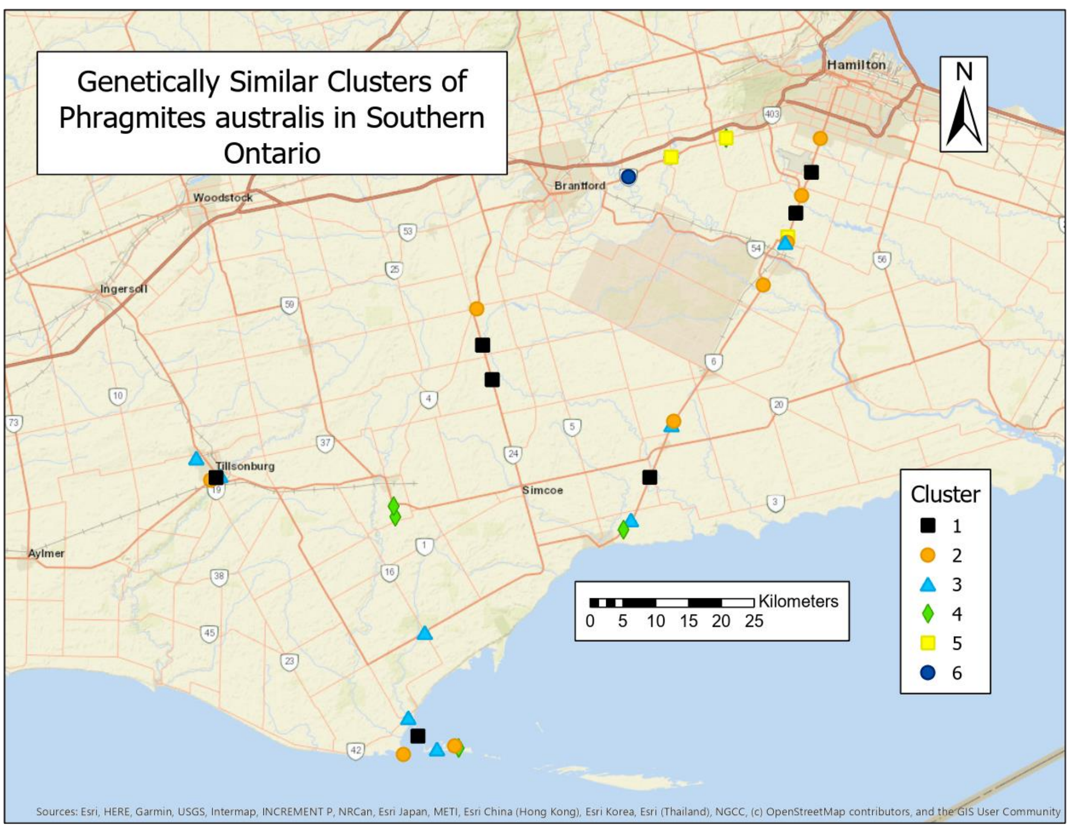

Figure 8 A map of our study region, showing the location and spread of genetic clusters identified among populations of Phragmites.

Discussion

We sought to quantify competitive outcomes between the invasive Phragmites haplotype M, and native plants that may be used to restore landscapes adjacent to roads and wetlands. We asked whether or not some native plants may be considered as a biocontrol to exclude new Phragmites invasion through seed dispersal. We also measured the effect of soil moisture and salinity on the outcome of competition. Finally, we asked whether population structure in the Hamilton-Norfolk region a result of seed dispersal (sexual reproduction), or root fragment transfer (vegetative reproduction).

Competitive Environment

The effect of competition on Phragmites varied across the competitive environments we studied. Communities of multiple species were generally more successful at supressing growth in Phragmites, with the most competitive species being *B. frondosa*. Our results corroborate the findings of others who concluded that the native annual *B. frondosa* is more competitive against Phragmites than other native plants of similar habitats (Byun, de Blois et al. 2013). In our studies*, B. frondosa* showed the greatest potential to reduce Phragmites biomass in both of greenhouse and field settings. The growth habit of *B. frondosa* is related to its life history as an annual plant; it is able to take advantage of resources more quickly which helps it to shade out seedlings of slower establishing perennials such as Phragmites. When species share very similar niches, the outcome of invasion may depend on the relative fitness, or growth performance between the invader and resident native species (MacDougall, Gilbert et al. 2009). This suggests that native plants with high growth rates (weedy habits) such as annuals and biennials may be the best suited native plants for repelling invasions at wetland restoration sites. However, in coastal regions, Phragmites displaces native wetland annuals such as *Bidens cernua* (L.) which also rely on fluctuating water levels and exposed mud banks to germinate (Tulbure and Johnston 2010).

We also found that communities of mixed grasses and forbs were at least as competitive as the best single species competitors, and in the field study where density was controlled, multi-species competitive environments reduced Phragmites more than single species competitive environments. The communities containing the halophyte *S. sempervirens* also reduced Phragmites biomass considerably, in all field conditions, but only dry greenhouse conditions. These findings are consistent with other experiments that found communities of mixed functional groups were more competitive against Phragmites than monocultures (Peter and Burdick 2010). This additional effect may be driven by niche complementarity (Kennedy, Naeem et al. 2002) allowing each competitor to allocate more resources to competition.

Increased competitive ability of all species could be conferred through facilitation from the most stress-tolerant species, although the traits related to stress tolerance are not necessarily related to the traits that effect the outcome of competition in a community (Funk, Cleland et al. 2008).

Phragmites seeds may not be able to establish in existing dense vegetation or standing water (Tulbure and Johnston 2010). Although we found Phragmites seedlings could tolerate growing with a low number of competitors, it did not accumulate biomass quickly, even in the control conditions. Its invasive habit may emerge over time, as it gains a fitness advantage over resident plants of similar niches (MacDougall, Gilbert et al. 2009), which may also less tolerant of changes in soil moisture and salinity.

We did not anticipate that Phragmites would grow larger when planted with *R. laciniata* in field conditions than when planted without it. This result is notable because *R. laciniata* was the third species planted in combination with either *B. frondosa* or *S. sempervirens* in the community environments, which were more competitive than single species competitive environments of the same density. Though we found polycultures were more competitive than monocultures, other studies have found that diversity increases the chance of invasion. In an agricultural field setting, crop polycultures were more likely to create favorable microsites for weedy invaders (Palmer and Maurer 1997).

Phragmites is known to invade wetlands with high floristic diversity such as Long Point marsh. Therefore, discovering evidence for facilitation rather than competition may not be surprising. Here we provide further evidence that some plant communities may be more vulnerable to invasion than others depending on the species present, and that some native plants may even facilitate invasion as others resist it (Byun, de Blois et al. 2013, Gallien and Carboni 2017).

Moisture & Salinity

The effect of soil moisture on competitive effect was most pronounced in the greenhouse comparisons, though the effect was not statistically significant. Here Phragmites biomass was lowest in dry environments, even in the absence of competition. In the greenhouse Phragmites was least affected by moisture when growing with the strongest competitors. Successful management of this invasive species, and the recovery of resident plants has previously shown to be dependant on moisture availability (Rohal, Cranney et al. 2019).

In the field, Phragmites grew largest at high salinity levels, regardless of competitor (Figure 2). This is not surprising since salt tolerance is suspected to drive invasion of Phragmites (Vasquez, Glenn et al. 2005), and the native competitors used here are primarily plants of freshwater wetlands (Voss 1972)However, we also found that the outcome of competition may be driven by a complex interaction between soil parameters and competitive environment, especially at lower salinity, with Phragmites gaining an advantage as salt levels rise. Since Phragmites did not grow larger in saline environments in our greenhouse study, we conclude that their advantage at high salinity is derived from tolerance traits, rather than growth performance or other fitness traits, consistent with physiological studies of this species (Vasquez, Glenn et al. 2005, Guan, Yu et al. 2016).

Phragmites was able to tolerate extremes in salt and moisture better than some of the species it may out-compete in the wild including *M. fistulosa*, *P. virgatum* and *R. laciniata*. Phragmites can reduce local plant diversity through altering soil properties as well as through competitive growth traits (Uddin and Robinson 2017), and so some species will be more sensitive to those changes than others.

Salt- tolerant species should be considered in restorations with caution. For example, *S. sempervirens* is not historically native to the Great Lakes region, but it has spread inland from the Gulf of the St. Lawrence River along salty roadways. Though it has the potential to spread, it also grows quickly, has moderate suppressive effects on Phragmites compared to the native wetland edge perennials we studied that are not salt tolerant. Furthermore, *Solidago* species are important nectar source for fall bees and butterflies and could provide an ecologically superior option for salt contaminated roadsides that are otherwise dominated by Phragmites, and where no native wetland plants will thrive.

While competitive effect of some native species may help in suppressing Phragmites seedlings, most of the seedlings we studied in competition did not actually die over the time that we studied them, in either greenhouse or field setting. Future works should follow such competitive outcomes for multiple seasons. My study was not able to measure how well seedlings in competition overwinter, or if competitive effects would be the same in subsequent years. Given that Phragmites can tolerate harsh conditions and spread vegetatively by root and stem fragments, the level of competitive effect needed to fully eliminate Phragmites once established is unclear. Managing invaded areas by cutting and removing standing biomass may be a more realistic way to support baseline biodiversity, than eradication (Carlson, Kowalski et al. 2009).

Seed Dispersal

Though some authors note low seed viability in northern populations of Phragmites *australis (Maheu-Giroux and de Blois 2006)*, handling methods during seed stratification and germination may affect these outcomes (Ekstam and Forseby 2007). We found that the majority of filled, undamaged seeds were capable of germinating, though we extracted and stratified the seeds in a slightly different method than these studies, so results may be difficult to compare.
While Phragmites is capable of producing upwards of 350-800 seeds per ramet (Belzile, Labbé et al. 2009) and though seeds are capable of long-distance dispersal, the majority of seed rain settles within a short distance (McCormick, Brooks et al. 2016). Since our evidence shows related populations are often far apart, we suggest that these populations are a result of dispersal through human vectors. Seeds can easily be caught in machinery, clothing, soils, or plant materials and transported long distances.

Large variation within distinct genetic clusters implies that sexual reproduction is responsible for the majority of new Phragmites invasions, and not vegetative transfer, as has been hypothesized by others. We also found that genetic clusters do not congregate on the landscape, signifying long-distance,
spread along the roadsides by seed.

Implications for Practice

Our study indicates that some native plants are better than others at suppressing new Phragmites seedlings. The best species for this purpose would be native annuals that gain size and accumulate biomass quickly. Our study also implies that Phragmites has a competitive advantage in saline conditions, even against fast growing annuals. Future research should focus on identifying native annuals with high salt tolerance, and greatest seed production.

- Cover crop seed mixes for newly constructed roadsides near Phragmites should include *B. frondosa, S. sempervirens, V. hastata, E. riparius,* and *S. maritima,*
- More research is needed to identify a broader suite of native species that are similar to the species listed above.
- Overall, *P. virgatum, M. fistulosa and R. laciniata* were not strong competitors in the roadside conditions that we simulated.
- Diversity may help resist invasion, but we found forb dominated communities were more competitive than grass dominated communities.

- We recommend using species that will grow at least 1m in the summer, and provides horizontal shading, likely a broadleaf (dicot) annual or biennial.
- We recommend using ubiquitous, ecologically benign, salt- tolerant species as an ecological bridge to help revegetate salty roadside ditches.
- Explore the use of salt-free de-icing solutions for Ontario's highways to slow the spread of Phragmites.
- A lack of geographic clustering suggests that reducing long-distance spread should be a high priority management action; this can be accomplished by mowing seed heads before they mature and adhering to clean-equipment protocols to limit vehicle facilitated spread.

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CHAPTER 5 Opportunities and challenges for Intervention Ecology

Data that support best practices for ecological restoration in Ontario are limited, particularly in the realm of native, herbaceous plant revegetation. By contributing to the regionally specific literature in Ontario, my research aims to improve the efficacy of seed-based restoration, by testing common methods and their assumptions, and by asking how human intervention can influence restoration trajectory. I asked if seed origin matters to restoration outcomes. I asked if installation method matters to restoration outcomes, and if bee communities actually benefit from these native plant additions. I also asked If restored communities can ever hold their own against invasive species. Below I use a summary of my findings to answer these questions, and to outline the practical implications of my work.

I did not observe evidence for local adaptation among populations of *Monarda fistulosa* at a regional scale, and therefore I was able to reject my original hypothesis. Instead, we found a strong affect of origin, interpreted as maternal effect on seed quality. I also found a strong effect of site, such that seedlings from all origins performed best at the same site, which was also the site where the best performing seedlings came from, Kelso. Together, this evidence implies that three of the four populations studied are performing sub-optimally in their home site. This is consistent with previous studies which found Ontario population to be significantly maladapted in their growth traits (Hamelin 2012). Though my study was limited to one species, it still contributes to best-practices in seed-transfer by highlighting the value of reciprocal transplant as a first step in determining appropriate genotypes for re-introduction. More reciprocal transplant studies are needed for better climate change risk assessments and to better inform seed transfer for assisted geneflow (Aitken and Whitlock 2013). Species-specific data is lacking for many populations that may be at risk from climate change (Maschinski, Baggs et al. 2006, McLachlan, Hellmann et al. 2007, Beardmore and Winder 2011) , and in most cases we do not know if populations will benefit more from genetic rescue or through the maintenance of locally specialized traits (Havens 2015, Olwell 2015, St. Clair, Dunwiddie et al. 2020).

My study of roadside meadow recreation provides evidence that adjustments in seed installation method, can actually impact restoration outcomes. However, we observed that differences in target cover fade over time. As hypothesized, floristic quality varied between seed installation treatments, measured three years after restoration. Surprisingly, high floristic quality, and high levels of target cover were associated with different seed-installation methods. While a greater initial vegetative cover may justify the additional costs of a high seeding rate, long-term cover reaches the same carrying capacity independent of seeding method and may actually prevent conservative species in the seed mix from establishing. This implies that even small variations in seeding method can impact restoration outcomes, and that no single approach is best for all restoration outcomes. Similar studies in other regions have also found a trade-off between cover, diversity and seed mix cost, though these experiments were not conducted at roadside, and did not employ hydroseed methods (Burton, Burton et al. 2006, Wilkerson, Ward et al. 2014). Some intervention methods may favor certain restoration objectives at the cost of others. Practitioners and policy makers are urged to consider how restoration targets interact with each other through the intervention approaches that we choose.

Within my roadside restoration study, I had the opportunity to scientifically test whether revegetating roadsides with native wildflowers can actually create better quality habitat for pollinating insects, with the expectation that roadsides revegetated with native wildflowers would support a greater abundance and diversity of bees compared to roadsides dominated by Eurasian pasture grasses and opportunistic, exotic weeds. I found a greater bee abundance after three years in restored sites, compared to unrestored sites, however, this pattern was only observed at one of two research locations. I conclude that native plant abundance is one of several nested factors that influences the diversity and abundance of bees at the roadside. Regional differences may overwhelm any benefit to the community that local-scale habitat creation provides (Keilsohn, Narango et al. 2018, Angelella, McCullough et al. 2021). We also did not detect greater taxonomic diversity in these restored sites. Low capture rates in unrestored sites and in bad years may under-represent the true diversity in these communities.

Finally, in a test of resistance, we found that few native plants occupying a similar niche to Phragmites were strong competitors against this invasive species. Therefore, I cannot accept the hypothesis that native plants are generally able to resist invasion by Phragmites; however, I did observe considerable variation in the response of Phragmites to competitive environment. As hypothesized, soil moisture significantly impacted the outcome of competition, but only in the greenhouse trial. Similarly, we accept the hypothesis that soil salinity can impact the outcome of competition, as observed in our field phytometer trial. As predicted by previous studies (Byun, de Blois et al. 2013), the annual plant, *Bidens frondosa* was the strongest and fastest growing competitor. The salt-tolerant coastal goldenrod *Solidago sempervirens* also showed some consistent resistance to Phragmites by reducing its biomass even in saline environments where Phragmites has a competitive advantage. However, very few competitive outcomes resulted in the death of Phragmites, at least in the duration of our study. Native counterparts like *Monarda fistulosa* perished at a higher rate in the shared growing conditions, and *Rudbeckia hirta* seemed to increase Phragmites biomass slightly. While plant community-driven biocontrol needs further exploration as an effective solution, insect bio-controls have recently been approved for use and may be more effective than competing vegetation (Blossey, Endriss et al. 2019).

For the Practitioner

While these results reveal complexity that I did predict, they may be valuable case studies for ecological practitioners, nonetheless. The following recommendations are based on my experimental results and review of best practices attached (Appendix 1).

When sourcing plant material for the restoration of grassland species, we do not recommend relying solely on a local provenance, without first performing a performance trail of your provenance options. Some populations may no longer be well-adapted to their current conditions, and therefore produce lower quality seeds. Despite the presence of a thriving wild population, local conditions may not be ideal for germination of seeds, and that species may not represent an ideal candidate for restoration at that site.

When restoring grassland through seed addition, consider how seed handling and seed installation methods effect the success of restoration. To achieve green and stable conditions more quickly, we recommend seeding in two phases at a low rate (15kg/ha), particularly where cost of seed is a concern, in small areas, or on steep slopes. However, if seed availability is not a constraint, and the project is larger, we recommend hydro-seeding in one phase at a high rate, up to 30kg/ha. All native grassland seed should be installed between October and March, under the supervision of an experienced hydro-seed operator, as well as restoration ecologist with a stake in the revegetation.

At this time, we cannot either recommend or discourage the planting of pollinator strips along roadsides. While native plant addition does seem to attract more bees and provide foraging habitat for more bees, it remains unclear whether these sites act as populations sources or sinks. The resources might be better spent on restoration targeted to land with fewer risks to insects, assuming this can be found. Bee populations in regions with more intensive land use, and lower natural connectivity appear to respond more slowly to restoration, and in our study, their abundance did not significantly increase over three years following restoration.

Unfortunately, we concluded that resistance among native plants to invasion from Phragmites is likely futile, though native annuals may be our best line of defence. Perhaps my study focused to narrowly on evaluating native competitors that are readily displaced by Phragmites in a wild setting. It may not be surprising that Phragmites was not out completed by these species, one-on-one after one growing season. The only suggestion we have beyond further investigation is to employ fast growing, native, annual plants in restoration to resist invasion. We found *Bidens frondosa* had the greatest competitive effect, but similar species should be identified and studied.

Study Limitations, Conceptual Issues & Future Work

In each study, we found evidence that human intervention into restored plant communities can have meaningful ecological impact, at least in the short term. However, constraints on experimental design did create limitations in the strength of each experiment. Furthermore, some restoration outcomes are influenced by factors we cannot control for, and restoration targets based in human valuation of ecosystem components can be odds for philosophical, rather than biological reasons.

The study of local adaptation in *M. fistulosa* was constrained by several factors. First, due to limited capacity, the experiment included a relatively small sample size, with only ten seedlings of each of four origins reciprocally transplanted among those four sites. Also, though we measured fitness traits, such as total aboveground biomass and seed production, physiological traits and belowground biomass mass may tell a significantly different story, though these traits were not measured. Further work should also consider measuring environmental variables such as soil particle size, pH, cation exchange capacity, or average soil moisture content (Liu, Wang et al. 2020). This data would have been valuable for interpreting site difference in our study.

We found no support for a universal 'local-is best' approach to seed sourcing as a form of ecological intervention. Instead, we found that restoration performance was a function of site characteristics, and quality of seed source independently, and not on how well seeds were matched to their origin. However, this pattern may be case specific, and our study is narrowly focused on a single species in only four watersheds. While guidelines exist for some rare plants, and for trees important to forestry, some practitioners question the value of generalized, seed-transfer policies that either promote or restrict transfer. I agree with others who have concluded that without species-specific data, these policies can sometime do more harm than good (Maschinski, Wright et al. 2013), and also see good reason to conserve local genotypes in the process of assisted gene flow (Leimu, Vergeer et al. 2010, Walters and Berger 2019). Future work should focus on identifying healthy, robust source populations to be used in restoration. More work is needed to detect where local specializations, including adaptively plastic genotypes occur and match these traits with future habitats under climate change. Conservation of specialised genotypes may be helped by assisted migration within or toward these regions (Maschinski, Falk et al. 2012, Iverson and McKenzie 2013, Valladares, Matesanz et al. 2014, Rehfeldt, Leites et al. 2018).

The study of roadside meadow seeding methods revealed that slight variation in restoration methods can impact the structure of a re-created plant community, both in terms of biomass but also floristic quality. However, these two metrics alone may poorly reflect restoration success. Quantifying floral resources, both in abundance and diversity might be a better way to judge the ability of a meadow restoration to provision pollen and nectar resources. However, measuring FQI and diversity metrics does inform local plant diversity, which is predictive of insect diversity in many cases, FQI may be an important metric for plant conservation, especially when roadside restorations recreate rare ecosystem, such as tall grass prairie. Further ecosystem function such and soil sequestration or flood mitigations could be measured in response to roadside grassland restoration, but these questions were outside the scope of my work.

I was also able to also measure how revegetation with native plants influenced the native bee community and found in some cases this type of intervention has a positive effect on local bees by attracting a greater number of them. However, some research has shown that roadsides are dangerous places for bees to be attracted to due to high mortality documented along revegetated highways (Baxter-Gilbert, Riley et al. 2015, Keilsohn, Narango et al. 2018). Because we were not able to actually measure the population dynamics of bees attracted, we can only assume that the additional bees we attracted reflect additional bees in the population being supported by additional resources, and not simply an 'oasis' effect of creating a dense patch of co-blooming resources. We can also only assume that the bees we caught would not have otherwise collided with a car, before having the chance to reproduce. These are large and potentially serious assumptions about how we measure bee diversity, but in a highly fragmented and degrade landscape such as the sub-urban southwest of Ontario, it is likely that habitat availability is a much stronger limiting factor in the decline of bee populations than is road mortality. The relative contribution of each factor has not been weighed sufficiently to advocate in favor of roadside meadow restoration or against it.

Habitat restoration is motivated by a desire to both return species diversity to the landscape, but also increase ecosystem functioning. However, the relationship between these two variables is more poorly studied than many conservationists realize, and field ecology suffers unreconciled philosophical issues (Hargrove and Pickering 1992, Fahrig and McGill 2019, Newman 2020). In some cases, the majority of ecosystem function is provided by a small fraction of species in the system, and many low-diversity systems are nonetheless stable and productive (Bullock, Aronson et al. 2011, Montoya, Rogers et al. 2012). Though native seed addition did attract more bees in some of our restored meadows, I was not able to tell but if the increase can be attributed to increased floral diversity generally, an increase in native plant diversity, the presence of the most abundant native plant species, the second most abundant native plant, the process of preparing the site, or applying the slurry of paper hydro-mulch. Similarly, I have been cautious not to interpret a greater diversity of bees as a benefit to local pollination services, as I do not know if species are providing equal services, or if bee populations are actually increasing.

I also studied how well native plants can compete with the invasive Phragmites in an effort to understand if we can revegetate landscapes with native plants that repel invasion. I found that resistance, may in fact, be futile. I measured the response of Phragmites to one-on-one competition with native species in a variety of mimicked roadside environments and found that stressful growing conditions limited Phragmites more than competitors who were less well-suited to those growing conditions. However, my studies did not manipulate competitor density beyond having two different competitors growing with Phragmites. Species that appear to be poor competitors one-on-one with Phragmites, for example, may still be capable of outcompeting Phragmites at higher densities, Although, we might find that fast growing native specie, such as Bidens are actually less competitive at high density, due to increased intraspecific competition for resources that Phragmites is limited by. Future work might point more specifically to optimal seeding densities for different native species that can provide effective biocontrol (Von Holle and Simberloff 2005, Burton, Burton et al. 2006, Middleton, Bever et al. 2010, Wilkerson, Ward et al. 2014)

It may not be surprising that I found little evidence to support the hypothesis that native plant seedlings can totally outcompete Phragmites seedlings when their densities are similar. Why should we expect plants from communities often invaded by Phragmites to be strong competitors against Phragmites? Management remains the best line of defence-- reducing existing populations and preventing them from dispersing. However, given the persistence of seeds in the soil, it remains unclear whether heavily invaded sites or regions can ever be fully cleared of Phragmites. Intervention can be costly, and if funding for invasive control relies on the public perception of success, then competition with a continually remerging seedbank may become more important.

Some suggest that eradication of invasive species is misguided, and that while science can describe how or why invasions occur, the role of science is not to decide if invasions are good or bad, or if they ought to be controlled (Brown and Sax 2004). Others hypothesize that invasive plants fill novel niches in altered landscapes and provide an ecological bridge to remediate degraded sites. Though invasive plants reduce biodiversity and disrupt mutualisms (Tylianakis 2008), some philosophers of ecology note that decay, as well as sudden catastrophic change are woven into natural cycles (Bodin and Wiman 2004). Insofar as we recognize the value of nature to human flourishing, we will continue to manage the decline of biodiversity through restoration, intervention, and revitalization in all forms. This work will benefit from being informed by long-term, species-specific data, and by testing scientific hypotheses about how ecosystems form and function.

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Appendices

Appendix 1 Revegetating Ontario roadsides with native plants: a jurisdictional scan and literature review to support best practices

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> "Even for the most seasoned revegetation professional, achieving success is not guaranteed."
> (Armstrong et al, 2011)

Introduction

Background

There are over 16600 km of provincial highways in Ontario (MTO 2017). Most roads are flanked vegetated green space, as part of the road easement, or adjacent land use. This roadside verge may be a harsh environment, supporting only stress-tolerant agronomic ground covers and non-native weeds. Nevertheless, they have become increasingly important natural spaces for wildlife, especially in urban and suburban regions where roadsides serve as important conduits for migration (Penone et al. 2011, Jakobsson et al. 2014). Though roads can restrict the movement of wildlife across the landscape, and fragment habitats, roads can also be rich in plant diversity, and can serve as valuable pollinator habitat (Forman & Alexander 1998, Ries et al. 2001). Roadside restoration is an opportunity to reconcile a human need for transportation, with supporting rare prairie-grassland habitat in Ontario, along existing infrastructure corridors (Lundholm & Richardson 2010, Kostyack et al. 2011). Plants that tend to thrive on roadsides will tolerate stressful growing conditions such as pollution, disturbance and weed pressure.

Pollution- Airborne NaCl from de-icing salt can travel up to 120 m from the road and cause leaf injury, but typical NaCl accumulation occurs only within the first 5m of the roadside, where it is toxic to many plants, particularly trees (Forman & Alexander 1998, Aanen et al 1991). Both native and non-native species can be adapted to salt, though native salt tolerant species are naturally rare in the environment and/or are very short-statured, and therefore more easily invaded or more quickly succeeded. Many do not have other auto-ecological traits that make salt-tolerant weeds pervasive, like adaptation to disturbance, or increased biomass and seed production.

Highways can impact surrounding land up to 200m away from the road, through NOX and mineral nutrient pollution form exhaust, agriculture, and roadwork. Heavy metals such as lead can be found in plant tissues up to 5m form the road edge, but in soils up to 25m (Forman & Alexander 1998, Angold 1997). Nitrogen pollution may further facilitate the spread of invasive plants (Aanen et al 1991). Native grassland species are often adapted to nutrient poor environments, and so lose their competitive ability in soils with high nitrogen deposition (Panetta & Hopkins 1991).

Weed Competition- Roadside soils may also be compacted, or exposed to high winds, and may easily erode on steep slopes. These spaces are dominated by plants with growth traits that allow them to withstand and thrive in disturbed or compacted soils. Repeatedly disturbed roadsides can be dominated by non-native species including common annuals and biennials of waste places (e.g., Vipers Bugloss, Chicory, Teasel etc.). Some verges have been planted and managed with exotic species including forage crops (e.g., Crown Vetch, Birds Foot Trefoil, White Glover, Alfalfa), pasture grasses, used for erosion control, along with ornamentals escaped from cultivation (Timothy, Smooth Brome, Canary Reed Grass, Tall Fescue, Miscanthus) as well as ornamental trees and shrubs (e.g. White Poplar, Norway Maple, Red Pine, Buckthorn).

It is surprising that non-native species with a high invasive ranking (I-RANK, Randall, et al. 2008,) that are still planted on roadsides in Ontario today (FHWA 2017b). Planted exotic trees have been observed to spread into adjacent habitats in half of the sites they were planted (Forman & Alexander 1998, Forman & Deblinger 1998). Invasive exotic species, such as Phragmites can use roadways as motorists do, to travel across the landscape (Wilcox 1989). However, certain high-quality habitat fragments do remain within present highway and country road easements, and these communities can serve as reference models for new habitat creation, and as expansion loci, if possible, without degrading the pre-existing native plant populations.

The interest and need to restore native plants on roadsides must be reconciled with the physical and ecological barriers identified above. This review will explore the strategies that jurisdictions outside of Ontario have adopted to meet the contemporary goals of Integrated Roadside Vegetation Management. We will summarize the best practices use to restore and recreate native plant communities. In Ontario, where few such projects have been completed successfully, we can learn from the work of our peers in distinct, but comparable jurisdictions, and aim to apply some of their methods, while researching and developing context-specific solutions for Ontario roadsides.

Incorporating native trees and shrubs has been the focus of roadside landscaping in Ontario because they are long -lived and can require less maintenance and sequester more carbon than traditional herbaceous cover (Scrivener 2012). However, establishing native plant communities on roadsides has the potential to save even more in long-term maintenance costs, including weed management, and snow plowing, while adding to the ecological value of road corridors.

Native prairie plants are hardy, long-lived or readily self-seeding perennials that are well-adapted to harsh, open environments. Other benefits of native revegetation include:

- native plants perform well in poor soils.
- deep grass root systems provide excellent erosion prevention.
- dense foliage reduces erosion by intercepting runoff and increasing infiltration.
- decaying foliage adds organic matter to the soil, making it more absorbent.
- deep roots allow prairie plants to tolerate drought and high salinity
- tall-grass prairie species can shade out many adventitious weeds.
- large dense grasses provide natural snow fencing.
- diverse plantings adapt to a wide range of site conditions.
- provides habitat for songbirds, and small mammals.
- provides critical habitat for agricultural crop pollinators.
- enhances, through colour and form, the natural beauty of the road corridor
- restores a piece of the region's natural and cultural heritage (Brandt et al., 2011).

Scope

This document aims to synthesize the reports of others on this subject, as well as the guiding documents of jurisdictions studied. We will describe the various frameworks and best management practices which allow the United States, and other jurisdictions, to manage roadside habitats for increased ecological value.

The *Program Development* portion of this report is primarily a chronological summary of the development of successful roadside restoration programs in the USA, recognizing Integrated Roadside Vegetation Management (IRVM) as fundamental to all of them. We will explore similar programs that currently exist in Canada.

The *Review of Best Practices* portion of this document offers methods recommended through the various guiding documents used by the jurisdictions above. These have been organized into seven components of restoration and presented as follows:

1) site selection,
2) stakeholders, organization, and funding,
3) seed provenance and species selection,
4) site preparation,
5) seed mixes and installation methods,
6) weed control and maintenance, and
7) performance evaluations.

We end with summary table of roadside restoration considerations, or stages of development. A rough cost comparison is also included, and finally our top ten recommendations to support further roadside revegetation with indigenous plants.

Literature Search- We began by conducting an Internet search for the phrases 'roadside re-vegetation/restoration', 'roadside native plants/seeds', 'native highway planting/seeding', 'salt tolerant native plants', 'grassland green infrastructure' and several other terms, through online archives such as JSTOR, Web of Science, Google Scholar and others. Additional personal communications were provided from several experts through unpublished reports, or planning documents, as well as in person conversation.

*Jurisdiction*s- We will draw examples from US States with established public programs and public-private partnerships that deliver roadsides vegetation programs, using native plants— Iowa, Minnesota, Wisconsin, Michigan, Idaho and others. There are varying levels of collaboration between NGOS, state and federal agencies in these projects, depending on region. Canadian examples will also be explored from Saskatchewan, Alberta, Yukon Territory as well as recent developments in Ontario.

Part A Program Development

US Roadside Revegetation Before IRVM

The first nod towards ecologically managed roadsides in the US was in the early 1930s, when the Texas State Department of Transportation began incorporating native plants on highway sides because of their tolerance to local conditions, and to support wildlife (Markwardt, 2005; Landis 2005). Elsewhere, prior to the 1960s, there was little thought placed on roadside revegetation and management in the United States. Highway verges were maintained as the "nation's front yard" (Harper-Lore & Wilson, 1999), with a focus on keeping them mowed, weed-free lawns. In 1965, the Highway Beautification Act formalized an initiative begun by the former first lady, Lady Bird Johnson. The Act includes, for the first time, the planting of wildflowers, alongside trash removal, and advertisement regulation, as part of maintaining roadside spaces. Unfortunately, early projects did not focus on seed provenance. Lack of planning, and matching species to site conditions, along with inadequate funding for maintenance meant that many of these early projects failed (Johnson 1970, Hann 2010).

In 1973 the FHWA (Federal Highway Administration) launched a program called Operation Wildflower. Transportation departments were encouraged to partner with regional horticultural and botanical societies to incorporate wildflower seeds into highway verges. The program continues today with limited funding for project costs but is not mandatory in any US jurisdiction.

The first mandatory regulation in the US, requiring native plants be used in roadside revegetation was the Surface Transportation and Uniform Relocation Assistance Act (STURAA 1987). However, this act only requires that a small portion, a minimum .25% of the funding, is allocated to purchasing native plant stock for every landscaping project on federal highways. Because the funding is allocated to highway landscaping, it can only be applied to additional aesthetic projects and the creation of new wildflower verges, and not on the management of invasive species, or maintenance of existing native plant habitats (Harper-Lore & Wilson, 1999; Haan, 2010)

The motivation to incorporate native plantings have been many, including erosion and runoff control, improved water quality, improved habitat for plants and animals, reduced need for herbicide, reduced glare, drifting snow and increased sightlines for drivers (Johnson 2008). Though some states continue to develop ecologically minded roadside revegetation strategies, other states have not developed a native seed industry to supply these types of projects, and others report that invasive plants present too great an obstacle to restore native communities on roadsides (Henderson, 2000; Haan, 2010).

In 1991, only 4 of the 14 prairie states actively practicing prairie restoration participating in roadside revegetation with native species. At the time, departments of transportation cited inadequate dissemination of restoration guidelines. Since then, many jurisdictions have focused on creating regionally appropriate guides (Harrington 1991, FHWA 2017a).

IRVM & State Programs

Iowa

Leading states have formal, state-sponsored programs to enhance roadsides with native plants. In 1988, the state of Iowa developed an Integrated Roadside Vegetation Management (IRVM) approach to include environmental and cultural considerations of as well as economic and safety concerns to the management of roadside verges. By addressing pre-existing regulations, laws, obligations and constraints facing roadside habitats in a single integrated plan, IRVM aims to meet several environmental, safety, and economic goals at once. It does so by focusing on solutions that will be sustainable in the long-term. Elements of a typical IRVM plan include shrub control, tree trimming and cutting, mulching, fertilizing, erosion control, herbicide application, rodent and insect control, prescribed burning or mowing, planting and finally native seed harvesting. Further discussion on setting up an IRVM program is included in the *Stakeholders, Organization and Funding* section of the *Review* below. The IRVM office is located and associated with the Prairie Centre at the University of Northern Iowa (Johnson, 2008).

A companion program was launched in the same year, called the Iowa Living Roadway Trust Fund. The LRTF is intended to support IRVM for eligible projects, including native seeding, plant salvage and noxious weed control. This program is funded by the Iowa Department of Natural Resources through the Resource Enhancement and Protection Fund (REAP), but augmented by the Road Tax fund, and access fees. Nearly $12 million in funding was provided to IRVM related projects in Iowa between 1990 and 2009 (LRTF, 2009). Between 1988 and 2011 just over 100, 000 acres of both state and county roadsides have been planted with seed mixes containing 15 to 45 indigenous grassland species.

Iowa has since published an updated *IRVM Technical Manual* for roadside prairie restoration within the state (Brandt et al., 2011). The *IRVM Technical Manual* is an ideal guide for developing programs aimed at integrating native species into roadside restoration projects. A "toolbox" of fundamental principles in IRVM is offered that suggests plantings should be diverse, to discourage weeds, and to increase productivity through niche partitioning and complementarity. Cyclical burning is cited as the most cost-effective means of maintaining grassland on roadsides and controlling weeds. Emphasis is placed on developing partnerships between transportation departments and landowners are included in the toolbox as well. Coordinated efforts are critical to control noxious weeds and to reduce local disturbance that may promote invasive species. Recommendations are also given for initiating an IRVM plan at the county level, identifying the following items funded by the Living Roadways Trust Fund:

- roadside inventories
- seeding equipment
- discs, harrows, packers
- native seed and storage space
- prescribed burns and equipment
- public education (workshops and signage)

The manual also covers best practices for the major components of roadside restoration which will be discussed in more detail later in the *Review* but includes information on native seed sourcing (including a chapter on salvaging seed from historic grasslands), seed installation, erosion control, weed control, and prescribed burning (Brandt et al 2011).

Minnesota

Following suit, the state of Minnesota adopted elements of IRVM, with a focus on native revegetation and noxious weed control in their guide to best practices for roadside vegetation management (Johnson, 2008). This guide further highlights the need to manage roadside vegetation as snow fencing, considering visibility and driver safety. It suggests including a public relations plan, focusing on promoting the benefits of IRVM, and connecting infrastructure improvement projects with researchers. Promoting source-identified native grasses and forbs, Minnesota had at the time established a Native Wildflower and Grass Producers Association which recommend species and methods to establishing prairie grasses that will be discussed below. The Department of Natural Resources now manages a directory of native plant suppliers.

The guide cites several examples from within Minnesota where IRVM practices were successfully incorporated to promote native vegetation. Native grasses were used in one instance, along with biological insect control to reduce competition from Canada Thistle (*Cirsium arvense*), a pervasive and noxious weed. The Minnesota Department of Transportation has successfully used controlled burns to maintain native vegetation along wide highway easements in both rural and metropolitan areas. Furthermore, these roadside grasslands are harvested (and mowed at the same time), in order to supplement local DOT seeding projects (Johnson, 2008). Special considerations are paid to conserving extant rare plant populations on project sites. Populations of rare plants adjacent to the roadside can be enhanced by restoring nearby roadside habitat (Johnson, 2008).

Minnesota actively promotes private landowners convert unused, mowed lawn space along roadsides to native prairie though the Roadsides for Wildlife Program which produces several quick-fact reference sheets for grassland restoration best practices. Methods are generally the same as those described in the IRVM technical manual. See www.dnr.state.mn.us for more information.
The guide also includes results of a driver's survey. Not surprisingly, participants agreed that roadsides comprised of mixed prairie and wildflower species were most appealing to look at and appeared well maintained. The same survey found that mowed sod grasses with no other vegetation were unattractive in all settings used.

Wisconsin

Helping to prioritize and expand ideal restoration opportunities, Wisconsin employs the Native Wildflower Banking Program. In urban areas, or those with verges too narrow for ecological restoration, federal STURAA funding for wildflower planting, can be banked and used to augment future wildflower plantings in more appropriate regions. Roadsides are required to be planted with native trees, and native forbs where possible, especially in rural areas, and those near high quality habitats. The Bureau of Highway Operations (BHO) funds roadside inventories to identify native communities for salvage. Where plants cannot be saved on site, they are moved to a suitable site nearby.

Roadside restoration contracts in Wisconsin explicitly require that the installer have a minimum five years of experience with installing native grassland seed. However, the establishment periods for native seeding contracts are extended, due to the slow establishment of prairie species. During this period, re-seeding is not required, but mowing and weed control are. Long-term management is the responsibility of regional operations staff. Burning is ideal, but mowing is also effective. The Wisconsin Department of Transportation emphasizes that mowing must be accompanied by litter removal; otherwise mowing has little effect on grassland maintenance (WDT 2006). The Wisconsin model is very similar to the IRVM model, with an emphasis on herbicide reduction, conservation of grassland species, providing safe roadsides, and management of existing prairie and grassland remnants. Several best practices from the *Special Native Seeding Provisions* portion of the *Roadway Standards* of the WDT will be elaborated our *Review* below.

Michigan

In 2003, the state of Michigan also began to consider the use of native plants in roadside revegetation. An executive directive was given to the Department of Transportation to use an interdisciplinary, collaborative and context-sensitive approach to roadside vegetation management where possible, but this is not enforced (Office of the Governor, 2003; Haan, 2010).

Much like Ontario, native prairie grassland occurs only the southern Peninsula of Michigan and may therefore be used as restoration models for native roadside revegetation in the southern extent of these neighboring jurisdictions only (Haan, 2010). However, because these habitats are rare, managed roadside restoration provides a unique opportunity for compensation and reconciliation of these early successional communities and species.

By studying experimental native roadside seeding, Hann (2010 and 2011) presented recommendations for site selection, and matching species with several soil parameters in southern Michigan. Recommendations will be discussed below. Despite this work, and although Michigan has a well-developed native plant, seed and restoration industry, Department of Natural Resources as well as a Native Wildflower Society and Native Plant Producers association actively involved in seed salvage, native roadside seeding appears still to be infrequent in Michigan. However, the Michigan Department of Transportation did report to be incorporating a diversity of shrubs grasses, vines and wildflowers into a nine mile stretch of major highway, citing climate change-readiness and erosion control as motivating benefits of this approach (MDOT, 2010).

Others

Sponsored by the National Institute for Advanced Transportation Technology, the Idaho Transportation Department and the University of Idaho published a list of native plants particularly well suited to the unique roadside conditions, and ecoregions of Idaho (Robson & Kingery, 2006). This document also includes a review of topsoil stockpiling techniques for the restoration practitioner.

Delaware also uses many native plants and grass in roadsides plantings. To better guide staff, regional activists and legislators, the Delaware DOT produced *Enhancing Delaware Highways*, with help from the Delaware Center for Horticulture, the University of Delaware and the National Urban and Community Forestry Council. This guide largely focuses on maintenance of existing vegetation but does include grassland seeding methods and a chapter on local seed collecting and regional seed sourcing (Barton et al. 2002).

The state of Tennessee has also investigated the use of native revegetation in highways sides, conducting seeding trials and a jurisdictional review, similar to this one. The report concludes that native seeding is not only viable for highways sides in Tennessee but an ideal alternative. Best practices are similar to those offered by the IRVM office (Swan, Cripps et al. 1993).

The National Wildlife Federation has also been working with cities in Ohio Indiana, Missouri, Nebraska and Arkansas to create certified Community Wildlife Habitat by planting roadsides with corridors of native plants. These projects are applied for by public interest groups.

Most recently, the state of New Jersey passed a bill requiring state road revegetation to include only native plant species. Those that supported the creation of this bill cited cost savings, and hardiness, in the face of environmental change (S-227/A-963, Cocozza 2017).

Federal Highway Administration Technical Guides

Building on established state-level collaboration and learning from ongoing projects, the FHWA published a 2007 report entitled *Roadside Revegetation: An Integrated Approach to Establishing Native Plants*. It presents a full technical working guide of best practices to achieve habitat restoration in roadside conditions (Steinfeld et al 2007). The following is brief outline of their recommended approach, while specific key components is highlighted in the *Review of Best Practices* below. The FHWA offers a five-phase project cycle for any roadside restoration: initiation, planning, implementation, monitoring/management, adapt and improve, and finally the initiation of new approaches and future work.

1. In the first phase stakeholders and cooperators are identified, along with their anticipated roles. Record keeping standards are identified, as well as project objectives and specific milestone timelines are proposed. During initiation, benchmarks of performance must be developed for use in the monitoring phase. These may include identifying acceptable standards of species establishment and diversity or percent soil cover.

2. Planning for a restoration project requires the collection of project contacts, physical site data such as soil structure, construction and supply cost estimates, as well as any environmental and cultural impact assessments and associated permits. In this second phase the revegetation objectives should be defined, including the edification of reference model sites, and the units of revegetation within the project site.

3. Implementation begins with site treatment and preparation, as well as seed source contracting. Once the installation of soils, seed, mulches, fertilizer, protections and amendments are completed, quality control must be assured.

4. When entering the monitoring phase, it is critical to evaluate the original objectives, and to update any management strategies to reflect any unforeseen changes to the initial plan. Management towards these objectives may include invasive plant control, erosion control, over-seeding, prescribed burns or controlled grazing/mowing.

5. Finally, the project is evaluated, and further work is organized, or lessons learned are used to inform associated projects in the future (Steinfeld et al 2007).

The FHWA now supports native roadside revegetation, nation-wide through their *Roadside Vegetation Program* as part of their current *Environmental Review Toolkit*. Support for native seeding on federally funded highways in any state still remains from STURAA (1987), but programing is administered at the state level, with some states generating additional support through Adopt-A-Highway, and Roadsides-for-Wildlife programs, and Intermodal Surface Transportation Efficiency Act (ISTEA) enhancement projects. Two major publications have since been released by the FHWA which provide some of the most comprehensive materials to inform restoration projects in all states.

The first, *Roadside Use of Native Plants* provides a collection of introductory essays from experts on fundamental principles of site preparation, species selection, public relations, using and communicating ecological concepts, specifying native plants in contract designs, and many other topics. It includes a state-by-state listing of native species appropriate for landscape use, federally listed endangered species, dominant community types, as well as state revegetation expert contacts and native plant and suppliers. Finally, this guide includes relevant policy regarding invasive and naïve species, exception from STURAA provided by seed banking (i.e. compensation planting), in more favorable sites (FHWA 2017a).

The second guiding document is *Vegetation Management: An Ecoregional Approach,* which builds on experience in the Midwest and other examples with IRVM. The ecoregion model allows land restoration projects to be managed more efficiently by categorizing projects based on climate, soils, and native vegetation communities, and not by State boundaries alone (FHWA 2017b). Provided, are maps of the ecoregions of each state, as well as an example of a Ecoregional roadside vegetation management plan for the Sand Hills region of Nebraska. Components of this Ecoregional Plan include:

- Climate description
- General soil types
- Site Hydrology
- Existing and nearby plant communities
- Unique landscapes and habitats
- Sociological and historic features
- Land use and Major communities
- Transportation types
- Corridor objective
- Urban and metropolitan objectives
- Rural objectives
- Aesthetic considerations

US Domestic Scan Report Summary- FHWA, 2011

As momentum towards ecologically restored roadsides grew, and more projects were completed, the FHWA began a domestic review of successful revegetation projects that used native species. The scan was conducted onsite at each project location by a team of six re-vegetation experts. Five projects were located near the west coast, and two near the east coast, which would be most representative of conditions in southern Ontario. Though no two projects were identical, their success was attributed to just a few key elements, that include early planning, clear project objectives, and stakeholder collaboration, commitment to monitoring and maintenance, as well as commitment on the part of the contractor, to successful revegetation with native species (Armstrong et al, 2011). Below is a brief summary of the projects included in this report.

Route 9, Vermont

Because this designated Vermont Scenic Highway is held to a high design standard, daily oversight was provided by a landscape consultant with expertise in native plantings, working closely with both the landscape architect and construction team. Innovative solutions to planting steep slopes of stone fill over mineral soil were developed by the Vermont Agency of Transportation. Combination of jute netting, and Geo-grid was used to stabilize slopes and prevent erosion while a native seed mix established. The seed mix was sown into a 12" layer of salvaged local topsoil placed over the stone fill. They project team concluded that supplemental watering was not necessary and did not contribute to project success. Nearly 100% native plant coverage, plus offsite wetland mitigation meant this project achieved its restoration goals.

Cascade Lakes Pass, New York

The challenge for this project was to mitigate the effects of ice salt and sand on the adjacent lake, and native White Birch trees. Working with state park and environmental agencies, the New York State Department of Transportation provided $20,000 of funding as well as the necessary equipment, through a state program called the Green and Blue Highways Initiative. The steep and narrow slope between the road and lake was made more hospitable to native, salt tolerant plants through the installation of rolled coconut fibre, coir log 'planting benches', along the upper bank, filled with imported topsoil. Though several native species provided an effective and salt tolerant buffer (Sweet Oxeye, Little Blue Stem, Tamarack, Grey Goldenrod, Prairie Chord Grass, Wild Bergamot, Sand Cherry), White Birch was not successful, and additional weed species were brought onsite with the topsoil.

Blaine Road (Nestucca River), Oregon

Bordering a designated state Scenic Waterway, and part of the National Wild and Scenic River System, this restoration project was part of an impact reduction strategy for the second phase of a road upgrade, prompted by public outcry to the work done in the first phase. The US Forestry provided the restoration plan in consultation with the US Fish and Wildlife Service, and the US Army Corps Engineers who mandated re-vegetation rate of 12 plants per 100 square feet of stone slope. Slopes leading down to the river were covered first with blow-in compost, and planted with potted, locally source-identified, native species. Steep slopes above the road were reinforced using MSE (Mechanically Stabilized Earth) retaining walls, planted with native grasses and shrubs. To prevent sediment from flowing off the road, composts berms were created along roadsides that face the river. All three elements were successful, but stakeholders identified minor changes to the retaining wall design, including narrow standing ledges, would have allowed the plants to be more easily installed. Early communication and analysis of objectives and project impacts led the complex team to a successful decision to use MSE rather than a bioengineered retaining wall.

Sandlake Road, Oregon

Assessing the role of blown-in compost (mulch) on opposite sides of this highway suggests that adding compost to a hydro seed mixture alone can have dramatic beneficial effects on growth and establishment of target grass species. What began as an engineering oversight, ended up yielding useful information. Though the seed-only restoration did not necessarily fail, the doubled cost of including compost, more than doubled the success of the plantings. This project with administered directly by the FHWA.

Setter's Road, Idaho

After a nearby roadside seeding failed to establish and prevent erosion into the adjacent lake, the Idaho Transportation Department began the restoration of a regularly scheduled road widening, by creating slope conditions that were more favorable to seed establishment. This was achieved by importing rough stone and covering the slope 12" deep in a rock armor base, hydro mulched and seeded. This project utilized only woody shrub species, and annual cover crop grasses to achieve green and stable in the first year. It was concluded that the annual grasses did not contribute to slope stabilization, but that the rock armor base was successful in doing so. The shrubs took up to 3 years to germinate, and some were buried too deep in rock crevices and shifted sediment to germinate. In hindsight, the project leads report that a perennial grass mixture would have been a better option.

Smith Creek, Idaho

In order to improve road alignment along this stretch of predominantly prairie highway, extensive cuts and fills were made to the right of ways, requiring the restoration of over 55ha, which followed standard Idaho Transportation Department design specification, executed by a private consulting firm. The project was funding by the FHWA. Some problems with slope-stabilization arose after the initial restoration, which were solved collaboratively between FHWA, the ITD and the design consultants. A separate native seed mix was used for slopes and ditches than was used for flat ground and was applied between the fall and spring to allow seeds to stratify. The cause of shifting slopes in the case was the placement of topsoil over a clay soil slope, which would easily saturate and erode with precipitation. In the third year of the project, coarse rock was placed into these sections, as described above, allowing seeds to settle long enough to germinate. All other areas were successfully colonized by the native grass seed mixture.

Native Plant Research Project (Worley), Idaho

A decommission segment of a re-aligned highway interchange became the host site for ongoing prairie restoration research project with the University of Idaho. The restoration was established with hydro seeding and planting of native container stock as well, with the purpose of evaluating candidate roadside restoration species, and evaluating noxious weed control methods. Topsoil was removed prior to seeding, and the seed mix was designed collaboratively between ITD and University of Idaho professors, based closely on nearby reference sites, a critical step that was not normally incorporated into roadside revegetation projects at the time in Idaho. Twenty transects were constructed through the restoration to measure plant cover. After the first year the site was covered 13% by target native species, and 14% by non-target species. This project is ongoing, and while visual assessments of percent cover are imperfect and simplistic, the FHWA has concluded they are sufficient for determining project success (Armstrong et al, 2011).

The FHWA domestic scan highlights the importance of a clear re-vegetation plan with the following elements: background information, roles and responsibilities of cooperators and contractors, revegetation objectives, relevant constraints, laws, policies and regulations, inventory or local noxious weeds, summary of soil characteristics, project schedule, a defined monitoring approach, and the different vegetation units that will be restored and monitored.

Even with a clear plan, the FHWA domestic scan discusses common deficiencies in revegetation that were encountered during the projects above. Most are associated with public perception, valuation of native revegetation and contract management. The most striking of these issues include attempts to cut costs by contractors who may not have the same level of commitment to native revegetation as the design consultant or government agency specifying a native approach. Other general comments made by the authors of the scan were that there is a general lack of seed, and diversity of species required to restore most candidate roadside projects, suggesting that the FHWA cannot rely solely on the private agricultural sector to provide native seed to public roadway improvement projects. Also, times to complete some stages of project work, such as site preparation, were underestimated by the contractors involved. Weed established was also identified as a major barrier in urban and disturbed agricultural regions. Despite careful planning and installation, the authors remind us that precipitation levels ultimately dictate restoration success. If one of the first three years is drier than average, direct seeded restorations will likely fail (Armstrong et al, 2011).

US Seed Supply

The supply of native, locally sourced seeds to supply roadside restoration is limited, but growing. The Western Federal Division of the FHWA has been working closely with the United States Department of Agriculture's Forest Service to promote the use of local wild-type seed that is procured through a Seed Increase Contract. This integral process was developed in 2001 through the National Fire Plan Strategy which aims to secure and safeguard local sources of native plant material in case of natural disaster (Steinfeld et al 2007). Through this process, wild seed is collected by USDA biologists, and provided to the contracted grower to scale-up through agricultural practices, and to provide bulk seed material for restoration. *Seed Increase Contracts* are initiated several years prior to commencement of any large restoration project, to allow time for appropriate seed mixes to be scaled-up and processed (Landis et al, 2005).

Conservation Plant Materials Centers (PMC) are regional store houses of source identified seed, managed by the Federal Department of Agriculture, though Natural Resources Conservation Service. These PMCs serve the federal restoration needs of their individual ecoregions, while acting as a hub for native plant research and production innovation (Arthur & Gartshore 2004)

Furthermore, the *Plant Conservation Alliance* (PCA) has also made great advances in the collection, and safekeeping of native plant seeds, working with a broad spectrum of seed producers from commercial farmers to botanical gardens. The PCA is an agent of the Bureau of Land Management (BLM), and made up of representatives of six federal and state agencies, as well as 288 cooperating groups from every sector including, first nations communities, universities, native plant producers, natural resource extraction companies, pest management programs, and horticultural societies. In order to support the demand for native plants and seeds, the PCA is working on the following action plan:

- encourage the use of native plants in landscaping and restoration
- provide notice of seed needs through regional working groups to producers
- develop federal capacity for native seed production
- develop nation directory of restoration experts
- develop national and regional native plant supplier directories
- creation of federal native plant procurement task force
- maintain a network of universities, botanical gardens, and NGOs for seed conservation and accessioning (PCA 2012)

The PCA and BLM currently run a seed salvage and conservation program called Seeds of Success. Originally established in partnership with the Royal Botanical Gardens Kew and the BLM, in 2008 the program was ratified as the official national native seed conservation program, bringing together, through a memorandum of understanding, the BLM, Chicago Botanical Garden, Lady Bird Johnson Wildflower Centre, New England Wildflower Society, New

York City Department of Parks and Recreation, North Carolina Botanical Garden, as well as the Zoological Society of San Diego (SOS 2008).

They have also released a ground-breaking proposal to identify, scale up, produce, and store native seed at the national scale, for use in large-scale restorations, such as range-land fires. This five-year program is budgeted, at over three-hundred million dollars. Presently, none of the proposed actions have been implemented (Olwell & Bosak 2015)

Canadian Programs

Saskatchewan

Until recently, the Saskatchewan Ministry of Highways and Infrastructure relied heavily on traditional agronomic cover species. These species were problematic, because they would invade local ecosystems beyond the roadside, facilitate agronomic weeds, and draw wildlife to browse on the forage crops. In 2006, the Ministry outlined steps towards Effective Environmental Stewardship of highways in their 2008 Performance Plan. This has prompted the growth of a native grassland seed industry to supply native revegetation projects coordinated through eh Ministry of Highways and Infrastructure as well as the provincial Ministry of Environment and guided in part by the Native Plant Society of Saskatchewan, founded in 1995 (Neufeld 2008).

Native roadside revegetation in the province is driven by partnerships, developed by the Saskatchewan Wetland Conservation Corporation, developing harvesting and processing guides for seed producers, and acquiring additional seed resources and project funding from a broad array of government agencies, NGOs and Corporations including TD, Ducks Unlimited, World Wildlife Fund, and the EcoACTION program though Environment Canada (Neufeld 2008, SWCC 2002). While there seems to be no mandatory requirement for native revegetation in the province, prairie restoration is frequent in Saskatchewan, where the Ministry of the Environment and the Ministry of Agriculture both produce guides to prairie restoration methods, as do other groups and businesses.

Alberta

Another province with a well-developed restoration industry, the Province of Alberta has recently started incorporating native seed mixes into roadside restorations as well. Numerous agencies came together in 1997 to from for the Alberta Native Plant Working Group in order steer the sustainable propagation, harvesting and prescription of native plants in the province. Examples of stakeholders represented in this Working Groups include the Canadian Seed Trade

Association, Alberta Native Plant Council, Alberta Research Council, and Alberta Environment and Parks. General Prairie seeding guidelines are given and are similar to those developed in the US.

Alberta also requires that any ecological restoation project, whether roadside or not, must use seed derived from plants in the same Natural Region in Alberta that the project is in, or a similar region in a neighboring province or state. Regional cultivars may also be used, but only when that cultivar was derived from alocal genotype, and is named based on it's county origin. If germination rates are under 70% in pre-seeding trials, or if the local native cultivar is of unknown origin, those native seeds should be susbstituted (Smreciu 2002).

Yukon Territory

Not surprisingly, this northern Canadian Territory has a well-developed native revegetation manual for guiding various infrastructure improvement projects, including roadways. It offers several Key Messages:
1) successful revegetation is incremental,
2) define project objectives,
3) measure site conditions,
4) retain and reuse organic materials,
5) condition the ground (through cultivation and/or fertilization, de-compaction),
6) choose site appropriate species,
7) discuss priority for local genotypes (over cultivars, or non-native 'ecovars'),
8) timing and coordination are critical,
9) include follow-up monitoring.

Though site conditions may be dramatically different between Ontario and the Yukon, innovative solutions to shared problems may still be applicable. For example, the use of large woody debris such as logs and stumps as a mulch to stabilize moderate woodland slopes. Though these grubbed stockpiles appear untidy or unsafe at first to some, they provide microclimates ideal for native tree seedlings to germinate and are a cost-effective erosion control.

However, the Yukon's local seed production industry is not yet well developed, and most projects use species native to the Yukon but derived from more southern stock. As a result, local-sourced seed is not a requirement. While planting native species is still an improvement, there is debate whether infrastructure restoration should be restored only with non-invasive, non-native cover crops (Matheus & Omtzigt 2012). The Yukon Revegetation Manual also includes a chapter on hand harvesting and propagation of native seed and encourages more seed collection and collaboration to take advantage of limited agricultural capacity.

Ontario

The present standard seeding specifications offered by the Ontario Ministry of Transportation include only an Old Field Mix, containing several aster and goldenrod species (OPSS 2014). While this group of forbs are important food sources for pollinators, and are often found in old fields, they are slow to establish, and require winter or fall sowing for optimal results. This mix is also not fully characteristic of native grasslands of the southwestern, and most developed part of the province. Though "native grass" is as good practice for erosion control, but none of the specified mixes contain native grass species. There is interest in adding a more diverse palate of fast-growing native grasses and forbs to some of the specified MTO mixes (Berketo 2016 personal communication). Species recommendations are made based on the following *Review* and ongoing experiments with the team at McMaster.

In 2004 a study by Arthur & Gartshore on the feasibility of native species plantings on roadsides was prepared for Environment Canada, which reviews many of the same jurisdictions and best practices as we do here. The authors differentiate Ecologically Integrated Vegetation Management from typical IRVM by treating native plant conservation as central to roadside vegetation management, including the expansion of ecologically critical habitat into roadsides.

The report notes that while native species have likely adapted to their local conditions, they likely also expand their ranges through natural migration events to track their optimal environment as it shifts over time. The authors also recognize that global warming may "necessitate new thinking related to normal species ranges and approaches to species conservation" which will affect species selection (Arthur & Gartshore 2004).

The report sites two previous feasibility studies for Ontario, by Elmhurst & Cain (1990) and Cain (1997). Both recommend the use of native wildflowers and prairie grass mixes for revegetating roadsides. Species recommended by Cain (1997) included several rare prairie plants, which establish readily from seed, but occur in restricted habitats and are not recommended by Arthur & Gartshore (2004). Most of the experimental plots established in the 1990s failed due to weed competition, erosion, and drought conditions. Sites sown with old-field hay mulch performed best and had fewest weeds (Cain 1990;1997). However, Arthur & Gartshore (2004) note that it is standard practice for MTO to fertilize new plantings, which benefits weeds over native species by removing the competitive advantage of native species in low nutrient environments.

With funding from TD Friends of the environment, the Ontario Horticulture Association published a guide to creating a road-side pollination patch, in units of six by twelve meters. The guide is simple, yet comprehensive and includes several small-scale site preparation methods for hobbyists. Public interest groups are encouraged to approach different levels of government to access permission to work on and restore public roadsides. Presently, there is a planting component to the provincial Adopt-a-Highway program for any group wishing to establish a native plant community on existing provincial roadsides. Applicants must contact the Adapt-a Highway District Representative, or the Roadside Vegetation Management Unit with the following information (Dunk et al. 2010):

- specific highway corridor you wish to adopt
- project duration (minimum three years)
- name of interest group
- authorized group representative
- number of volunteers
- proposed working dates

The conversation about native roadside revegetation in Ontario has been ongoing since the late 1970s (Anderson & Lewis-Watts 1978), but recently several large projects have incorporated native prairie plantings. Approximately 170-acre Tallgrass prairie was successfully created along highway 40 in Lambton County, with the hopes of saving maintenance costs, and creating a living snow fence. This restoration also links significant Carolinian habitat, and hosts over 45 different introduced prairie species, including uncommon and regionally rare species. The project was instigated by the Sydenham Field Naturalists, and the Rural Lambton Stewardship Network, and completed with the support of MTO and Tallgrass Ontario (Rankin 2014)

One of the largest roadside prairie restorations undertaken in Ontario to date has been the Herb Gray Parkway project, adjacent to the Ojibwa Prairie, a combination of First Nations land, and Provincial Park land, with road easements belonging to the province dissect several sections of the prairie habitat. Because of the locations rich natural and cultural heritage, special provisions were put in place to protect nearby habitats, salvage rare plants, and enhance new roadsides with native plants (Burley, 2013).

Newly constructed highways verges, and compensation habitat on the 407E extension have incorporated seed from indigenous and regionally sourced plants. Native plants have been used in habitat creation for Species at Risk mitigation associated with this project, as well as for slop stabilization, and landscape plantings (Arthur 2013). Plant materials were required to originate from the local seed zone, or adjacent zones (34, 37 and 32). Regionally rare plants and species at risk were salvaged from construction sites. The project's restoration ecologist successfully worked with site supervisors to coordinate salvage efforts, and to retain high quality fragments to serve as seed sources to supplement restoration materials in future and nearby projects. In doing so, the plant salvage team managed to discover and protect regionally rare species that were not identified during the environmental assessment. A performance evaluation will be conducted by the McMaster research team in 2017 and 2018 on several sections of roadside seeded with native grassland species. We also propose to compile the results of Restoration Site Monitoring Reports and Vegetation Restoration Annual Reports, which are to be completed for the first five years following restoration by the contractor (Arthur 2013). This data will be used to generate baseline success data and to compare sites and strategies.

Many other guides to prairie restoration and habitat creation for off-road applications exist as well, but a complete review of these resources is beyond the scope of this review. However, consult the following table for a list of additional resources on habitat restoration, seed collection, and native plants.

Table 1. List of some grassland restoration and stewardship groups, native plant societies and research groups involved in habitat creation projects.

US Department of Agriculture, Natural Resource Conservation Services	https://www.nrcs.usda.gov/wps/portal/nrcs/site/national/home/
Tallgrass Ontario	http://www.tallgrassontario.org/
Land Stewardship and Habitat Restoration Program, OMNRF	https://www.ontario.ca/page/land-stewardship-and-habitat-restoration-program-guidelines#section-0
Plant Conservation Alliance	https://www.nps.gov/plants/
Carolinian Canada Coalition	https://caroliniancanada.ca/
North American Native Plant Society	http://www.nanps.org/
Ontario Vegetation Management Association	http://www.ovma.ca/
National Roadside Vegetation Management Association	http://www.nrvma.org/
Tallgrass Prairie Centre	https://tallgrassprairiecenter.org/
Society for Ecological Restoration	http://chapter.ser.org/ontario/
Ontario Plant Restoration Alliance	www.ontariopra.com

Part B Review of Best Practices

The following discussion will compare, and contrast strategies developed by the jurisdictions above, synthesizing procedures used in Iowa, Wisconsin, Saskatchewan, Alberta, and by the FHWA. Sections are organized by the major components of a restoration project, with a particular focus on field methods that promote native re-vegetation of roadside environments. Examples are drawn from the multiple technical guides and best management practices developed by the jurisdictions summarized in the previous section.

Site Selection

Native revegetation is frequently prioritized when the road is bordered by environmentally significant habitat, and/or a water course. The purpose of many native revegetation projects is to help protect and enhance the integrity of adjacent plant communities by eliminating the potential for a non-native species to escape the roadside verge. Roadside vegetation management, in these instances, may also focus on plant and seed salvage, as well as minimizing disturbance to pre-existing native species in the road right of way. Using native species to revegetate sloping roadsides next to waterways, on the other hand, can provide a sturdy, permanent, sustainable solution to runoff, erosion, and sedimentation (Brandt et al 2011).

Site characteristics must first be studied, in order to move forward. Decisions on plant material, soil, grading, stabilizers, and installation methods should all be based on prior site characterization. This can include:

- *Site Description*- relevant site characters include slope aspects, qualitative description of site sediments, measure of soil structure, type and amount of organic matter, survey of undisturbed plant communities in several areas near the restoration.
- *Detailed Soils Testing- pH, Calcium carbonate, Electrical Conductivity, Organic content, and levels of Nitrogen, Phosphorus, Potassium, and Sulfur. (*Matheus *& Omtzigt 2012)*

In cases where road construction must be compensated with habitat creation, sites may be selected that are adjacent to the road, or some distance away, in order to help meet the goals of other conservation projects, such as providing critical habitat for species at risk. Compensation sites are less likely to be long and narrow but could be accomplished by following many of the best practices of IVRM (407.

Many highway restorations occur on land owned by the transportation department or ministry. However, often these corridors will transect private lands in addition to public land-conservation areas, rivers, and parks. Therefore, stakeholders will vary from project to project, and in some instances should include representatives of local environmental NGOs, as well as cultural and community groups.

Stakeholders, Organization & Funding

One way to involve all stakeholders is to establish a steering committee which will provide oversight to any revegetation strategy, such as Integrated Roadside Vegetation Management (IRVM). An IRVM Program Aims to:

- Maintain a safe and effective road network.
- Provide ecologically suitable vegetation management.
- Utilize the immense space within roadside corridors to full potential.
- Prevent soil erosion.
- Control undesirable noxious weeds.
- Reduce reliance on herbicides.
- Plant the best-adapted species assemblages (Brandt, et al, 2011)

Once the IRVM Steering Committee is created, they work closely with the dedicated IRVM Manager, or other designated staff to deliver the program objectives and solve problems along the way.

Prior to the initialization of any revegetation project, clear project goals, and individual expectations must be scheduled, including but not limited to- contracting the agricultural scaling-up of source-identified plant material, or timely procurement of existing, regionally appropriate plant stock. Roadside inventories must be completed to identify native habitat fragments and other priority restoration areas. Project budgets must be finalized an approved by all parties prior to any work being started and must include a minimum of 3–5-year maintenance and monitoring period, with a long-term strategy to provide continued upkeep. Efforts should be made in advance by the contractor to collaborate with the revegetation consultant in order to minimize impacts to pre-existing native species, retain stripped topsoil and coarse woody debris for use in the final revegetation phase (Brandt, et al, 2011).

Funding in the United States typically is provided through state-generated funds. However, all state transportation departments must spend one quarter of one percent of federal high funding to purchase native plant material for roadside restoration. Some projects are augmented by contributions of local environmental advocacy groups, along with horticultural and agricultural community groups. In Iowa, counties are encouraged to hire dedicated roadside vegetation managers to take advantage of program support from the IRVM group at the University of Northern Iowa seed materials and funding through the Living Roadway Trust Fund, and the IRVM and coordinate native roadside revegetation. However, if this is not possible other engineering staff with an interest in ecological restoration may serve as the liaison between the county and the LRTF program (Brandt et. al. 2011). Funding for native roadside restoration in Ontario has been provided by the OMTO, and Infrastructure Ontario, with contributions made by the Windsor Essex Mobility Group as a joint venture with Parkway

Infrastructure Constructors, to finance the Rt. Hon. Herb Gray Parkway, including compensatory restorations and road corridor plantings (Lura Consulting, 2017). Native re-vegetation was required for many sections of the 407 EDR in Ontario in part because it will extend through the Oak Ridges Moraine, an ecologically significant region, north-east of Toronto.

Plant Provenance

In order to better conserve genetic diversity within a region, and to better match plant material to local conditions, many jurisdictions have regulations for the use of wild source-identified native seed, relaying on the development of seed provenance tracking and labelling programs, such as the Native Selections program (formerly the Iowa Ecotype Project) started in 1990 by the Tallgrass Prairie Center (Brandt et al 2011). The Association of Official Seed Certifying Agencies (AOSCA) supported this by publishing a set of source-identified standards in 1994 (Arthur & Gartshore 2004). Yellow-tag certified native seed is collected from sites through the state, to promote a broad genetic base. Some counties also manage their own county-source identified seed, which is tracked separately by county as Local Ecotype seed.

The Tallgrass Prairie Centre also administers a handy online Seed Calculator, which will help practitioners prescribe species assemblages, and rates based on county, soil and moisture properties, timing, planting method and cost restraints. This tool is meant for calculating large - off road restorations, but could also be applied wide, mesic, and relatively flat roadsides. Extreme roadside conditions may consider other factors when choosing species and application rates. Developing an interactive website like this would be a valuable first step in guiding roadside managers to include native grassland restoration on Ontario roadsides.

In Ontario, no such formal seed-provenance tracking program exists, and although Ontario source-identified plant material is frequently specified in restoration contracts within provincial parks and conservation areas, there is no system in place to enforce these rules or to audit the accuracy of native plant provenance reporting (Arthur, 2017; personal communication). Ontario Tree Seed Zones were developed by the Ontario Ministry of natural Resources (OMNRF, 2010) to allow foresters and tree producers to make more informed decisions.

However, several native plant producers in the province do keep seed provenance records for the wild sourced plants. The St. Williams Nursery & Ecology Centre follows a Lot Code System, which tracks plants by Ontario Tree Seed Zone (including forbs), date collected, and collector. Several specific locations within a seed zone are often pooled within single Lot Code, unless the species is uncommon or at-risk, but the specific county records are kept on file for each Lot Code.

While these seed zones are based on climate data, and the results of assisted migration trials in several tree species, Tree Seed Zones may not be biologically meaningful for all species, especially short-lived herbaceous plants. It is important to note that the scale and degree of local adaptation varies among species, as do the conditions driving the adaptation These conditions inevitably change over time, which means adaptation is an ongoing, dynamic process, and not a fixed effect; in other words, the characters that are best adapted for present conditions are not likely to remain the best characters for conditions in the future given a changing environment. This is especially relevant in light of global climate change, shifting historical species ranges north (Leimu & Fischer, 2008; Leimu et al., 2010).

Plants must constantly adapt to their environment as it changes around them. Even where local adaptation as evolved, the changing environment implies that that the locally adapted genotypes of today may not be the best adapted to future conditions, even the same locations. Therefore, the goal for conservation should be to maintain the capacity for populations to adapt new genotypes in response to future environmental change, rather than conserving an individual population with a target genotype (Tadeusz & Ebert, 2004; Lavergne et al., 2010).

When sourcing plants for restoration, a review by Sgro et al., (2011) recommends that by choosing half of the plants from within the local population and half of the plants coming from various distances away from the restoration location. This potential can increase genetic diversity within restorations, making it more likely that the mix will include ecotypes that will thrive even in environmental conditions atypical of the seed zone.

Narrow seed transfer zones for uncommon prairie plants reinforces the fragmentation imposed on plant communities by roads and other human development. Restricting the transfer of regionally rare plants across narrow zone, such as watershed boundaries, hinders gene flow via reduced migration, and promotes inbreeding. We agree with Maschinski et al. (2013) that “the local is best paradigm may be dooming many reintroductions to failure.”

Species Assemblage

It is likely that only a subset of any seed mix will be perfectly suited to thrive in each roadside microclimate, though efforts should be made to match species requirements with site conditions. Skousen & Venable (2008) observed only Big Blue Stem, Brown Eyed Susan, and Indian Grass in restored West Virginia roadsides, but did not observe Butterfly Milkweed, Beardtongue or Goldenrod, which were also seeded. They concluded also that a cool season grass functional group was required to provide high native species cover in the early project stages.

Native species assemblages ought to be informed by reference communities of native plants near the project site. The Ecological Land Classification for Southern Ontario provides a starting point to classify broadly, which habitat types occur in an around a restoration project (Lee et al. 1998). Vegetation surveys may highlight appropriate restoration species (see mix recommendations below), that may be scaled up from local seed, given enough lead time (2-3 years). A well-developed example from Ontario, of regionally tailored restoration reference models can be found in Arthur (2013). Planting templates were based ELCs from the Durham region, as well as the Norfolk Sand Plain, for novel roadside habitats where local reference communities did not match physical conditions of heavily altered roadsides. This planning document pragmatically recognizes that “Ecological restoration models must be sufficiently flexible to account for the stochastic character inherent in natural systems and to allow for a range of probabilistic outcomes that are neither completely deterministic nor static.”

Though species will vary by jurisdiction, any grassland seed mix should include species from the following functional groups (Brandt et al. 2011):

Quick Establishing- perennials and biennials that germinate quickly without a length cold-moist stratification period, and grow quickly as well, providing green and stable conditions, serving as a nurse-crop to other target species, and potentially flowering within the first season. These are must-have for any native grassland restoration and should be considered for all projects with suitable conditions. Ontario examples:

- Brown Eyed Susan (*Rudbeckia hirta*)
- Canada Wild Rye (*Elymus canadensis*)
- Sand Dropseed *(Sporobolus cryptandrus*)

Warm Season Grasses- These characteristic prairie species will eventually come to dominate the site without maintenance. They provide superior erosion control and are drought tolerant, thriving in summer heat, and providing fall colour, natural winter snow fencing, and habitat for mammals and birds. Though some prairie grass species are tall, sometimes well over 1m, they need only be excluded from restorations near intersections and driveways for safety reasons. Not including warm season species like Indian Grass or big Blue Stem could reduce the adaptability of the restoration in the long term (Brant et al, 2011). Ontario examples:

- Big Blue Stem (*Andropogon gerardii*)
- Yellow Indian Grass (*Sorghastrum nutans*)
- Switchgrass (*Panicum virgatum*)
- Prairie Cordgrass (*Spartina pectinata*)

Cool Season Graminoids- Because many cool season grasses are also quick to establish, they may be used as nurse crops or cover crop to prevent erosion. Because they emerge early in the season, they provide late winter and spring erosion control, as well as habitat for ground-nesting birds, which warm season grasses cannot. Ontario examples:

- Virginia Wild Rye (*Elymus virginicus*)
- Riverbank Rye (*Elymus riparius*)
- Fox Sedge (*Carex vulpinoidea*)
- Soft Rush (*Juncus effuses*)

Legumes- as nitrogen fixers, legumes provide a key ecological function in grassland communities. Though there are several dozen species of native legume in Ontario, and though their seeds are typically easy to cultivate and germinate, few species have been trialed as a bulk-restoration species in Ontario. Many are rare in the regions they occur but may be appropriate for roadside grassland creation with its home region, or specific soils. Ontario examples:

- Showy Trefoil (*Desmodium canadense*)
- Round Headed Bushclover (*Lespedeza hirta*)
- Canada Milkvetch (*Astragalus canadensis*)
- Hairy Bushclover (*Lespedeza hirta*)
- Blue Sundial Lupine (*Lupinus perennis*)

Showy & Easy- Having a mix of conservative and liberal species will likely add longevity and wildlife value to the restoration site. A minimum of 15 species has been recommended, with an even mix of grass and forbs (Brandt et al. 2011). The foundation of the planting, along with the quick establishing species, will be common, robust species that are reliable in variable growing conditions. Ontario examples:

- Wild Bergamot (*Monarda fistulosa*)
- Gray Goldenrod (*Solidago nemoralis*)
- Sweet Oxeye (*Heliopsis helianthoides*)
- New England Aster (*Symphyotrichum novae-angliae*)
- Common Milkweed (*Asclepias syriaca*)
- Foxglove Beardtongue (*Penstemon digitalis*)

Showy & Conservative- For conservation value, uncommon species, and even species-at risk may be considered for roadside revegetation. If a project is adjacent to high quality habitat, or is sufficiently large with favorable conditions, then conservative species may be prescribed, by a native re-vegetation expert. It is a misconception that uncommon species are less robust than common species. Many establish readily from seed when sown in the proper conditions. They may just require a narrower range of habitat to thrive. Ontario examples:

- Pale Coneflower (*Echinacea pallida*)
- Spiked blazing star (*Liatris spicata*)
- Yellow Hyssop (*Agastache nepetoides*)
- Great St. John's Wort (*Hypericum ascyron*)

Early Bloomers- though providing blooms for wildlife and colour is relatively easy in the summer and fall, few native grassland species bloom early in the season, and are easy to produce as bulk seed. Wild Strawberry (*Fragaria virginiana*), or Prairie Smoke (*Geum triflorum*), or Thimble Weed (*Anemone cylindrica)* for example, are possible early blooming species but are very costly to produce in bulk quantities and are not highly competitive plants. Spring ephemerals like Trillium species typically occupy woodland habitat and do not produce seed that can be dried and stored. Producing bulk woodland forb seed would also be a monumental challenge. None the less, a few later spring flowering species may be successful in many applications.

- Foxglove Beardtongue (*Penstemon digitalis*)
- Hairy Beardtongue (*Penstemon hirsutus*)
- Golden Alexanders (*Ziza aurea*)
- Wild Columbine (*Aquilegia canadensis*)

Wet Species- where shallow water or seasonally wet conditions dominate, the seed mix should be tailored to include marsh, swamp, and moist meadow species (Brandt et al, 2011). Because many wetland species require specific moisture levels to germinate, there are generally fewer wetland species that are recommended for successful watercourse restoration. Banks may need to be reinforced with live woody shrub stakes, and containerized stock. See Site Preparation below for more examples of slope stabilization methods.

Salt Tolerant Species-In many roadside areas in Ontario, the presence of de-icing salt pollution will prevent native seedlings from establishing. However, a few native species can tolerate high salt concentrations, including prairie grasses, whose deep roots reach deep into the ground, beyond the extend of salt pollution. Ontario examples:

- Canada Rye (*Elymus canadensis*)
- Indian Grass (*Sorghastrum nutans*)
- Little Blue Stem (*Schizachyrium scoparium*),
- Side Oats Grama (*Bouteloua curtipendula*),
- Brown Eyed Susan (*Rudbeckia hirta*)
- Bushclover (*Lespedeza spp; Johnson, 2008*)

Road Shoulder Species- Furthermore, it may be impractical to use a native grassland mix on the road shoulder which may receive repeated annual mowing. Non-native ground cover mixes like Perennial Rye and Alfalfa are recommended for Iowa road shoulders (Brandt, et al, 2011). However, based on the auto ecological traits of some native species, and using their habitats as a reference model for the road shoulder, we have identified several alvars, and sand-barren species which may tolerate road shoulder conditions, and might be tested in conjunction with a non-native low-growing groundcover. Ontario examples:

- Hairy Beardtongue (*Penstemon hirsutus*)
- Blue Eyed Grass (*Sisyrinchium montanum*)
- Upland White Goldenrod (*Solidago ptarmicoides*)
- Hoary Vervain (*Verbena stricta*)
- Small Pussy-Toes (*Antennaria neglecta*)
- Sand Dropseed (*Sporobolus cryptandrus*)
- Switch Grass (*Panicum virgatum*)

Wisconsin requires that native seed mixes be derived from wild-type populations within state, or from a select few counties in neighboring states. WDT has incorporation native species into tradition cover crop mixes for urban use and erosion control, but also specify more diverse grassland mixtures for wider, more viable project, comprised entries of native species. Native seed mixes are prescribed by the restoration designer where appropriate. WDT recommends many of the same species above, including prairie grasses and forbs found in Ontario.

WDT uses Big Blue Stem, Side Oats Gramma, Canada Wild Rye, Indian Grass and Little Blue Stem most often, with the following species for heavier loam soils-- Canada Anemone, New England Aster, Wild Bergamot, Showy Trefoil, Wild Geranium, Grey Headed Coneflower, Golden Alexander, Sky Blue Aster, Pale Coneflower, Stuff Goldenrod and Hoary Vervain; and the following forbs for sandy soils-- Butterfly Milkweed, New England Aster, Flowering Spurge, Wild Lupine, Spotted Bee-Balm, Grey Headed Coneflower, Sky Blue Aster, Pale Coneflower, Stiff Goldenrod and Hoary Vervain. Oddly, Brown Eyed Susan is only specified in the Salt tolerant Native Mix, which also includes the prairie grasses mentioned above (WDT 2017).

Site Preparation

Most roadside revegetation projects are prompted by regular road widening or realignment needs, or through the construction of a new road. In these instances, site preparation should focus on seed bed preparation, final grading, and selection of fill. Rough sites should not be rolled to remove large clods on slopes, to retain microsites for seed germination (WDT 2017). In some cases, sub-soils should be rather than native topsoil which may have a rich weed seed bank. If non-native species are present, including pasture grasses, they should be controlled to reduce competition with the target seed mix (Brandt et al 2011).

Few case studies could be found where existing non-native groundcover was completely removed and attempts were made to replace this cover with native vegetation (but see Haan, 2010; and the Native Plant Research Project in Worley Idaho, Armstrong et al, 2011). In fact, after comparing restorations of newly constructed roadsides and previously established ones, Skousen & Venable (2008) concluded that the costs of sufficiently removing non-native cover (herbicide and tillage), would be too labor intensive, and costly for most state highway departments to fund, and native grassland creation could be focused on degraded, open sites.

Photo: preparing seed bed by roto-tilling glyphosate treated red and tall fescue for MTO Native Seeding Trials, 2016.

Where existing vegetation must be converted to prairie, sods must be killed and possibly removed. Spring is an ideal time to apply a 2% solution of glyphosate to cool season weeds and grasses, but this can be done anytime the plants are actively growing and less than 12" tall. Several separate applications may be required to kill thick sods (Brant et al. 2011). Existing vegetation should be mowed approximately 6 weeks before seeding, in either spring or fall, to allow **at least** two weeks between mowing and spraying, and another two weeks between spraying and seeding (WDT 2017). No-till drilling is the easiest way to install seed into dead weed stubble (Brandt et al 2011), but the site can also be shallowly tilled. In some cases, topsoil was removed prior to seeding, which reduced weed recruitment and nitrogen levels, increasing soil calcium, more similar to the restoration reference fen habitat (Tallowin & Smith, 2001). On roadsides particularly, soils my need to be further de-compacted by disking and harrowing prior to seeding (Matheus & Omtzigt 2012)

Perhaps the most difficult re-vegetation projects involve stabilizing slopes to prevent erosion. Non-vegetated slopes present significant environmental and road safety concerns, and so roadside restoration projects cannot be approved for completion until vegetation is established. Successful innovative solutions have been implemented in order to physically reinforce the slope it's self in order to ensure that the applied seed mixes can settle long enough to germinate without washing down slope, and offsite with precipitation. See Armstrong et all 2011 in the scan above for examples of bioengineered and mechanical terracing, to help hold soils on site for seeds to germinate in.

Seed Mixes & Installation Methods

The ratio of grasses to wildflowers (forbs) in the mix will depend on project goals and planting location but should contain a minimum of 25% forbs for adequate long-term stability. Weeds may take advantage of these unoccupied ecological niches. If the goal is to create habitat to support additional diversity of insects, birds, and the plants themselves, the ratio of species present in the mix should be closer to 50% grass and 50% forb, or even more wildflowers to add aesthetics to highly visible intersections.

Though seeding rates may be specified by weight per area, the more precise method is to prescribe a specific number of seeds per area (Brandt et al. 2011). In order to prescribe seed using seeds per square metre, for example, you need to know the number of seeds per gram of pure live seed, the percentage of pure live seed in the lot, and the average germination rate in a sub sample of the seed lot. See Table: for the calculation of kg installed based on specified seeds per square meter (Smreciu 2002). These details may not be readily available from all see suppliers in Ontario.

Table 2: seeding rate conversion (Smrciu 2002)

Target Plant Density	No. Seeds in a Gram	Pure Live Seed %	Establishment %	Conversion g/m^2 to kg/ha
$\frac{\text{plants}}{m^2}$ X	$\frac{\text{1 gram}}{\text{no. seeds}}$ X	$\frac{1}{\text{PLS \%}}$ X	$\frac{1}{\text{est \%}}$ X	$\frac{\text{1 kg}}{\text{1000 g}}$ X $\frac{10{,}000\ m^2}{\text{1 ha}}$

Without these details, composing a seed mix can be "more of an art than a science," but there are a few simple suggestions offered by the Alberta program. First, restoration practitioners should aim for an average of 10 established plants per square metre, but these figures should be based on reference sites. They caution us to avoid using high seeding rates that may lead to fast growing species choking out other native seedlings, but to increase rates on slopes and erodible sites. However, Alberta is the only jurisdiction we reviewed that encourages higher rates for broadcast seeding than drill seeding (Smreciu 2002).

Photo: Native Wildflower Seed Mix, showing Green Headed Coneflower, Sweet Oxeye, Switch Grass, Showy Trefoil, Brown Eyed Susan, Grain Sedge, Evening Primrose

Not all species can be processed using convention seed cleaning equipment, so some native seed will contain a high proportion of inert, non-seed material, such as extra-floral appendages- awns, pappus, chaff- leaf and stem fragments, and soil dust. Seed suppliers should, at a minimum provide the ratio of Pure Live Seed (PLS) in all lots. Germination rate may also be reported but is only required for a few native species which are also important agronomic crops, such as Slender Wheat Grass (*Elymus trachycaulus*). Purity is also required for all seed sold under Canadian Seeds Act. Even native seed mixes should contain minimum amounts of non-target species (less than 1% noxious weeds), with 0% invasive plant seeds (Smreciu 2002, Government of Canada 1996, Arthur 2015 personal communication).

The IRVM Technical Manual recommends 2.25kg/ha of wildflower (forb) seed, and about 8.5kg/ha of grass seed for Level sites, which is equivalent to approximately 108 seeds per square metre. These rates should be increased by 50% for a 3:1 slope and should be doubled for a 2:1 slope. Rates should be increased a further 15-30% if hydro-seeding in a mulch slurry, to account for seed "hung-up" in the equipment. Fine wildflower seed can easily get lodges in small crevices, including corrugated hoses. Seed stored be kept cool at 50% humidity or less. Most prairie seed will remain viable at 50% humidity for at least at a year at temperatures between 0-10 degrees Celsius (Brandt et al. 2011).

The WDT (2017) specifies different rates for different mixes but vary between approximately 18kg/ha for native seed mixtures, up to 130kg/ha for simple non-native cover mixes used on gravelly road edges and steep slopes.

Even when applied in the Yukon, native grassland seeding rates are similar, at approximately 15kg/ha which equals roughly 1500 Pure Live Seeds, of a grass-only seed mix (Matheus & Omtzigt 2012; grass seed is typically larger than wildflower seed).

Any proposed mix of diverse native species does not represent the "final plant quantities to be found on a restored site at some future point in time", but rather installation quantities that, if combined with successful installation methods, site preparation and adaptive monitoring will achieve a self-sustaining, native assemblage, ecologically tailored to microclimates within the restoration site (Arthur 2013).

Installation methods should be determined prior to the prescription of native seed. Not all sites can be prepared or seeded in ideal ways for native seed mixes to establish. Enough time should be allowed between the procurement of the seeds and the installation in order to test methods and trial seed application and investigate any additional seed testing. Below is a summary of the primary seed installation methods:

Broadcast – Broadcasting is the ideal method for many fine seeded species on even ground. De bearded grass seed flows easily though a broadcaster, but the chute may need to be modified to allow coarser, unclean seed to pass. Hand broadcasting is also an option for small sites. For ease, mix native mix with a carrier, like sand, peat, or a nurse crop. Large sites should be harrowed after broadcasting, but all installation, methods benefit from packing after planting (Brandt et al 2011)

Drill- No-till mechanical seed drilling, using a Truax or equivalent drill, is the ideal native seed installation method for level roadsides. Drilling can increase seed establishment and speed up germination. It's recommended to set the rate to slightly below your target amount, due to unevenness in the terrain. The bumpy the course, the lower the rate should be. Most drills will have separate chutes for large and small seeds, coarse and fine seeds. Do not plant native prairie seed more than .7 cm approximately ¼ inch. (Brandt et al, 2011)

Hydro-seed- Using water and mulch to blast the seed mix onsite, a 1500-gallon hydro-seeder can cover .15 hectares in one load. It can be time consuming to refill the tank, and some locations may be far from accessible water to fill the tank. The cost of a good quality seed mulch with tackier can cost as much as the seed mix itself, and some "shadow area" behind uneven ground may be missed. About 2250kg/ha of mulch should be applied to a slope of 3:1. The seed mix should be applied first, and the hydro mulch applied second in a separate pass. This ensures better seed to soil contact. This prevents the seedlings establish roots into the mulch, and not the soil. Though it may seem more efficient to complete in a single step, the two-step method is reportedly more successful (Brandt et al. 2011). Seed should not remain mixed with water and mulch inside the tank for more than one hour (WDT 2017).

Photo: Hydro-seeding native seed mix with fibre mulch, with one and two stage treatments, for MTO roadside seeding trials

Project timing is critical for successful seedling establishment. Winter seeding can work well, taking advantage of freeze-thaw settling the seed to an ideal depth. Opportunities may be brief, and winter seeding on slopes should not be attempted. Early spring is ideal for planting warm season grasses using any method. Dormant seeding in late fall and early winter is ideal for forbs. Midsummer seeding is not typically recommended due to high temperatures, but can succeed though drilled application, increasing the rate by 25%, and mulch over to keep soil cooler (Brandt et al, 2011). WDT (2017) requires all seeding much be completed between October 15th and June 15th, with no seeding in warm summer months.

Cover crops are often recommended to provide quick and easy plant cover, especially on slopes and sensitive areas. Cover crops can be sown before, after or alongside a native seeds mix. Application varies with location, timing, and soil properties.
Nurse companion planting rates:

- Spring: 38kg Oats and 5.5kg Annual Rye per hectare.
- Summer: 38kg oats and 11kg Annual Rye per hectare.
- Fall: 32kg Winter Wheat per hectare (Brandt et al 2011).

Erosion control planting, followed by native seed, cover crop rates are:

- Summer: 38kg Oats, 11kg Annual Rye and 11kg White Millet per hectare.
- Fall: 64kg Winter Wheat/ha (Brant et al. 2001).

WDT (2017) recommends Annual Oats before June and Winter Wheat after September. However, if native establishment is a priority on slopes, then annual cover crops should make up no more than 10% of the total seed mixture, to prevent them from out-competing native the species (Matheus & Omtzigt 2012).
Cover cropping can be extended over the first several years, to help exclude weeds during the long establishment phase of native plantings. This can be done through Ecological Bridging, which utilizes the life history strategies of a very carefully chose groups of short-lived non-native and native plants. Typically, this mix will include a very fast-growing annual non-native like Weeping Lovegrass to provide cover the first year (Arthur & Gartshore 2004). A short-lived native biennial such as Evening Primrose will form large flat rosettes in the first year, blocking a large surface area from weeds. Most plants will flower in the second year, and re-seed so that the third year following also has Evening Primrose rosettes to block fast growing weeds. Native perennials like Vervain, Bergamot and Beardtongue will spend their first year as a tiny seedling, unaffected by the shading weeds or cover crop. In the second year, they begin to flower and start producing seed alongside the biennial. These perennials come to dominate in in the third year, just as the long-lived, slow growing perennials start to establish.

Some projects may require additional planting of containerized plant stock, bare root saplings, seedling plugs, or live steak cuttings. Often steep slopes may have to be reinforced with established plant material prior to, or in lieu of seeding (Armstrong et al, 2011). Where the ground to very rocky, and few native species establish well from seed, shrub cutting s may be successfully and economically installed using hay-mulch matting to control erosion (Mallik & Karim 2008).

Weed Control & Maintenance

New plantings benefit from regular mowing through the first year, between two and four times to a height of about 8inches. For the first years, it may be difficult to assess the success of your planting because there may be many non-target weedy species present. Some of these like *Panicum capillare* and *Ambrosia artemisiifolia* are actually native and may be beneficial to the developing native e seedlings.

Weeds that establish within the restoration may have to be controlled periodically. This is especially crucial in the early stages of seedling development, and if there are disturbance events. Weeds can be effectively controlled by mowing the seed heads before they develop for several consecutive years. Spot spray rosettes in between (Brandt et al, 2011). The WDT (2017) recommends mowing twice in the first year. The first mowing should clip plants to 6inces once they reach 12 inches.

Ideally long-term maintenance of a successful native grassland restoration would only require periodic spot spraying of noxious weeds. Identifying source populations in nearby environments may be required to root out problem species permanently. Regional weed control programs are ideal, and the MTO is presently collaborating with researchers and the Ontario Invasive Plant Council to stop the spread of invasive plants along provincial highways. See Figure 1 for a list of control methods for common roadside weeds.

Prescribed burning is an alternative to chemical and mechanical control of weeds, since few are as well adapted to tolerating spring fire as native grassland species. Though fire on busy highways may seem at first like a distraction to drivers and potential safety concern, other jurisdictions have accomplished this through the development of a detailed health and safety plan, and a focus on public relations and education (Johnson, 2008). Burning is more economical than any other option of roadside grassland maintenance. It also reduces our reliance on herbicides like glyphosate. Grassland burning can be contracted to professional companies, but some natural resources and barks departments currently have burn team. Otherwise see Brandt et al. (2011) for detailed directions on creating a burn plan.

Long term success of a restoration seeding will depend on the level of commitment made to monitoring and adaptively responding to weed outbreaks and establishment failures.

Fig 1: Control methods for some noxious weeds of roadsides *(FHWA 2017b)*

AUTUMN OLIVE, (Elaeagnus umbellata)
- Chemical control: triclopyr, glyphosate, dicamba and 2,4-D.
- Non-chemical: Pull seedlings; cut trees and treat, or girdle.

BIRDSFOOT TREFOIL, (Lotus corniculatus)
- Chemical control: aminopyralid, clopyralid, 2,4-D, triclopyr.
- Resistant to glyphosate.
- Non-chemical: repeated clipping reduces seeds and weakens roots.

CANADA THISTLE, (Cirsium arvense)
- Chemical control: aminopyralid, chlor sulfuron, clopyralid (+), dicamba, glyphosate, metsufuron methyl, picloram, triclopy +
- Non-chemical: repeated mechanical methods; multiple mowings, sheep and goat grazing reduces seed production, well-timed burning with other; competitive plantings, and several bio-controls.

COMMON BUCKTHORN, (Rhamnus cathartica)
- Chemical control: glyphosate, triclopyr, fosamine, picloram, or hexazinone.
- Non-chemical: pulling, mowing with herbicide of re-sprouts.

COMMON REED, (Phragmites australis)
- Chemical control: glyphosate, imazapyr, fluazifop-P.
- Non-chemical: cutting and flooding possible on some sites.

CROWNVETCH/TRAILING, (Coronilla varia)
- Chemical control: aminopyralid, clopyralid, 2,4-D, glyphosate, metsulfuronmethyl, and triclopyr.
- Non-chemical: remove manually or by cutting or burning, then treat cut stems; revetetation, late spring prescribed burns, grazing in combination only.

JAPANESE KNOTWEED, (Polygonum cuspidatum/Fallopia japonica)
- Chemical control: glyphosate, triclopyr, or imazapyr.
- Non-chemical: mowing followed by herbicide. MULTIFLORA ROSE, (Rosa multiflora)
- Chemical control: glyphosate, triclopyr, dicamba, fosamine, metsulfuron, picloram, or tebuthiuron.
- Non-chemical: 3-6 mowings or cuttings annually, a biocontrol shows promise.

REED CANARYGRASS, (Phalaris arundinacea)
- Chemical control: fluazifop, glyphosate, or sulfometuron.
- Non-chemical: early season grazing, prescribed fire, and repeated mowings are effective with chemical follow-up;

RUSSIAN OLIVE, (Elaeagnus angustifolia)
- Chemical control: triclopyr, glyphosate, imazapyr, metsulfuron, via foliar, soilapplied, cut stump, hack and squirt, or basal applications
- Non-chemical: pulling of seedlings and saplings up to 3.5" with follow-up, mowing or cutting with herbicide, prescribed hot burn, and natural controls.

SMOOTH BROME, (Bromus inermis)
- Chemical control: fluazifop, glyphosate, imazapic, pronamide.
- Non-chemical: A single well-timed mowing in boot stage, burning can control spread but not eliminate smooth brome.

SPOTTED KNAPWEED, (Centaurea biebersteinii)
- Chemical control: aminopyralid, clopyralid (+), 2.4-D, dicamba, picloram.
- Non-chemical: mowing at late bud, avoid fertilizers, minimize disturbances, sheep and goat grazing long term, and a number of biocontrols.

TALL FESCUE, (Festuca arundinacea)
- Chemical control: glyphosate, imazapic, and imazapyr.
- Non-chemical: several mowings plus herbicide, several spring burns.

TARTARIAN HONEYSUCKLE, (Lonicera tatarica)
- Chemical control: glyphosate, triclopyr, fosamine, or metsulfuron.
  Non-chemical: pulling or grubbing, clip in spring and summer, or spring prescribed burn in fire-adaptedcommunities

Performance Evaluation

Native seedlings tend to establish more slowly than conventional cover crops, with natives contributing nearly zero percent cover until the third year of establishment (Skousen & Venable 2008).

Restorations may be evaluated in several ways, but should be judged based on their similarity, or fidelity to reference remnant grassland in species composition, structure, function, and longevity. Generally, prairie restoration exhibits lower species richness than remnants, a decline in native species richness over time and a greater proportion of exotic species than remnants. However, we can predict which restorations will be most successful by plotting the trajectories of various metrics of fidelity. Several robust measures are described below.

Overall, due to differences in life history strategy, warm season grasses will typically come to dominate, reducing cover from broad-leaved plants, and also reducing species richness, and conservatism. Conservative species have been associated in restoration sites with higher burn frequency and higher soil bulk density. This is unexpected because conservative species are not associated with frequent fires in remnant habitats, and floristic quality typically increases in drier grasslands. Ultimately, restorations that receive a higher diversity of native seed will more likely retain some conservative species and a higher floristic quality (Hansen & Gibson, 2013).

The Society of Ecological Restoration (SER 2004) suggests the following characteristics should be studied in the evaluation of restoration performance:

- Diversity and structure reflect a reference model.
- Frequency of indigenous species
- Presence of critical, stabilizing functional groups.
- Capacity to maintain reproductive populations.
- Functioning of ecosystem services
- Integration with surrounding landscape
- Reduction of threats to fidelity
- Resilience to disturbances
- Self-sustainability

Measures of species richness and abundance, and quantifying interactions in different tropic levels can help to estimate ecosystem resilience. Nutrient cycling is often estimated by measuring the soil nutrient availability. Diversity measures are easiest to report and most reported. Species richness (number of species) is a simple way to assess a community, but functional diversity can also be measured by assigning species to guilds. Percent cover, height, and biomass are common and relatively easy metrics to report as well. Processes such as mycorrhizal integration, herbivory and decomposition rates can be scored through more in-depth analysis. Processes like pollination, and dispersal may only be indirectly measured by estimating insect visitor diversity, frequency of insect visitors, and passive restoration rates of target species (Ruiz-Jaen & Aide 2005).

Common indices of diversity include the Shannon and Simpson indexes, as well as various estimates of Evenness. Either biomass or cover may be used to calculate these values in forb and shrub communities, because these measurements are correlated, but biomass tends to give more precise estimate, and capture greater niche diversity (Chiarucci et al. 1999).

The *Floristic Quality Index* is one common method of quantifying species richness in a plant community. FQI values can be compared between restoration sites and their reference habitats as the project progresses. There are several ways to calculate FQI, based on the nature of the plant community type being studied. Roadsides communities are often dense, and therefore total plant cover exceeds 100% visual cover due to overlapping canopies (3). Refer to the table below from the US Geological Survey Fact Sheet for a full outline of methodology: (https://pubs.usgs.gov/fs/2011/3044/pdf/FS11-3044.pdf). A Floristic Quality Assessment is available for Ontario, providing coefficients of conservatism for target native species (Oldham, 1995).

Here we include, as an example of best practices, details from the performance evaluation used for the Herb Gray Parkways revegetation, in Ontario (Burley, 2013). Drawing from our review of best practices above, we have provided an analysis of these requirements. Monitoring during the Establishment Phase was conducted on newly seeded areas for two full growing seasons after installation. Performance standards are as follows:

> *"1. Successful germination of the nurse crop (where applied).*
> *2. Successful germination of specified permanent seed species (appropriate species shall be visually evident by 60 days); and*
> *3. Uniform and evenly dispersed cover of seeded species (i.e., no significant bare areas). In addition:*
> *4. At the end of the first full growing season, a majority of the mandatory species, depending on the timing of seeding and the variable stratification requirements of species within the prairie-based seed mixes, shall be visually evident; and non-specified vegetation shall not exceed 30% of seeded earth area; and*
> *5. At the end of the second full growing season, a majority of the mandatory species shall be visually evident relative to the target typology in any area; and non-specified vegetation shall not exceed 20% of seeded earth area."*

Analysis: Given that native wildflower seeds ought to be seeded in late fall or winter (dormant seeding), for best results, we do not find the requirement for germination of native seed after 60 days to be appropriate. Many wildflower species germinate best after 60 to 90 days of cold moist conditions (stratification). Furthermore, if establishment is being judged by visual cover, we must recognize that native species will not contribute the majority cover in the first year. Often, non-native annuals, with faster seasonal growth rates will contribute most to cover, even in restorations that are later determined to be successful. Therefore, we do not support the requirement for non-target cover to be less than 30% at the end of the first growing season and would predict that even the best prepared and installed areas would fail to meet this standard. Furthermore, research suggests that while cover crops significantly reduce the presence of perennial weeds in the medium to long term, they are not effective at significantly suppressing annual weeds in the short term (Wilsey & Bong, 2007).

The required Management Phase monitoring include qualitative assessments such as health of plantings, and measurements such as FQI. Measurements are to be made annual for the first five years, and then once every five years. The performance standards below were used together to judge if the restorations were on trajectory towards a reference model:

> *"1. Restored typologies contain a characteristic assemblage of the species that occur in the reference ecosystem typology and provide an appropriate and representative community structure.*
> *2. Restored typologies consist of native species to the greatest extent…*
> *3. Restored typologies contain those vegetation functional groups necessary for the continued development and/or stability of the ecosystem or, if they are not present, the missing groups have the potential to colonize the typology by natural means.*
> *4. Restored typologies appear to function normally for their ecological stage of development, and the physical environment appears capable of sustaining reproducing populations of the species necessary for continued development (or stability) along the desired successional trajectory.*
> *5. Restored typologies are suitably integrated into the larger ecological matrix of preserved Species at Risk habitats and the Parkway Landscape Plan.*
> *6. Restored typologies are sufficiently resilient to endure the normal, periodic environmental stressors that naturally maintain the structure and function of the targeted ecosystems; and*
> *7. Restored typologies appear to be self-sustaining and have the potential to persist indefinitely under existing environmental conditions; or in the case of tallgrass prairie and savannah typologies, appear to be self-sustaining although still subject to long-term management to halt succession."*

Analysis: Recreating exact references assemblages on newly constructed roadsides may not be a practical goal, as these sites may differ physically from the references sites and represent a novel habitat type. Rather, the goal should be to restore a level of native biodiversity over a given area (species-area curve) that is representative of a model plant assemblage. It may be effective to differentiate newly constructed roadside restorations from restorations within natural areas and on naturally occurring soils. Also, assessing ecosystem function is recommended as a means of evaluation, but no methodologies are put forward. This could be improved by comparing annual net primary productivity, or pollinator diversity between restoration communities and reference communities.

Ongoing adaptive management is based on these above assessments. Problem areas are identified when they that do not meet most of the criteria above. Remedies may include the maintenance of early successional assemblages, site alteration or re-seeding, as well as the mitigation of external pressures, such as disturbance.

Ongoing monitoring and management should focus on tracking not only relative species abundances, but also important ecological functions and characteristics. For successful restorations, these metrics, or Performance Standards, should be compared to the reference model in order to gauge restoration success for any regulatory compliance monitoring (Clewell & Aronson 2007, Arthur 2013).

Conclusions

Nearly 25% of Canada's population lives in south-western Ontario, within the northern limits of the Carolinian Life Zone, occupying no more than .25% of the county's land mass. Unfortunately, over 90% of the natural vegetation cover has been lost in this region (CCC 2017). Green spaces, including highway rights-of-way may be better able to contribute to the restoration of our ecosystems by re-vegetating them with a much wider diversity of grassland, species, shrubs, and plants of open habitats like dune and alvars.

Other jurisdictions, in similar climates have incorporated grassland species into roadside planting for decades, and a rich literature of best management practices for native roadside revegetation exists to guide the practitioner in Ontario. Through the scan and review above, we have presented the strategies, both in the field and in the boardroom, to ensure safety, aesthetic, and conservation goals are met. Below is a summary checklist of the major components of a native roadside restoration project. An example of relative restorations costs is also included, followed by final recommendations to support native roadside plantings, and ensure project success.

Summary Table

Roadside Restoration with Native Seed Development Plan Summary Table		
1 Site Selection	a	restoration of constucted or altered roadside, or of established verge
	b	restoration borders significant habitat, plant community or watercourse.
	c	restoration buffers roadside ditch from private land use
2 Stakeholders	a	identfy priavate stake holders: neighbors, famrers, restoration industry
	b	identfity governement stakeholders: municipalities, ministiries
	c	identify NGO stakeholders: environmental charities, plant societies
3 Organization	a	form IIVRM stakeholder committee on a region or project basis
	b	determine long term objectives
	c	assign scheduled duties to IRVM committ
	d	compile budget, permits, site characteristics, seed materials development
4 Funding	a	regular road widening, MTO funding, and/or
	b	habitat and species protection, MNRF funded, and/or
	c	beautification, natural heritage conservation, municipal or provincial funding and/or
	d	R&D, industry and university funded (various sponsors) and/or
	e	environmental/ecological benefit, NGO funded (and others etc...)
5 Plant Provenance	a	non-nativecover species only; extreme slopes, toporary construction sites
	b	locally sourced native species, with limited non-native cover crops; most roadsides
	c	commercial eco-vars and nurse crops; narrow, urban roads, residential areas
	d	wild collected seed from within project region; conservation or SAR seedings.
6 Species Selection	a	quick establishing species (Canada Rye, Brown Eyed Susan, Evening Primrose)
	b	warm season grasses (Big Blue Stem, Switch Grass, Side Oasts Gramma)
	c	cool season graminoids (Virginia Rye, Riverbank Rye, Fox Sedge)
	d	common wildflowers (Wild Bergamot, Milkweed, Foxglove Beardtongue)
	e	fast growing forbs (Showy Trefoil, Sweet Oxeye, Hoary Vervain
	f	slow growing forbs (Early Goldenrod, Sky Blue Aster, Round Headed Bushclover)
	g	salt tolerant species (Indian Grass, Brown Eyed Susan, Little Blue Stem, Virginai Rye)
	h	uncommon wildflowers (Virginia Mountain Mint, Pale Coneflwoer, Hairy Bushclover)
	i	species for wet conditions (Blue Vervain, Blue Lobelai, Swamp Milkweed)
	j	species for dry conditions (Sand Dropseed, Slender Wheat Grass, Hairy Beardtongue)
7 Site Preparation	a	mow if necessary
	b	spray twice, 6 weeks apart
	c	cultivate, till, harrow, decompress
	d	roll seed bed
8 Seed Mixes	a	12-18kg/ha, double for hydroseeding, double again if seeding slopes greater than 3:1
	b	cover crop, native or non-native
	c	clean or unclean, application rate adjust for proportion PLS
9 Installation	a	Hand Broadcast
	b	Mechanical Broadcast & Harrow
	c	Drill Seed
	d	Hydro-seed in 2 phases, or hand-seed and hydro mulch
10 Weed Control	a	Hand Pull or mow seed heads
	b	Spor Spray with herbicide
11 Maintenance	a	mow or graze with goats
	b	burn periodically (5-8 years)
12 Evaluation	a	species germinated by end of first groing season, rate of green up/gemination
	b	percent cover, FQI, biomass production etc compared to reference (third season)

Sample Costs

The cost of native prairie seed is typically higher than non-native cover crop seed and this high seed cost has been cited as a barrier to revegetating with native seed (Arthur & Gartshore 2004). However, when site preparation, installation and establishment maintenance costs are considered as well, the cost of native seed contributes minimally to the overall project cost. Below are three examples of hypothetical restoration plans, and associated costs.

1. (Per hectare)	
Site Prep- Sprayx1	$5200
Site Prep- Till	$2800
Installation (Hydro-Seed)	$8000
Native Seed	$1800
Mowingx1	$3000
	$20800

2. (Per hectare)	
Site Prep- Sprayx1	$5200
Site Prep- Till	$2800
Installation (Hydro-Seed)	$8000
Standard Seed	$350
Mowingx1	$3000
	$19350

3. (Per hectare)	
Site Prep-Excavate	$5200
Installation (Seed Drill)	$2500
Native Seed	$1800
Mowingx1	$3000
	$1250

This suggests that failed non-native plantings can cost far more than a successful native planting, if timed and installed properly. The high cost of site preparation and seed installation warrants precision and careful monitoring of these action items.

Prices for these action items may vary from contractor to contractor, and will be affected by site location, site condition (weed pressure) and accessibility. The estimate above is based on costs for the experimental seeding installed along highways 3 and 7 in 2016 which are being conducted in conjunction with this review. Price quotes come from the St. Williams Nursery & Ecology Centre, as well as Green Stream. Installation and site preparation costs may be slightly overestimated when scaled up beyond the 500m^2 experimental plots, due to efficiencies of scale, and the high costs associated with floating heavy equipment to remote sites. In comparison, the 401/21 demonstration prairie planting in 1999 cost roughly $7400 per hectare, but this was installed with a no-till seed drill, this does not include mowing or maintenance during the establishment period (Arthur & Gartshore 2004).

Recommendations

To help incorporate more native plantings into Ontario highway sides, the following recommendations to the Ministry of Transportation have been highlighted based on our review of successful programs elsewhere.

1. Focus quality control on ensuring best practices for native seed installation are followed, to minimize seeding failure including drill seeding where possible, two-phase hydro seeding/mulching, winter sowing wildflowers and spring sowing grasses separately, sowing at the right time of year, using minimal nurse crop where possible.
2. Encourage counties to identify, scale up and produce local native grassland seed; educate county roads departments on native revegetation; support the expansion of the native seed production industry in Ontario.
3. Establish and study trial roadside grassland seedlings across the province to fine-tune regional best practices and species mixes.
4. Provide professional development on roadside native revegetation through workshops with conservation agencies that are engaged in habitat creation projects, soils and seeds experts, and revegetation specialists.
5. Initiate Seed Production Contracts at least 2-3 years prior to the restoration phase of any large roadside project, especially outside of seed zones 34, 37 and 38.
6. Continue to rely on non-native cool season mixes, and/or small shrubs on steep slopes and dense clay soils where native grassland species are less likely to establish from seed. Not every roadside is conducive to direct seed installation.
7. Develop an IVRM-inspired program to address both native revegetation and habitat creation, but also maintenance and salvage methods of existing roadside fragments; develop an Ontario-specific roadside native revegetation guide; assign native vegetation management duties to existing staff or dedicated IRVM managers.
8. Conduct driver surveys on their experience with the vegetated roadside environment during car travel, or on the ground.
9. Investigate alternatives to NaCl based de-icing salts and promote natural snow fencing with prairie grasses or native shrubs.
10. Collaborate with regional conservation agencies and community interest groups to conduct an inventory of roadside native plant remnants on provincial highways in southwestern Ontario. These remnants can serve as seed sources and expansion nodes for habitat restoration.

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Appendix 1.1 *Example Native Seed Mixes for Ontario Roadsides*

Standard Roadside, Standard Old Field, High Visibility Sand, High Visibility Clay, Norfolk, Windsor, GTA

* do not include near intersections or driveways

** rare species should be included with consultation from regional expert.

	SA	SB	HVS	HVC	MM	EG1	EG2	EG3
Species	%	%	%	%	%	%	%	%
*Big Blue Stem**		8	10	10	5	15	18	15
Little blue Stem		10	10	8		8	10	
*Indian Grass**	15	10	10	10		15	15	10
Switch Grass	5	5	4	5	10	5	5	5
Sand Dropseed		1	2			3		2
Slendwer Wheat Grass		10	20			15		5
Canada Wild Rye	14	10	7	7	5	15	10	10
Virginia Wild Rye	20	20	7	17	20		15	15
Riverbank Wild Rye	14			10	20			10
Fringed Brome	5				10			5
Fox Sedge	3	3		3	5		3	
Soft Stemmed Rush	1		1	1	2	1	1	
Brown Eyed Susan	3	3	5	5	3	3	3	5
Evening Primrose	2	3	5	5	2	2	1	2
Wild Bergamot	2	1	1	1	2	1	1	1
Showy Trefoil	1	2	2	1		1	1	1
Sweet Oxeye	1		1	1	2	1	1	
Grey Headed Coneflwoer	1	1	1				1	
Green Headed Coneflower				1	1		1	
Hoary Vervain		1		1			1	1
Bushclover			1			1	1	1
Virginia Mountain Mint	1	1	0.5	0.5	0.5	1	0.5	0.5
Hairy Beardtongue		1	1	1				1
Foxglove Beardtongue	1	1	1	2	1	1	1	1
Common Milkweed	1	1	1	1	0.5	1	0.5	1
Butterfly Milkweed			0.5			1	1	1
Swamp Milkweed	1			1	1			
Blue Vervain	1			1	1	1	1	
Blue Lobelia			0.5	0.5	1	0.5	0.5	0.5
Purple Stemmed Aster	1			0.5	1			0.5
Panicle Aster	0.5			0.5	1			
New England Aster	1	1	1	0.5	1	0.5	0.5	0.5
Heath Aster	0.5	0.5	1		1	0.5		
Stiff Goldenrod	1	1		0.5			0.5	
Rough Goldenrod					1			
Early Goldenrod			0.5			0.5	0.5	0.5
Grey Goldenrod	1	1	0.5	0.5	1	0.5	0.5	1
Upland White Aster	1	1	0.5			0.5		0.5
Tall Cinquefoil	1	1	0.5			0.5		1
Sky Blue Aster		1	0.5	0.5		0.5	0.5	1
Smooth Aster			0.5	0.5		0.5	0.5	
Arrow Leaved Aster	1	1	0.5			0.5		1
Wild Columbine			0.5			0.5		0.5
Golden Alexanders				0.5	1		1	
Smooth Pussy Toes		0.5	0.5	0.5		0.5		0.5
*Dwarf Blazing Star***			1			1		
*Spiked Blazin Star***				1			1	
*Blue Lupine***			1			1		
*Great St. Johns Wort***				0.5	1	0.5	0.5	
*Swamp Rose Mallow***						0.5	1	
*Pale Coneflower***				1			1	
	100	100	100	100	100	100	100	100