EVALUATING ROADSIDE RESTORATION SUCCESS

EVALUATING THE SUCCESS OF ONTARIO ROADSIDE RESTORATIONS – AN ECOSYSTEM APPROACH

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LAY ABSTRACT

Ecological restoration is crucial for reversing global biodiversity loss. Roadsides may be suitable for the restoration of grassland ecosystems, but few studies have comprehensively evaluated the long-term success of roadside restorations. I assessed the plant community, bee community, soil carbon and plant-fungal relationships at roadside restorations along three Southern Ontario highways and compared these measures to unrestored roadsides and a remnant grassland. Restoration increased native plant diversity, though not to remnant levels. Bee communities varied mostly by highway, though bee abundance was positively correlated with plant diversity. Soil carbon in roadside sites was similar to the remnant but did not differ among restored and unrestored sites. Plant response to soil fungi collected from roadside sites varied significantly by site. Thus, roadside restoration can benefit some ecosystem components, but more intervention may be required to restore ecosystem function. This study highlights the importance of evaluating restoration success in a comprehensive manner.

ABSTRACT

Ecological restoration, or assisting the recovery of damaged ecosystems, is recognized as a crucial activity for reversing biodiversity loss across the globe. Roadside rights-of-way may be suitable areas for the restoration of endangered grassland communities, because they occupy significant areas of underutilized land, are managed as early successional plant communities, and may serve as corridors for wildlife movement and gene flow. However, though many roadside restoration projects have been undertaken in North America, few studies have evaluated their long-term success and most monitoring is narrow in scope. True restoration includes restoring an appropriate species composition, vegetation structure and ecosystem functions, and thus these ecosystem components must be measured when evaluating success. I assessed the plant community, bee community, soil carbon and plant-fungal relationships at roadside restorations of various ages along three major highways in Southern Ontario and compared these measures to unrestored roadsides and reference sites. I found that roadside restorations successfully increased native plant richness, though not to the level of a remnant grassland. Bee communities varied mostly by highway rather than site type, though bee abundance was positively correlated with plant diversity and bare ground. Soil carbon in roadside sites was similar to that of a remnant grassland but did not differ among restored and control sites. Plant growth response to arbuscular mycorrhizal fungi collected from roadside sites varied depending on the site and showed a weak negative correlation with site age. Taken together, these results suggest that roadside restoration can benefit some ecosystem components, but simply seeding native plants along roadsides may not be sufficient for improving ecosystem function. This study highlights the importance of evaluating success in a comprehensive manner that includes multiple ecosystem components.

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LIST OF ABBREVIATIONS

AM = Arbuscular mycorrhizal

FQI = Floristic Quality Index

MGR = Mycorrhizal growth response

MTO = Ministry of Transportation of Ontario

SER = Society for Ecological Restoration

DECLARATION OF ACADEMIC ACHIEVEMENT

I, Mara McHaffie, declare this thesis to be my own work. I am the sole author of this document. No part of this work has been published or submitted for publication or for a higher degree at another institution.

To the best of my knowledge, the content of this document does not infringe on anyone's copyright.

My supervisor, Dr. Susan Dudley, and the members of my supervisory committee, Dr. Jurek Kolasa and Dr. Jianping Xu, have provided guidance and support at all stages of this project. I completed all of the research work.

INTRODUCTION

Background

In order to address widescale ecosystem degradation and biodiversity loss, land managers across the globe are assisting damaged ecosystems in recovering their composition, functions and self-sustainability through the process of ecological restoration (SER 2004; McDonald et al. 2016). With over one million kilometers of roadway in Canada alone (Wojcik & Buchmann 2012), roadsides represent a vast and largely untapped source of land that could be restored to create habitat for wildlife, including native pollinators, and provide ecosystem functions such as carbon storage (Baer et al. 2002; De Deyn et al. 2011; Wojcik & Buchmann 2012; Gardiner et al. 2018; Wigginton & Meyerson 2018). Recognizing this potential, land managers have initiated a number of roadside restoration projects in North America over the last few decades (Durant 1994). However, despite having the goal of producing multiple ecosystem benefits, most roadside restoration projects have been primarily focused on native plant establishment (Bugg et al. 1997; Haan et al. 2012; Burley 2013; Arthur 2015). More comprehensive, long-term evaluations of these roadside restorations are necessary to ensure that they are successful in producing not only healthy native plant communities, but also thriving pollinator communities and important ecosystem functions.

Evaluating Restoration Success

Evaluating restoration success advances our scientific understanding of restoration ecology, ensures that resources are used effectively and confirms that benefits to nature are being realized. True ecological restoration aims to restore an ecosystem's species composition, functional groups, physical environment, biological processes, functions, connection to the landscape, and resiliency to stressors (Hobbs & Harris 2001; SER 2004; Ruiz-Jaen & Aide

2005). Thus, several of the key attributes of restored ecosystems, including indicators related to diversity, vegetation structure and ecosystem processes, should be assessed relative to historical or constructed references representing healthy ecosystems in order to comprehensively evaluate success (SER 2004; Ruiz-Jaen & Aide 2005; Hansen & Gibson 2014; McDonald et al. 2016). However, evaluation of ecological restoration is dominated by measures of floral diversity and abundance, with less focus on other taxa and important ecological processes and functions (Brudvig 2011; Wortley et al. 2013). However, the restoration of plant species composition does not necessarily lead to the restoration of other ecosystem attributes, so it is critical that evaluations of restoration success consider multiple ecosystem components beyond plant diversity (Hobbs & Harris 2001; Ruiz-Jaen & Aide 2005).

Roadsides

The potential for roadsides in restoration

Roadside rights-of-way have been proposed as potential areas for restoration that could connect larger habitat patches and offset the negative impacts of road construction. Roadside rights-of-way may be suitable for restoration because they occupy a considerable amount of land (over 600,000 hectares in Canada; Wojcik & Buchmann 2012), are unsuitable for most other land uses and, due to their linear nature, may be able to serve as habitat corridors, allowing wildlife and plant populations to move between fragmented habitat patches (Penone et al. 2012; Gardiner et al. 2018). Rights-of-way are traditionally maintained as disturbed, early-successional vegetation communities and may therefore be particularly suitable for the restoration of grassland ecosystems, which are critically endangered in North America (Rodger 1998; Gardiner et al. 2018). With less than 3% of Ontario's original range of tallgrass prairie remaining (Tallgrass Ontario 2017), there is considerable demand for suitable land for grassland restoration.

Restoring roadsides to native vegetation may also reduce management costs incurred through frequent mowing and pesticide use (Elmhirst & Cain 1990; Durant 1994; Wigginton & Meyerson 2018). Thus, the restoration of roadsides to prairie ecosystems may be beneficial from both an ecological and an economic perspective.

While roadsides may be suitable for grassland restoration, they also present challenges that make roadside restoration difficult. Compacted soil, erosion, road salt, air pollution and dust can hinder native plant establishment and favor the growth of aggressive exotic species (Forman & Alexander 1998; Bochet et al. 2010; Haan et al. 2012). Furthermore, there is concern that creating wildlife habitat close to major roads will lead to increased mortality due to vehicle strikes (Forman & Alexander 1998; Baxter-Gilbert et al. 2015; Muñoz et al. 2015; Keilsohn et al. 2018). Given the unique challenges of roadside environments, there is clearly a need to assess the success of roadside restorations to determine if roadsides are viable areas for ecosystem restoration. However, similar to other restoration projects (Wortley et al. 2013), monitoring of roadside restorations is often short-term and focused on plant establishment (Bugg et al. 1997; Haan et al. 2012; Burley 2013; Arthur 2015) without consideration for other taxa (but see Ries et al. 2001; Hopwood 2008) or ecosystem processes. A more comprehensive evaluation of roadside restorations that measures a variety of ecosystem components would help to determine the potential for grassland restoration along roadsides and to optimize roadside restoration practices to maximize success.

Extant roadside restorations

The initiation of several roadside restoration projects in Ontario over the last two decades provides an opportunity for a long-term, comprehensive evaluation of roadside restoration success. Native grassland plantings along Southern Ontario roadsides vary in age, with the oldest restorations dating back to at least 2002 and a number of new plantings completed in the last five years. This allows for the investigation of how restoration projects vary with age, and whether reference levels of diversity and functioning can not only be achieved but also sustained over longer time periods. Furthermore, while restoration efforts generally follow the same basic model of applying native plant seed to rights-of-way, projects have used a variety of different seed mixes, planting methods, and management regimes. This allows for insights into which practices have resulted in the most successful restoration of different ecosystem components. Additionally, varying geographic locations may reveal the influence of landscape-level factors on restoration and shed light on how generalizable restoration outcomes are in different landscapes. Finally, the presence of traditionally managed, 'unrestored' roadsides near restoration sites also allows for a comparison of ecosystem metrics between traditionally managed and restored roadsides. Thus, roadside restorations in Southern Ontario provide an excellent system in which to comprehensively evaluate restoration success over a variety of different study sites and management regimes.

Evaluating Vegetation Communities in Restorations and Roadsides

Vegetation metrics used in restoration studies

While the vegetation community should not be the sole focus of restoration monitoring, it does form the basis of food webs and provide structure and habitat to the ecosystem, and thus should be a component of any evaluation of restoration success (Young 2000). Assessment of the plant community should include metrics that related to both the diversity and structure of the vegetation (Ruiz-Jaen & Aide 2005). Measures of diversity include species richness and evenness as well as indices that incorporate both of these, such as Shannon-Wiener and Simpson's diversity (Kindscher & Tieszen 1998; Polley et al. 2005; Hackett et al. 2016).

Floristic Quality Index (FQI) can also be used as a measure of both diversity and vegetation 'quality' or function, as its calculation incorporates species richness as well a measure of species' fidelity to undisturbed sites (Taft et al. 1997; Swink & Wilhelm 1994; Bourdaghs et al. 2006). Measures of vegetation structure include cover, biomass and vegetation height (Ruiz-Jaen & Aide 2005). Comparing plant community metrics among restored, control and reference sites will reveal whether roadside restoration can not only increase plant diversity compared to control sites, but also whether they can achieve and maintain a level of diversity, composition and structure comparable to remnant ecosystems. Plant community measures can also be compared to measurements of other ecosystem components to determine relationships between the plant community and overall ecosystem health. This will be important in understanding the potential and the limitations of ecosystem restoration that primarily focuses on altering the plant community.

Relationships between plant communities and ecosystem functions

Restoration of the plant community certainly has the potential to lead to the restoration of other ecosystem components due to plants' position at the base of the trophic pyramid (Young 2000). Primary producers have strong impacts on ecosystem services relative to many other trophic groups and plant diversity is positively correlated with the delivery of regulating services, such as the regulation of soil carbon levels, and cultural services such as recreation (Soliveres et al. 2016). However, the combined effects of multiple trophic levels are stronger than the effect of any one trophic level alone (Soliveres et al. 2016) and while increasing plant diversity can positively affect food web complexity and the diversity of other taxa, the effect of plant diversity decreases with increasing trophic level (Scherber et al. 2010; Rzanny & Voigt 2012). Furthermore, environmental factors can also strongly influence ecosystem function (Soliveres et al. 2016), and the landscape context can influence the degree to which changes in plant diversity influence other taxa, such as pollinators (Scheper et al. 2013; Ballare et al. 2019). Thus, actively increasing plant diversity along roadsides does not guarantee the restoration of other ecosystem components. More direct measurements of other taxa and ecosystem functions are necessary to determine whether they can successfully be restored by increasing native plant diversity along roadsides.

Ecosystem Processes and Functions

While the evaluation of ecosystem processes and functions is clearly necessary to measure roadside restoration success, it is impossible to evaluate all aspects of any ecosystem due to financial and time constraints. Thus, measures of restoration success should be chosen based on their relevance in the ecosystem being restored and the project goals (Ruiz-Jaen & Aide 2005). Thus, when deciding on ecosystem components to consider in this study, I chose to include components that were measurable, known to play important roles in sustaining grassland ecosystems and providing ecosystem services, and commonly included in the goals of grassland and roadside restoration projects. The indicators I chose to measure were the abundance and diversity of bees, soil carbon storage and the interaction between plants and arbuscular mycorrhizal (AM) fungi.

Pollinators

Pollinators as indicators of restoration success

Bee abundance and diversity are useful indicators of restoration success because they can reveal whether restoring the plant community leads to the restoration of a higher trophic level and also because bees provide valuable pollination services. Pollination services are vital to the sustainability of restored ecosystems, as 88% of flowering plant species rely on animal pollination globally (Ollerton et al. 2011; Cariveau et al. 2020). Furthermore, bees are vital for the pollination of food crops. Pollination services are estimated to be worth US $200 billion per year, and native bees are increasingly being recognized for their substantial contributions to crop pollination (Klein et al. 2007; Blaauw & Isaacs 2014; Reilly et al. 2020). However, insect populations are in decline across the globe (Hallmann et al. 2017), and the threat posed to wild ecosystems and crop production by this decline has prompted the inclusion of pollinator habitat creation as a key goal of restoration projects and native plantings (Menz et al. 2011). As roadsides often border of agricultural fields and form long linear patches, restoring pollinator communities along roadsides may benefit crop production in adjacent fields (Blaauw & Isaacs 2014) and create greater habitat connectivity for the benefit of both wild bees and wild plants (Cariveau et al. 2020). Thus, restoration of the bee community is a key objective that should be assessed when evaluating roadside restoration success.

Pollinators along the roadside

There is some evidence that roadside plant community restoration can benefit pollinator communities. Roadsides containing a greater diversity and abundance of floral resources host a greater diversity of butterflies and burnets (Munguira & Thomas 1992; Ries et al. 2001) and are correlated with lower road mortality rates for these taxa (Skórka et al. 2013). Similarly, Hopwood (2008) found positive correlations between floral richness and abundance and bee diversity and abundance at roadside sites, with restored sites supporting a greater abundance and diversity of native bees. Furthermore, increasing floral resources in land adjacent to agricultural land can increase wild bee abundance (Blaauw & Isaacs 2014). However, very few studies have been conducted on bees in roadside environments (but see Hopwood 2008), so it is unclear if these patterns are generalizable across different landscapes. In agricultural environments, the effect of increasing plant diversity on bee communities can vary depending on landscape factors such as the amount of nearby natural habitat, connectivity and landscape heterogeneity (Steffan-Dewenter 2003; Holzschuh et al. 2007; Öckinger et al. 2018). Thus, it cannot be assumed that restoring healthy plant communities will lead to the restoration of a diverse pollinator community, and more research is needed in the roadside environment.

Soil Carbon Sequestration and Storage

Grasslands as carbon sinks

Soil carbon has been used as an indicator of grassland restoration success on numerous occasions because it is an indicator of the carbon cycling process (Ruiz-Jaen & Aide 2005) and because soil carbon sequestration may offset carbon emissions, mitigating climate change (Scurlock & Hall 1998). Globally, it is estimated that grasslands store 30% of the world's total soil organic carbon and sequester approximately 0.5 Gt of carbon per year (Scurlock & Hall 1998). However, conversion of grassland to conventionally tilled cropland has resulted in the loss of soil organic carbon (Davidson & Ackerman 1993; Senthilkumar et al. 2009; Abbas et al. 2020). Restoring agricultural land to grassland can help to reverse these losses and convert these lands from carbon sources to carbon sinks (Gebhart et al. 1994). The ability of restored grasslands to sequester carbon has been attributed to increases in plant diversity and the abundance of certain functional groups, including C_4 grasses and legumes, that increase total root biomass and lead to the incorporation of more carbon into the soil (Fornara & Tilman 2012; Lange et al. 2015; Mahaney et al. 2015; Yang et al. 2019). However, studies on carbon sequestration in restored grasslands have found mixed results, possibly due to differences in management (McGranahan et al. 2014), baseline carbon levels and soil texture (Senthilkumar et al. 2009). Thus, it is important to assess soil carbon when evaluating grassland restorations to ensure that the carbon sequestration process has been successfully restored.

Studies of prairie restoration and carbon storage

A number of studies have assessed carbon sequestration as a measure of grassland restoration success. Typically, ecosystem process such as carbon cycling are not measured directly but are measured indirectly using indicators such as soil carbon content (Ruiz-Jaen & Aide 2005). Research on prairie restoration undertaken on abandoned agricultural land found that while soil carbon content in restoration sites was usually greater than in agricultural fields, it was still significantly less than in remnant prairie sites, even thirty to fifty years after restoration commenced (Kindscher & Tieszen 1998; Potter et al. 1999; Fuhlendorf et al. 2002; Hansen & Gibson 2014). This suggests that the recovery of soil carbon pools may occur over longer time scales (Potter et al. 1999; Knops & Tilman 2000; Matamala et al. 2008). However, as agriculture can severely deplete soil organic matter (Knops & Tilman 2000) and there is evidence that changes in soil carbon may be dependent on baseline carbon levels (Senthilkumar et al. 2009), these findings may not be generalizable to restoration on other land uses. To my knowledge, there has been no investigation of soil carbon content in roadside prairie restorations. Thus, assessing soil carbon in roadside grassland restorations will help to determine whether similar changes in soil carbon following restoration can be expected in roadside environments.

Arbuscular Mycorrhizal Fungi:

Importance of AM fungi in grasslands

The symbiosis between plants and soil-inhabiting AM fungi may be an important indicator of restoration success because it is a key biological interaction in grassland ecosystems that has a strong influence on plant community composition and ecosystem functions (van der

Heijden et al. 1998; Rillig 2004). AM fungi are obligate symbionts that associate with 75% of plant species and colonize plant roots in order to obtain sugars from their plant hosts (Brundrett 2009). In return, AM fungi provide plants with increased access to water and nutrients, particularly phosphorus (Smith et al. 2010). While plant species vary in their response to colonization by AM fungi, many species that typically dominate late-successional grassland communities achieve much greater biomass in the presence of AM fungi, and have minimal growth and low survival when AM fungi is absent (Hetrick et al. 1988, 1990; Wilson & Hartnett 1998; Middleton & Bever 2012). AM fungi may also help to advance grassland succession by giving late-successional species an advantage over early successional species that do not respond as positively to AM fungi (Hetrick et al. 1989; Smith et al. 1999; Middleton & Bever 2012; Koziol & Bever 2015; Weremijewicz & Seto 2016). Thus, understanding interactions between plants and AM fungi may be critical for establishing successful grassland restorations.

Effects of disturbance on AM fungi

Despite the important role of plant-AM fungal interactions in grassland communities, grassland restoration is often attempted on land that has been highly disturbed and may therefore be lacking an abundance or diversity of AM fungi. Soil disturbance, such as that which occurs during road construction or the tillage of cropland, can greatly reduce AM fungal populations and the ability of AM fungi to colonize plant roots (Moorman & Reeves 1979; Jasper et al. 1989). Disturbance can also alter the AM fungal community composition, favouring more disturbance tolerant species while harming more sensitive species (Hart & Reader 2004). A reduction in AM fungal populations or changes in the composition of the AM fungal community due to disturbance could hamper the establishment of grassland species that depend on AM fungi and alter trajectory of the restored plant community (Smith et al. 1998; Wubs et al. 2016).

Restorations on disturbed land must therefore aim to restore plant-AM fungal interactions in order to achieve desirable and sustainable grassland plant communities.

Studies on AM fungi and restoration

The application of AM fungal inoculum to prairie restorations may overcome disturbance-induced changes to the AM fungal community and improve the establishment of native plant species (Smith et al. 1998; Middleton & Bever 2012; Koziol & Bever 2017). While some studies have found that inoculation can increase AM colonization and the cover of prairie plants in restoration sites (Smith et al. 1998; Maltz & Treseder 2015; Koziol et al. 2020), others have found that initial increases in colonization in inoculated soils did not persist over time or affect the prairie plant cover (White et al. 2008). Varying results may be explained by differences in soil nutrients (Johnson et al. 1997) and the initial abundance of AM fungi (White et al. 2008). Variation in the effects of inoculation may also be a result of the passive recovery of AM fungal communities through the natural recolonization of some disturbed sites by AM fungi (White et al. 2008), though few have investigated how plant-AM fungal interactions recover from disturbance over longer time scales (Maltz & Treseder 2015). Plant response to inoculation also varies depending on the source of inoculum, with inoculum from local natural prairie ecosystems generally outperforming commercial sources of inoculum (Rowe et al. 2007; Maltz & Treseder 2015; Middleton et al. 2015), though not in all studies (White et al. 2008). Given the clear context-dependent nature of plant response to AM fungal inoculation, more research is needed to understand when AM fungal inoculation is necessary for successful grassland restoration. Studying plant-AM fungal interactions in disturbed roadside sites of varying ages should improve our understanding of how plant-AM fungal interactions recover from disturbance and of the potential for AM fungi to improve the establishment of grassland plants in roadside restorations.

Thesis objectives

My objective was to undertake a comprehensive evaluation of the success of current roadside grassland restoration projects in Ontario in order to determine whether the establishment of native plant communities along roadsides led to not only increased native plant diversity, but also the restoration of the broader ecosystem. To investigate this, I measured the diversity, composition and structure of the vegetation community, the diversity and abundance of native bees, soil carbon content and plant response to AM fungi at roadside restorations, control and reference sites associated with three different highways in Southern Ontario. I asked the following questions:

1) Does restoring roadsides by planting them with native seed increase plant diversity and alter plant species composition so that restored roadsides are significantly different from unrestored control sites and closely resemble nearby reference sites? Does this pattern hold for different highways with unique geographies and histories?

2) Does restoring roadsides with native plants increase the abundance and diversity of bees so that bee diversity and abundance in restored roadsides are higher than the bee diversity and abundance of control sites and more similar to the levels of reference sites? Does this pattern hold for different highways with unique geographies and histories?

3) Are differences in bee diversity and abundance among roadside sites related to the plant diversity or bare ground available at the site?

4) Does restoring roadsides with native plants increase soil carbon storage beyond the levels found in control sites to levels similar to those of remnant grasslands?

5) Is the amount of soil carbon in grasslands correlated with the abundance of particular plant functional groups, particularly C4 grasses and legumes?

6) Does the growth response of native plants to AM fungi increase with time since disturbance?

METHODS

Study Sites

Thirteen roadside sites planted with native grassland species were located along three different highways in Southern Ontario: four sites along the Highway 40 'Prairie Passage' in Lambton County, four sites along the Rt. Hon. Herb Gray Parkway (hereafter referred to as the 'Herb Gray Parkway') in Essex County and five sites along the Highway 407 East Extension project (hereafter referred to as 'Highway 407') in Durham County (Figure 1). In each of the three localities, I also selected 'control' roadside sites that had been seeded with standard MTO seed mix, which consists primarily of non-native grasses and legumes. I also attempted to select 'reference' sites in each area that were representative of healthy grassland ecosystems that had not been degraded (SER 2004). Sites were selected so that they were at least 2km apart from each other where possible to ensure that the sites' bee communities were as independent from each other as possible. However, this minimum distance was not achieved for three of the Herb Gray Parkway restored sites and one pair of Highway 407 restored sites, though all of these sites were separated from nearby sites by major roadways (at least 6 lanes of traffic) or sound barriers. All roadside sites were defined as areas that were 42m in length (parallel to the road) and a maximum of 30m in width (or shorter, depending on the road width), with a buffer of 1m between the gravel edge of the road or the ditch (if a ditch was present next to the road) and the edge of the site. All reference sites, which were not located along roadsides, also measured 42m x 30m. A summary of site information is provided in Table 1. The sometimes sparse and incomplete nature of information on site history and management combined with a lack of replication of planting and management protocols precluded any statistical analysis on relative success of different methods, but potential impacts of these different methods will be reviewed in the Discussion.

Highway 40 Study Sites

Highway 40 restored sites were established between 2002 and 2010 by members of the Rural Lambton Stewardship Network with the intention of creating a 'Prairie Passage.' Restored sites were located adjacent to the existing two-lane roadway, which was constructed in the 1970s and runs through a rural area dominated by agriculture (Figure 2). The two oldest sites, Whitebread and Courtright, were established on land that had previously been seeded with a non-native seed mix when the road was originally constructed. Existing vegetation was sprayed with the herbicide glyphosate in the summer, native grass species were planted in the fall, and then native forbs were planted the following spring. Drill seeding was used to apply the seed to the sites. Controlled burns were conducted twice at the Whitebread site and likely also at the Courtright site, though no burns have been conducted since 2010. The two newer sites, Bickford and Courtright, were established on land that had previously been farmed for soybean. A 50/50 mix of native grass and forb seed was drill seeded into the ground among the soybean stubble in May 2010. These two sites were mowed in 2011 to reduce competition from non-target species but have not been mowed since then. I chose two 'control' sites located within the right-of-way along Highway 40 that had not been restored with native plants. The control sites had been seeded with a mainly non-native seed mix when the road was originally constructed. Although restored areas are interspersed among areas containing the original 'control' vegetation along this stretch of road and thus there is some opportunity for seed to travel between restored and control areas, I chose control sites that were separated from restored areas by at least two lanes of roadway. Furthermore, the control sites I chose lacked the prairie indicator species that had been established in restored areas. Since there were no remnant grasslands in the area, I selected as my reference sites two grassland restorations that were similar in age to the older roadside restorations and that had been established on conservation land outside the right-of-way. While these sites do not necessarily represent intact grasslands, comparisons between the roadside restorations and these references allowed me to determine if roadside restorations were as successful as restorations carried out on larger, less disturbed areas of land.

Herb Gray Parkway Study Sites

Herb Gray Parkway restored sites were established in spring 2015 on bare soil along the newly constructed six-lane roadway shortly after construction and grading were complete. The roadway runs through mainly suburban residential and commercial areas in Windsor (Figure 3). Due to the presence of endangered species in the area, special care was taken to implement and monitor the restorations to ensure successful habitat creation. For example, ecologists were responsible for removing endangered reptiles from the construction site, endangered plants were transplanted to new locations, and the creation of the seed mixes was carefully supervised. A variety of seed mixes, including a ‘pollinator seed mix’, were custom designed for this project by restoration ecologists and were applied to different areas along the roadway, along with nurse crops to stabilize the soil until native plants became established. Continual monitoring of the sites each year informed management strategies to maximize success. Management has included the use of herbicide, manual weed removal and mowing to reduce competitive pressure from non-target species. Where establishment was poor, some sites were reseeded or planted with an additional nurse crop. I was unable to locate control sites along the Herb Gray Parkway because the entire project was seeded with native species. Instead, I chose sites along a nearby roadway that had been seeded with a mainly non-native seed mix. These sites were located along the four-lane E.C. Row Expressway, which was constructed from 1971-1983 and which runs East-West, roughly perpendicular to and eventually crossing the Herb Gray Parkway. I was able to gain access to one remnant prairie site in the Windsor area (‘Chappus’), which I used as a reference site. This site is owned by the Ministry of Transportation of Ontario (MTO) and managed by Wood PLC, a contractor that is responsible for much of the restoration work associated with the Herb Gray Parkway project. The Chappus site was most recently burned in the spring of 2019.

Highway 407 Study Sites

Restored sites along Highway 407 were established in 2016 along the newly constructed four- and six-lane Highway 407 East Extension (Phase I), which includes the Highway 412 corridor. This roadway crosses through agricultural land and some natural areas (Figure 4). Native seed mixes were applied to designated sections of the roadside by hydroseeding, followed by the application of mulch. The seeding was carried out in many cases by the contractor that was responsible for the road construction, and seed was applied once each section had been graded, regardless of the time of year. While the restoration plan listed potential management actions, such as the use of mowing and herbicide to control invasive species and the use of nurse crops (Arthur 2015), I was not able to obtain specific information regarding which management actions were actually undertaken at each site. Since not all of the rights-of-way along the new highway were seeded with native species, I was able to locate control sites within the project that had been seeded with mainly non-native seed mix in the same year that the restored sites were seeded. While the control sites were interspersed among restorations along the length of the roadway so that native seeds could have in theory dispersed from restored to control sites, the control sites I selected did not contain native grassland indicator species that were present at most restored sites. I did not select reference sites in this area because none were available that were large enough to facilitate vegetation and bee sampling. Reference models used in the restoration plan had been constructed based on small remnant populations of indicator plant species and remnant sites in different counties (Arthur 2015).

Table 1. Site information for the 23 roadside and reference sites chosen for vegetation and bee sampling and, in some cases, soil collection. '40' = Highway 40, 'HG' = Herb Gray Parkway, '407' = 407 East Extension (Phase 1), including the 412. For restored and control sites, which were located within rights-of-way, 'Hwy' refers to the highway to which the right-of-way was adjacent, except for Herb Gray Parkway control sites, which were located within the right-of-way of a nearby highway (E.C. Row Expressway). For reference sites, 'Hwy' refers to the highway containing restored rights-of-way that was closest to these sites, which were not within a right-of-way. 'N/A' indicates information is not applicable to this site or was not available. The species present in each seed mix are listed in Appendix A.

Site	Coordinates	Hwy	Site Type	Date Planted	Seeding Method	Seed Mix	Site Prep	Adjacent Land Use	Previous Land Use	Management Actions
Whitebread	42.633205, -82.430889	40	restored	Fall 2002	Drill seeding	A	Sprayed with glyphosate	Agriculture	Right-of-way (non-native cool-season grasses)	Burned in 2007 & 2010
Courtright	42.823605, -82.416821	40	restored	Fall 2005	Drill seeding	A	Sprayed with glyphosate	Woodlot	Right-of-way (non-native cool-season grasses)	Likely burned between 2005 and 2010 (unconfirmed)
Bentpath	42.722987, -82.428415	40	restored	Spring 2010	Drill seeding	B	None	Agriculture	Farmed for soybean	Mowing
Bickford	42.765969, -82.420131	40	restored	Spring 2010	Drill seeding	B	None	Agriculture / Forest	Farmed for soybean	Mowing
Moore	42.843346, -82.415924	40	control	1975?	N/A	MTO mix	N/A	Agriculture	N/A	N/A
Oil Springs	42.795960, -82.417834	40	control	1975?	N/A	MTO mix	N/A	Woodlot	N/A	N/A
Property 80	42.739231, -82.346835	40	reference	2002	N/A	A	N/A	Agriculture / Wetland	Agriculture	N/A
Nicholls Memorial	42.723207, -82.354347	40	reference	2004	N/A	A	N/A	Agriculture / Wetland	N/A	N/A
Pulford	42.255290, -83.036916	HG	restored	April 2015	N/A	C	New construction (major disturbance and grading)	Urban trail system	N/A (new construction)	Reseeding, herbicide, manual weed removal

Site	Coordinates	Hwy	Site Type	Date Planted	Seeding Method	Seed Mix	Site Prep	Adjacent Land Use	Previous Land Use	Management Actions
Sandwich	42.240537, -83.011721	HG	restored	April 2015	N/A	D	New construction (major disturbance and grading)	Urban trail system	N/A (new construction)	Herbicide, planted additional nurse crop, reseeding, mowing
Pump Station 6	42.240364, -83.007540	HG	restored	May 2015	N/A	E	New construction (major disturbance and grading)	Urban trail system	N/A (new construction)	Herbicide, manual weed removal
Montgomery	42.239377, -83.008591	HG	restored	May 2015	N/A	D	New construction (major disturbance and grading)	Urban trail system	N/A (new construction)	Mowing, herbicide, reseeding, planted additional nurse crop
Dougall	42.276015, -83.013587	HG	control	1983?	N/A	MTO mix	N/A	Urban (commercial)	N/A	N/A
Walker	42.283611, -82.983039	HG	control	1983?	N/A	MTO mix	N/A	Urban (commercial)	N/A	N/A
Chappus	42.271054, -83.067193	HG	reference	N/A	N/A	N/A	N/A	Natural area	N/A	Burning
Pond 8E	43.926893, -79.086482	407	restored	2016	Hydroseeding (10kg/ha) with fibre mulch application	F	Herbicide application, placement of fill	Agriculture	N/A (new construction)	N/A
Pond 20E	43.952036, -78.989148	407	restored	2016	Hydroseeding (10kg/ha) with fibre mulch application	G	New construction (major disturbance and grading)	Agriculture	N/A (new construction)	N/A
Site 35	43.976107, -78.890105	407	restored	2016	Hydroseeding (10kg/ha) with fibre mulch application	H	New construction (major disturbance and grading)	Natural area (creek system) / Agriculture	N/A (new construction)	N/A

Site	Coordinates	Hwy	Site Type	Date Planted	Seeding Method	Seed Mix	Site Prep	Adjacent Land Use	Previous Land Use	Management Actions
Pond 44N	43.916895, -78.996618	407	restored	2016	Hydroseeding (10kg/ha) with fibre mulch application	I	New construction (major disturbance and grading)	Agriculture / Forest	N/A (new construction)	N/A
Pond 42S	43.943741, -79.006450	407	control	2016	Hydroseeding (10kg/ha) with fibre mulch application	G	New construction (major disturbance and grading), Placement of fill	Agriculture	N/A (new construction)	Partial regrading in 2019
Pond 11W	43.939679, -79.062257	407	control	2016	Hydroseeding (10kg/ha) with fibre mulch application	MTO mix	New construction (major disturbance and grading)	Agriculture	N/A (new construction)	N/A
Pond 45S	43.899187, -78.992712	407	control	2016	Hydroseeding (10kg/ha) with fibre mulch application	MTO mix	New construction (major disturbance and grading)	Agriculture	N/A (new construction)	N/A
Pond 28W	43.957008, -78.929522	407	control	2016	Hydroseeding (10kg/ha) with fibre mulch application	MTO mix	New construction (major disturbance and grading)	Agriculture	N/A (new construction)	N/A

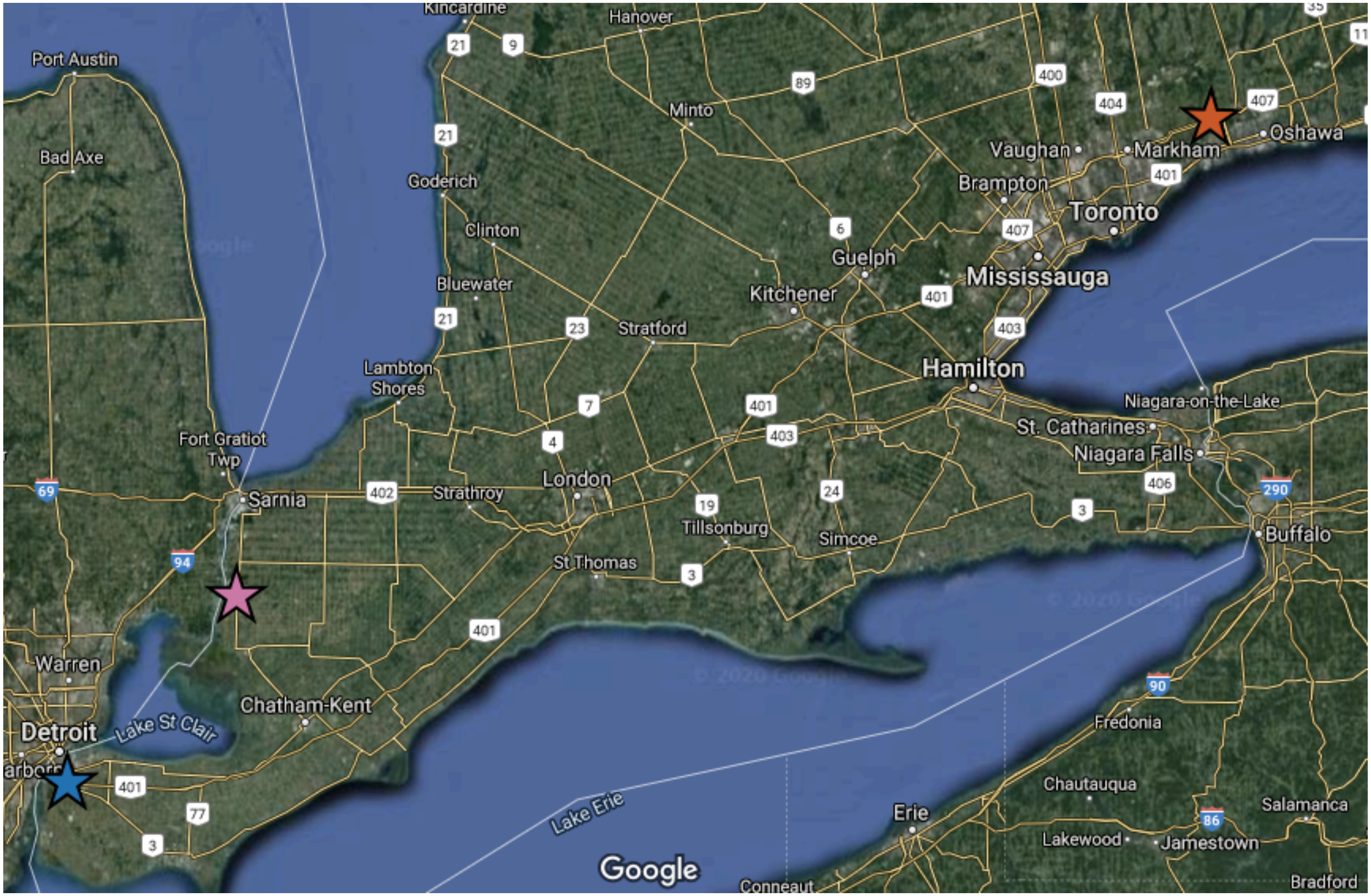

Figure 1. Locations of the three study areas in Southern Ontario. Herb Gray Parkway (blue) is located in Essex County, Highway 40 (pink) is located in Lambton County and Highway 407 (orange) is located in Durham County. This image was retrieved from Google Maps on August 22, 2020. Map data © 2020 Imagery © 2020 TerraMetrics.

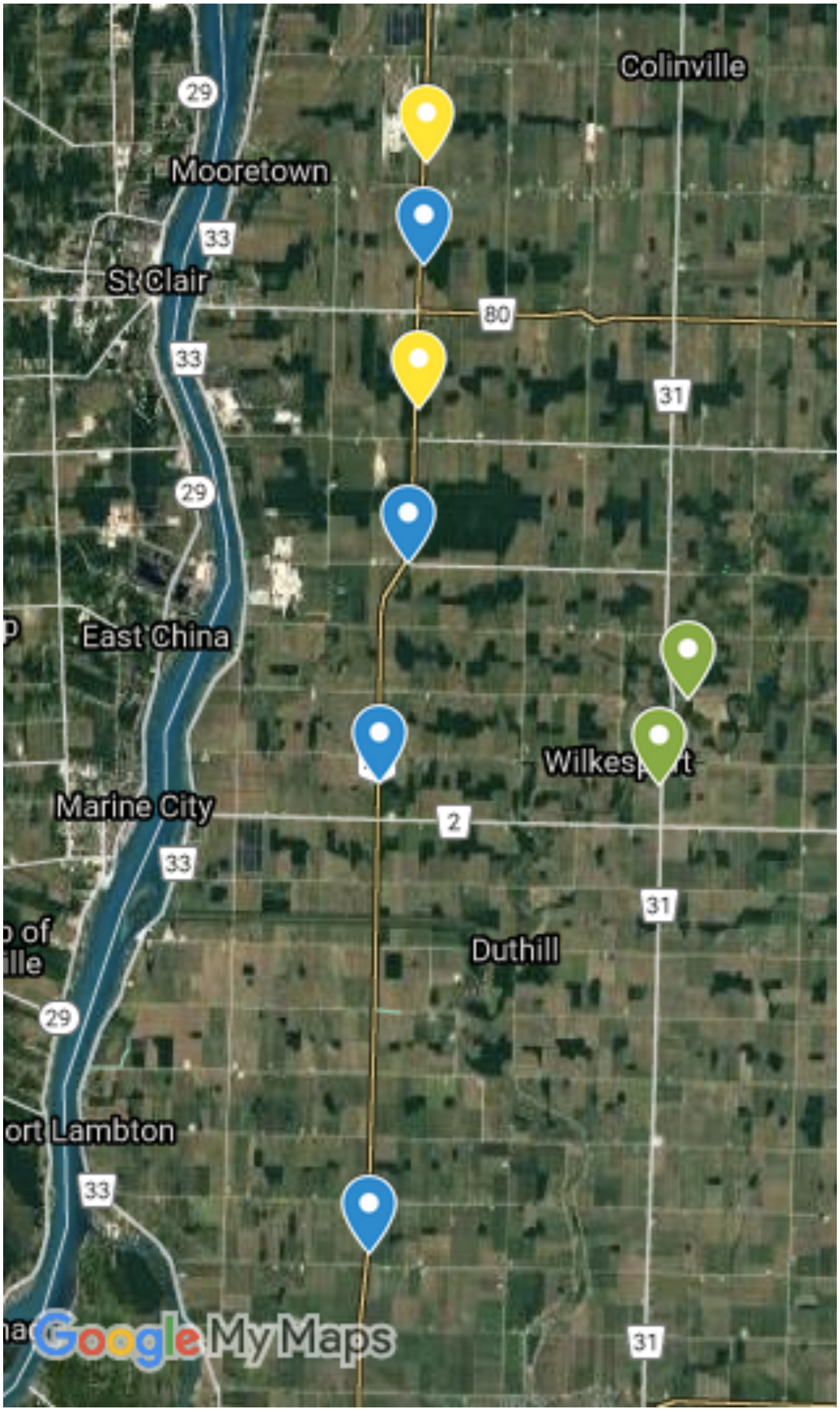

Figure 2. Locations of Highway 40 restored (blue), control (yellow) and reference (light green) sites. This image was retrieved from Google Maps on August 22, 2020. Map data © 2020 Imagery © 2020 TerraMetrics.

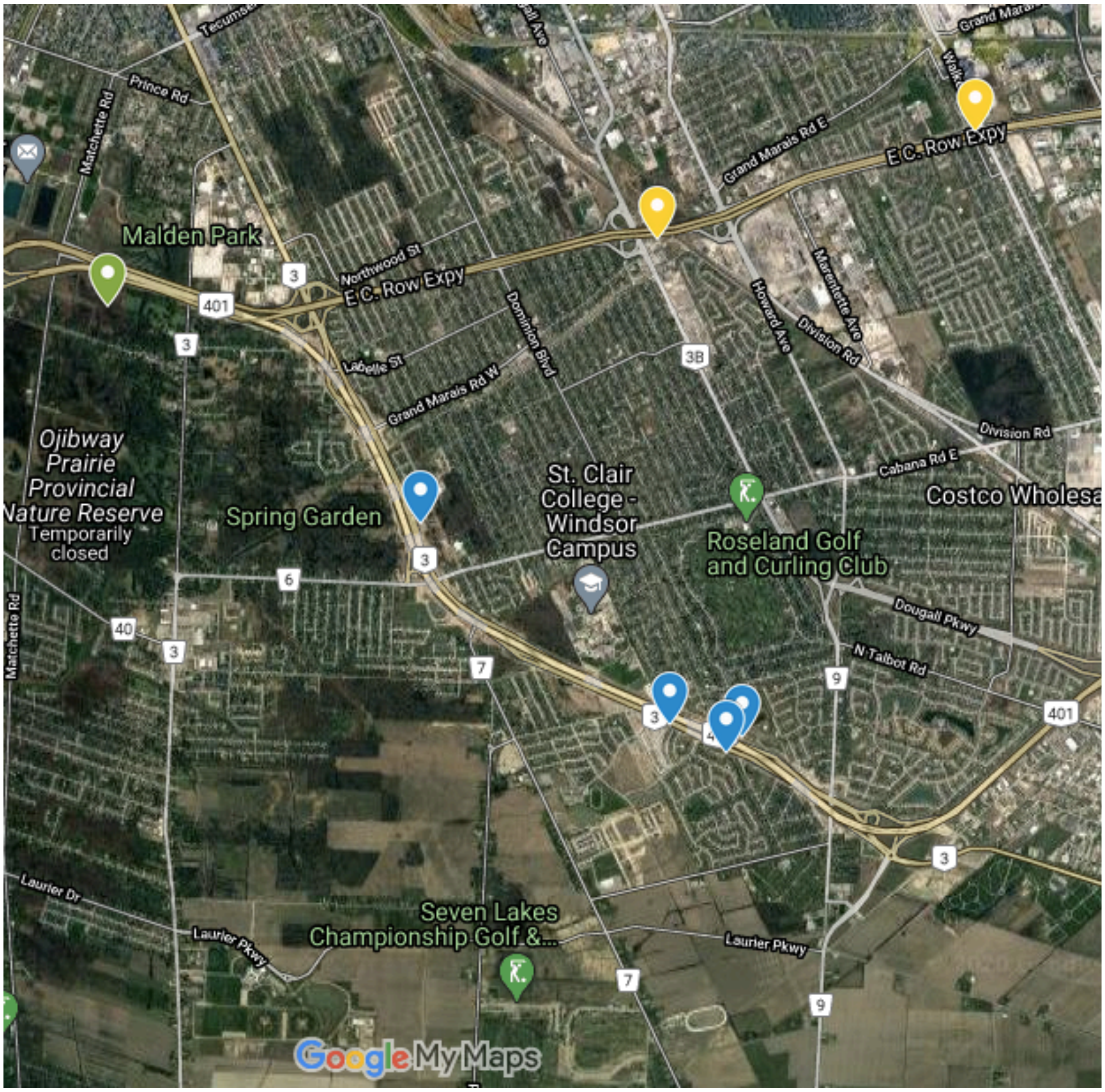

Figure 3. Locations of Herb Gray Parkway restored (blue), control (yellow) and reference (light green) sites. This image was retrieved from Google Maps on August 22, 2020. Map data © 2020 Imagery © 2020, CNES / Airbus, First Base Solutions, Landsat / Copernicus, Maxar Technologies, Sanborn, U.S. Geological Survey, USDA Farm Service Agency.

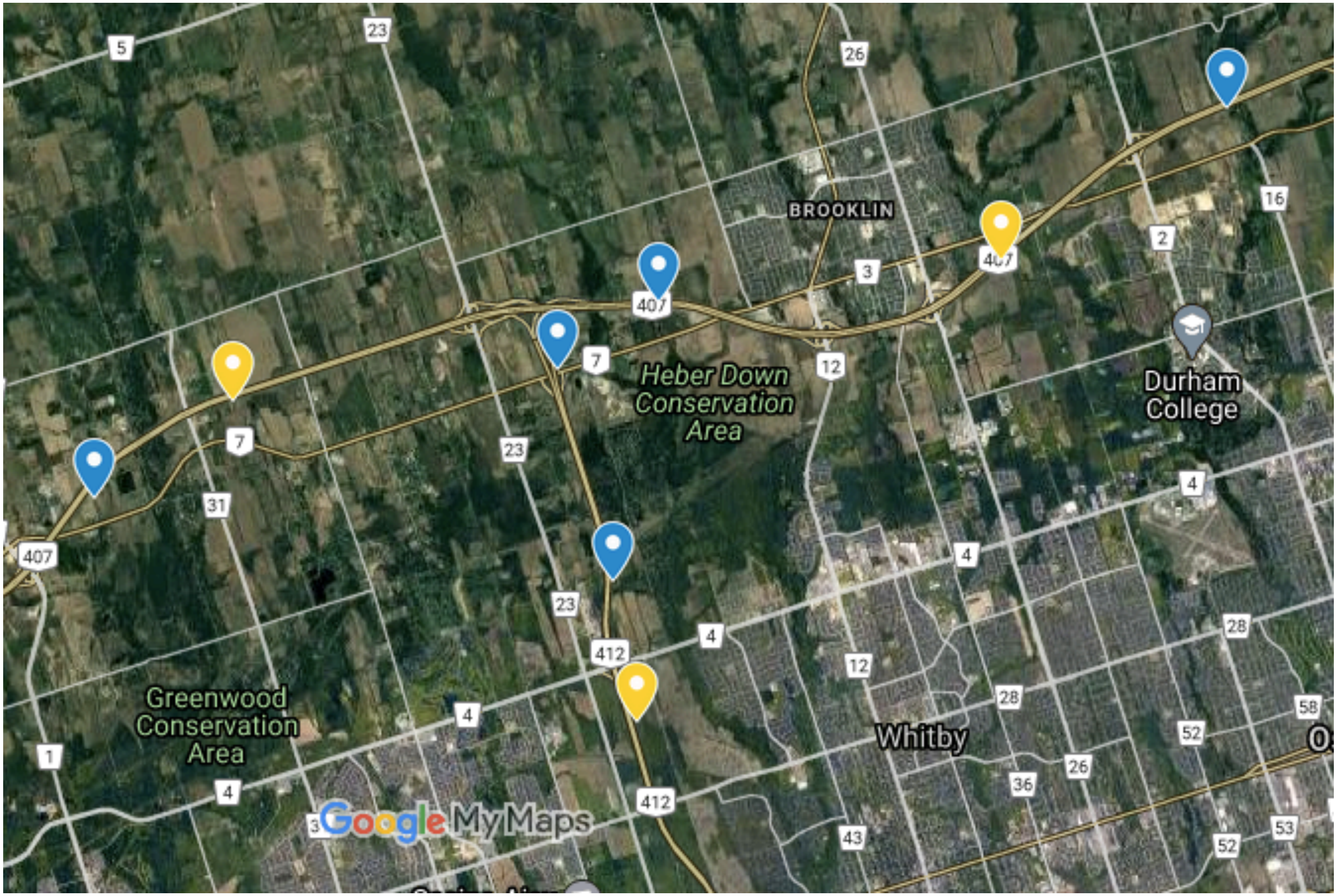

Figure 4. Locations of Highway 407 restored (blue) and control (yellow) sites. This image was retrieved from Google Maps on August 22, 2020. Map data © 2020 Imagery © 2020 TerraMetrics.

Vegetation Diversity and Composition

Vegetation surveys were completed at each site in order to assess their plant community diversity and vegetation structure. The plant community was assessed twice during the 2019 growing season to account for growth differences between species due to seasonality: once between June 17^{th} and July 3^{rd} and once between September 5^{th} and 16^{th}. The percentage of bare ground, which was defined as soil visible while looking down from standing height, and the percent cover of each plant species present in 1x1m quadrats was recorded using a modified Daubenmire scale (Daubenmire 1959; Brown & Bugg 2001) containing seven cover classes: 0-1%, 1-5%, 5-25%, 25-50%, 50-75%, 75-95% and 95-100%. The total cover of each quadrat was able to exceed 100% due to overlapping canopies. Fifteen quadrats were assessed at each site during each sampling period and were randomly located within three strata so that five quadrats were placed in each stratum. Sites were divided into strata based on distance from the road. For sites that were at least 30m wide, each stratum was 10m wide. For sites that were less than 30m wide, the width of the site was divided into three strata of equal width. Quadrats from the first survey were not relocated during the second sampling period – instead, new quadrats were placed in new random locations. In addition to recording the presence of species within quadrats, a 10-minute walk-through of each site, including areas outside but adjacent to the strict site boundaries (i.e. the verge edge and/or ditch), was completed after the quadrat sampling, during which any additional species not found in the quadrats were recorded. During each sampling round, all sites from the same county were surveyed within a 1-week period.

Bee Diversity, Abundance and Community Composition

Bees were surveyed using pan traps approximately once every 2 weeks on mainly sunny days with a daily high temperature exceeding 15°C and with a maximum sustained wind speed of less than 30km/hr (and usually < 20km/hr). Sampling started in early May and continued until the end of September, with Highway 40 and Highway 407 sites sampled 10 times and Herb Gray Parkway sites sampled 7-9 times. Sampling of some Herb Gray Parkway sites was not completed for some early sample periods due to logistical challenges. During each sample period, all sites near the same highway were sampled on the same day and sampling of sites from all highways was completed within a week, with the exception of the early September sample, which was delayed for Highway 40 sites (occurring 13 days after sampling at Herb Gray Parkway sites and 9 days after Highway 407 sites).

On each sampling day, 15 pan traps (5 yellow, 5 blue, 5 white) filled approximately ¾ full with water and unscented dish soap (5ml of soap per litre of water) were laid out at each site on the ground ~3m apart in a transect parallel to the road (Hopwood 2008; Westphal et al. 2008; NSERC-CANPOLIN 2009). The transect was placed roughly in the middle of the site, a minimum of 10m from the road and on level ground when possible. Vegetation was tamped down in a circular area approximately 1m in diameter prior to placing each trap on the ground to improve the visibility of the trap. Traps were laid out between approximately 8am and 10am and collected between approximately 4pm and 6pm on each sampling day. Invertebrates caught in the traps were stored in 70% isopropyl alcohol at room temperature until they could be sorted and identified to the genus level using a key by Packer et al. (2007).

Carbon Storage

Since carbon cycling is a process that occurs on a long timescale and previous studies have shown that carbon content in the soil requires decades to recover to the levels found in remnant grasslands (Knops & Tilman 2000), I focused my study of soil carbon content on the restoration sites at Highway 40, which were the oldest restoration sites in this study. During the vegetation surveys conducted in September 2019, a cylindrical soil core 2cm in diameter and 60cm deep was collected from the center of six randomly selected vegetation quadrats (two in each stratum) in each of the four roadside restoration sites. Each restored roadside site was paired with a nearby control site that was located in the right-of-way and had not been planted with native vegetation. Control sites were located across the road from each restoration site except for the Bickford control, which was located on the same side of the road as the restoration, in between the road and the edge of the restored site. Six quadrats were placed in these control sites to assess plant species' cover and collect soil cores using the same methodology as that applied to the restored sites. All soil cores were separated into 0-20cm, 20-40cm and 40-60cm depths, so that differences in soil carbon could be compared across different depths. Three bulk density cores, 5cm in diameter and 5cm in depth, were also collected from each of these sites in September 2019. The bulk density cores were collected next to the location where soil carbon samples were collected in one randomly selected quadrat per stratum. All soil samples were kept in an ice chest for transportation and in a 4°C refrigerator for storage. Samples were sent to the University of Guelph Agriculture and Food Laboratory for analysis of bulk density or total soil carbon, which was measured using a combustion method.

AM Fungi

Inoculum Sources and Soil Collection

I tested the response of a native plant to AM fungi in a greenhouse experiment using soil inoculum collected from roadside sites. Soil inoculum was collected from 11 of the study sites used for vegetation and bee sampling, selected so that they were representative of a variety of site types (restored, reference and control), site ages (time since restoration or, for control sites, time since road construction) and locations. The previously described sites from which soil was collected were Chappus, Whitebread, Courtright, Bentpath, Moore, Montgomery, Pulford, Walker, Site 35, Pond 44N and Pond 28W. Additionally, to capture a broader range of site ages, including very recently disturbed sites, soil was collected from seven newly constructed sites located in the right-of-way of the second phase of the Highway 407 East Extension project, which was located further East of the Phase I sites that were included in vegetation and bee sampling. Soil was collected from two sites planted in 2017, two sites planted in 2018, two sites planted in 2019 and from an unseeded stockpile. One site from each pair of sites completed in 2017-2019 had been seeded with a native seed mix according to landscape drawings, and the other site had been seeded with a mainly non-native MTO mix. The boundaries and strata of these new sites were delineated in the same manner as the other sites used in this study.

Between September 12th and October 3rd, 2019, I collected soil cores, 2cm in diameter and 15-20cm in depth, from the center of two randomly selected quadrats within each stratum for a total of six cores per site. The six soil cores were pooled and homogenized to create a single source of inoculum from each site. Soil inoculum was stored at 4°C until the experiment was initiated.

Study Species

I chose *Andropogon gerardii* as a study species for assessing root colonization and the effect of AM fungi on native plant growth. *A gerardii* is a native C_4 grass that is dominant in tallgrass prairie communities, and previous studies have shown that this species has a positive growth response to AM fungal colonization and is highly dependent on AM fungi for survival (Hetrick et al. 1988, 1989, 1990; Weremijewicz & Seto 2016). Thus, I expected that this species would be sensitive to differences among the AM fungal communities present within the different sources of soil inoculum. *A. gerardii* seeds were wild collected from a 5-year-old prairie restoration in Hamilton, Ontario on November 5^{th}, 2019. Since this site is a restoration, the *A. gerardii* population at this site is not a natural population but originates from seed produced at an Ontario native plant nursery by propagating seed collected from wild populations elsewhere in Southern Ontario. The seeds were stored at 4°C until planting.

Greenhouse Experimental Design and Set-Up

A. gerardii plants were grown from seed in a fully factorial greenhouse experiment where both soil treatment and inoculum source were varied. There were 2 mycorrhizal treatments: a ‘control’ treatment containing sterilized soil inoculum plus a microbial wash (no AM fungi) and an ‘AM fungal’ treatment containing live soil inoculum (with AM fungi). Soil inoculum for the control treatment was autoclaved at 132°C for 30 minutes. When a portion of autoclaved soil inoculum was mixed with deionized water and plated onto agar plates, no microbial growth was visible after 48 hours in an incubator. Microbial wash used in the control treatment was produced by suspending inoculum soil in water in a 1:4 volume:volume ratio for 24 hours with occasional mixing and then passing the solution through a 25 micron sieve to exclude AM fungal spores but allow smaller microbes to pass through (Ames et al. 1987; Koide & Li 1989; Bever 1994). A separate microbial wash was produced for each inoculum source. The microbial wash was intended to reintroduce other microbes that may be found in the live inoculum into the control pots to ensure that differences in plant growth between treatments could be attributed to the presence or absence of AM fungi (Ames et al. 1987; Koide & Li 1989; Bever 1994). There were 8 replicate pots per combination of mycorrhizal treatment (2 treatments) and inoculum source (18 sources) resulting in a total of 288 pots.

Planting and Maintenance

Seeds were sown in 3” (225ml) pots containing a sterilized background soil and either sterilized or live soil inoculum. The sterilized background soil consisted of a 2:1 mixture of Pro-Mix® BX + Mycorrhizae™ (Premier Tech Horticulture, Rivière-du-Loup, Quebec) and Quali Grow® All Purpose Sand (Quali-Grow Garden Products Inc., L’Orignal, Ontario) that had been autoclaved at 132°C for four 30-minute cycles. 150ml of the sterilized background soil was placed in the bottom of each pot, and an additional 75ml of sterilized background soil was then mixed with 10g of either sterilized (for the control treatment) or live (for the AM fungal treatment) soil inoculum and added to the top of the pot. Several uncleaned *A. gerardii* seeds were sown in each pot and lightly covered with the soil. Each pot then received 20ml of either microbial wash (for the control treatment) or distilled water (for the AM fungal treatment). Pots were placed on a single greenhouse bench in fully random locations and received 14 hours of light per day. The greenhouse daytime temperature ranged from 27-32°C and the nighttime temperature ranged from 15-20°C. Pots were watered to field saturation approximately every two days. Sixteen days after planting, pots were thinned so they each contained one individual. For two pots that contained no living individuals at that time, two individuals from a different pot containing the same soil treatment and inoculum source were transplanted into the pot, and then these pots were thinned to one individual after the transplant was deemed successful. After thinning, pots were weeded every few days to remove any new germinants and maintain a density of one individual per pot. Every week, pots were assigned to new random locations to minimize local environmental effects on growth. To avoid extreme nutrient limitation, six weeks after planting each pot received 35ml of 21-5-20 NPK Peters EXCEL® water soluble fertilizer with micronutrients (Scotts, Mississauga, Canada) that had been diluted to ¼ strength.

Harvest

After 86 days (approximately 12 weeks), the aboveground biomass in each pot was harvested by clipping shoots at the soil surface. The aboveground biomass was dried at 65°C for 20 hours and weighed as an indicator of plant performance. During harvest, the root systems of three randomly selected replicates in each soil treatment x inoculum source combination were also collected. The roots were washed and are being stored in 50% ethanol at 4°C until they can be cleared and stained to examine AM fungal colonization.

Calculations

Plant Diversity Metrics

The vegetation data collected was used to calculate the total and native plant species richness at each site and estimate the relative abundance of each species. Species' cover classes in each quadrat were assigned to the midpoint of each class (0-1 = 0.5%, 1-5 = 3%, 5-25 = 15%. 25-50 = 37.5%, 50-75 = 62.5%, 75-95 = 85%, 95=100 = 97.5%; Daubenmire 1959; Brown & Bugg 2001) and then summed across all fifteen quadrats for each site and sampling period before calculating relative abundance. Additional species recorded outside quadrats were included in calculations of site species richness and were assigned a total cover of 0.5% (equivalent to the midpoint of the lowest cover class) in calculations of relative abundance. Initial analysis (not shown) revealed that sampling period did not significantly affect any of the indices calculated using species' relative abundances, and so data from the two sampling periods were combined by assigning each species the higher of its June or September cover values and recalculating each species' relative abundance (McCain et al. 2011). The relative abundance of species in each site was then used to calculate Shannon-Wiener diversity (H), Simpson's diversity (1-D), Floristic Quality Index (FQI; Swink & Wilhelm 1994) and the proportion of vegetation cover that consisted of native species for each site. Shannon-Wiener diversity and Simpson's diversity were calculated for all species and for only native species. Diversity metrics can include different metrics of diversity, such as richness, evenness and function (Daly et al. 2018), so using multiple indices of diversity allowed us to investigate how restoration influenced these different components of diversity.

FQI was calculated using the formula $wFQI = wC \times \sqrt{S_n}$ (Bourdaghs et al. 2006) where S_n is the native species richness and wC is the abundance-weighted mean coefficient of conservatism based on coefficients of conservatism assigned to each plant species by Oldham et al. (1995). Only native plants were included in the calculation of wC. I chose to use an abundance-weighted FQI rather than the traditional FQI in order to reflect the ecologically meaningful difference between the presence of a few individuals of a species that may have been directly planted and the presence of a viable and thriving population. Plants that could not be assigned a coefficient of conservatism score because they had not been identified to the species level were excluded from the FQI calculation.

The cover values assigned to each species at each site were averaged across all sites of the same type at each highway to obtain an average percent cover for each species in each highway × site type combination. Species were then ordered according to their average percent cover to determine which species were the most abundant in each site type at each highway.

Bee Community and Bare Ground for Nesting

I summed the number of bee genera (‘bee genus richness’) and abundance of bees caught at each site across all samples to calculate the bee genus richness and bee abundance of each site across the entire season. However, samples from early May, late May and late June were not included in these calculations because samples were not collected at all highways during these periods and thus these sample periods could have biased the differences among highways. To obtain an estimate of the amount of bare ground available for ground-nesting bees at each site, I averaged the percentage of bare ground recorded in each of the 30 vegetation quadrats (15 in early summer and 15 in late summer).

Soil Carbon

Soil carbon was originally measured as a percent of total soil dry weight. However, in order to compare the total amount of soil carbon across sites, these measurements must be adjusted for different soil densities (Hansen & Gibson 2014). Thus, the mean bulk density for each site was calculated from the three bulk density cores and used to convert measures of soil carbon to units of g/m^2, allowing comparison between sites with different soil densities (Hansen & Gibson 2014). To assess the relationships between different plant functional groups and soil carbon, plant species were first divided into six functional groups: C_3 graminoids, C_4 graminoids, woody species, nitrogen-fixing forbs, other forbs and ferns and allies (modified from Arnone et al. 2011). I summed the covers of all species in each functional group to calculate the total cover of each functional group for each quadrat from which soil was collected.

Mycorrhizal Growth Response

To assess the influence of different sources AM fungi on the biomass of *A. gerardii*, I calculated the average mycorrhizal growth response (MGR) for each inoculum source using the equation $MGR = \ln(\frac{mean\ biomass\ of\ plants\ in\ AM\ fungal\ treatment}{mean\ biomass\ of\ plants\ in\ control\ treatment})$ (Hoeksema et al. 2010; Maherali 2014). An MGR greater than zero indicates that plants grew, on average, larger in the AM fungal treatment compared to the control treatment, while an MGR less than zero indicates that plants grew smaller in the AM fungal treatment compared to the control treatment. An MGR of zero indicates that plants were the same size in the AM fungal and control treatments.

Statistical Analyses

All statistical analyses were performed in R version 3.5.3 (R Core Team 2019).

The lm function was used to run two-way analyses of variance (ANOVAs) with an interaction to determine the effects of highway, site type (control vs. restored), and their interaction on total plant species richness, native plant species richness, total and native Shannon-Wiener diversity, total and native Simpson's diversity, the proportion of vegetation cover that consisted of native species, Floristic Quality Index, overall bee genus richness and overall bee abundance (across all sampling periods). I report type II sums of squares (Anova function in the car package) (Langsrud 2003), and model significance and adjusted R squared values were obtained using the summary function. For models in which there were significant main effects, I used the contrast function (emmeans package) with a Sidak adjustment to compare the restored sites from each highway to the control sites from the same highway. This allowed me to compare sites with similar histories and geographies and determine how successful restoration was at each individual highway. Reference sites were not included in these analyses due to a lack of replication of reference sites across all highways, but diversity measures for reference sites are included in results figures for comparison to restored sites. All means presented in results figures are estimated marginal means (emmeans function from the emmeans package). Residuals were checked for normality and homoscedasticity. Native plant species richness, FQI, proportion native cover and bee abundance were transformed using the natural logarithm to improve the homoscedasticity of residuals for the models in which they were the dependent variables.

The effects of total plant species richness, native plant species richness and mean percentage of bare ground on bee genus richness and bee abundance were evaluated using separate analyses of covariance (ANCOVAs) with type II sums of squares (with the lm function and the Anova function from the car package). Separate models were constructed for each of the continuous predictors (total plant species richness, native plant species richness and bare ground), and all models included highway as an additional independent variable. Site type was included as an additional independent variable for the bare ground models but was not included in the models for total and native plant species richness because site type is essentially a manipulation of plant richness, which was already included in the models. Bee abundance was natural log-transformed to improve the homoscedasticity of residuals. The interactions between the independent variables were not included in any of the final models because they were not significant for any model. For the model that examined the relationship between bee abundance and highway and total plant richness, one point had high leverage (Cook's Distance > 0.5) but removing this point from the analysis did not affect the results substantially, and so it was included in the analysis reported here. Only data on bee genera that are known to nest in the ground (Packer et al. 2007) were included in the analyses on the relationship between bare ground and bee abundance and genus richness.

To investigate relationships among sites in terms of their plant and bee community composition, sites were clustered using Ward's agglomerative method (hclust function in stats package). Two cluster analyses were completed, the first using the vegetation data and the second using the bee data. For each analysis, a Bray-Curtis dissimilarity matrix (which considers both species presence and relative abundance) was computed from either the site-level plant species cover data or bee catch data using the vegdist function (vegan package). A ln(x+1) transformation was computed on both the plant and bee datasets prior to computing the dissimilarity matrix in order to scale the data to reduce the influence of dominant species/genera. The dissimilarity matrices were then used as input for the clustering analyses.

The effects of site (location along Highway 40), site type (restored side of the road or control side of the road) and their interaction on soil carbon in the 0-20cm soil depth, 20-40cm depth, 40-60cm depths and all depths combined (0-60cm) were analyzed using two-way ANOVAs with type II sums of squares followed by a Tukey HSD post-hoc test. Separate models were constructed for each depth, since the goal was to compare soil carbon in different sites and site types rather that to compare carbon among depths. The Chappus reference site was not included in these models because it was the only site of the 'reference' site type and so it would have prevented interactions from being assessed. However, it is included in results figures for comparison.

To assess the relationships between different plant functional groups and soil carbon in different soil depths, I constructed multiple regression models (lm function) for each soil depth, in which the soil carbon in each sample was the dependent variable and the quadrat-level cover values for each functional group were the independent variables. Soil carbon was natural log-transformed to improve the homoscedasticity of residuals. The 'ferns and allies' functional group was not included in the models because it was only present at one site. No interactions among independent variables were included in the models.

To determine whether *A. gerardii* biomass varied depending on the presence or absence of AM fungi and the source of inoculum, I used a two-way ANOVA with type III sums of squares that including the main effects of soil treatment and inoculum source as well as their interaction. Type III sums of squares were used for this analysis because of the highly significant interaction between the independent variables. I used the contrast function (emmeans package) with a Sidak adjustment to compare the biomass of plants in the AM fungal treatment to the biomass of plants in the control treatment for each inoculum source. I then used the MGR for each inoculum source as the dependent variable in an ANCOVA that included the age (time since restoration, for restored sites, or time since construction, for control sites) and site type (restored or control) of the site from which inoculum was sourced as the independent variables. This model was designed to determine the effects of restoration and time since disturbance on the growth response of *A. gerardii* to the AM fungal community. The interaction between site age and site type was not included in the final model because it was not significant. Data for plants grown with inoculum collected from the Chappus site were not included in this model because site age was irrelevant for this site since it is a remnant and therefore was not constructed or restored. Three plants that died before the end of the experiment were omitted from these analyses, though including them produced similar results (not shown).

RESULTS

Plant Community Response to Restoration

Overall plant diversity response to restoration

There were significant effects of both site type and highway on several measures of plant diversity (Table 2). The Chappus remnant prairie had high levels of diversity across all metrics, with an impressive 99 plant species, 79 of which were native, and 94% native cover (Figure 5). Highway 40 references, which were restorations conducted on conservation lands, also had high levels of diversity, though they generally did not match the remnant prairie. Restored sites generally did not reach the level of diversity found in the remnant prairie, but they tended to have higher native diversity than control sites. Restoration significantly increased total and native plant richness, native Shannon-Wiener diversity, native Simpson’s diversity, and FQI, though the difference between restored and control sites varied among highways (Tables 2 & 3; Figure 5). Highway had a stronger effect on total plant diversity compared to native plant diversity, and restored sites along the Herb Gray Parkway generally had the highest plant diversity, while restored sites along Highway 407 had the lowest plant diversity (Figure 5; Table 2). The exception to this pattern was in Floristic Quality Index, which was highest for Highway 40 sites and lowest for Highway 407 sites (Figure 5H). The effects of both site type and highway varied depending on the diversity metric. In general, the significance of site type and highway effects decreased as the weighting of dominant species in diversity metrics increased. For example, highway had a marginally significant effect on total species richness and Shannon-Wiener diversity, but no effect on Simpson's diversity.

Plant diversity response to restoration at Highway 40

Highway 40 restored sites had equivalent native plant richness to two nearby reference sites, and were similar to the references in other metrics as well (Figure 5B,D,F). Furthermore, restored sites had significantly increased native plant species richness and FQI compared to control sites. Restored sites had 38 native species and an FQI of 14.8 on average, compared to 17 native species and an FQI of 3.0 in control sites. All other plant diversity metrics were also higher on average in restored sites compared to control sites, though the differences between restored and control sites were not significant. Restored and control sites were more similar in plant diversity metrics that included non-native species compared to metrics that only considered native species.

Plant diversity response to restoration at the Herb Gray Parkway

Restored sites along the Herb Gray Parkway were similar to the nearby Chappus reference site in measures of diversity that included non-native species, but they did not achieve the native diversity of the reference site. Restored sites contained an average of 44 native species and had an average FQI of 11.3, while Chappus contained 79 native species and had an FQI of 26.2 (Figure 5B,H). However, Herb Gray Parkway restorations showed the greatest improvement in plant diversity over control sites compared to the other two highways. Herb Gray Parkway restored sites had significantly greater total plant species richness, native richness, native Shannon-Wiener diversity and FQI compared to nearby control sites (Tables 2 & 3; Figure 5A,B,D,H), and consistently had higher diversity than the control sites for all other metrics, though the differences were not significant. Notably, restored sites had almost three times as many native species as control sites (Figure 5B).

Plant diversity response to restoration at Highway 407

Unlike the other highways that showed substantive increases in diversity in restoration sites, the Highway 407 restored and control sites had similar plant diversity (Figure 5). Highway 407 restored sites were not significantly different from the control sites in any metric of plant diversity and had an average of only 7 more native species compared to control sites (Table 3; Figure 5B). In fact, restored sites had slightly lower average diversity than control sites for some metrics, including the proportion of native plant cover (Figure 5C,E,G).

Response of plant community composition to restoration

In reference sites, the majority of dominant species were native and had moderate to high coefficient of conservatism scores (Table 4). In contrast, control sites were dominated by mostly non-native species plus a few disturbance-tolerant native species. Restored sites at Highway 40 and the Herb Gray Parkway were intermediate between control and reference sites in their dominant species composition. They contained several high-quality native species, but also some non-native, invasive species that dominated control sites. However, Highway 407 restored sites were dominated by non-native species and only one out of the top ten most abundant species was native.

In the cluster analysis on plant community composition data, all of the Herb Gray Parkway restorations and the two newer Highway 40 restorations (Bickford and Bentpath) clustered more closely with the reference sites than the control sites (Figure 6). The two oldest Highway 40 restoration sites (Whitebread and Courtright) clustered with the control sites from Highway 40 and the Herb Gray Parkway. In contrast, Highway 407 sites did not separate into clusters based on site types, and instead formed a single cluster that was distant from the sites at the other two highways.

Table 2. Analyses of variance for the effects of highway, site type and their interaction on plant community diversity metrics. F and p values are reported for type II sums of squares. Diversity metrics were calculated from species cover data collected during two vegetation surveys conducted during the 2019 growing season. Reference sites were not included in the analyses, therefore n = 20. H = Shannon-Wiener diversity, S = Simpson's diversity, FQI = Floristic Quality Index. Native richness, proportion native cover and FQI were natural log-transformed to meet model assumptions. P values less than 0.05 are bolded. Adjusted R^2 values for the models, in the order they are presented below, are as follows: 0.46, 0.43, 0.27, 0.30, 0.10, 0.17, 0.20 and 0.74.

Metric	Factor	df	F	P value
Total richness	Model	5,14	4.23	**0.0149**
	Highway	2	3.11	0.0765
	Type	1	8.91	**0.0098**
	Highway x Type	2	3.01	0.0818
	Residuals	14		
ln(Native richness)	Model	5,14	5.77	**0.0043**
	Highway	2	0.14	0.8719
	Type	1	21.49	**0.0004**
	Highway x Type	2	3.56	0.0561
	Residuals	14		
$H_{all\ species}$	Model	5,14	2.42	0.0884
	Highway	2	3.03	0.0804
	Type	1	2.10	0.1694
	Highway x Type	2	1.89	0.1874
	Residuals	14		
$H_{native\ species}$	Model	5,14	2.62	0.0710
	Highway	2	1.18	0.3361
	Type	1	8.45	**0.0115**
	Highway x Type	2	0.99	0.3972
	Residuals	14		
$S_{all\ species}$	Model	5,14	1.42	0.2273
	Highway	2	1.99	0.1730
	Type	1	0.30	0.5902
	Highway x Type	2	1.36	0.2893
	Residuals	14		
$S_{native\ species}$	Model	5,14	1.79	0.1793
	Highway	2	1.07	0.3709
	Type	1	5.06	**0.0410**
	Highway x Type	2	0.73	0.4979
	Residuals	14		
ln(Proportion native cover)	Model	5,14	1.95	0.1500
	Highway	2	1.86	0.1921
	Type	1	3.23	0.0937
	Highway x Type	2	1.38	0.2828
	Residuals	14		

ln(FQI)	Model	5,14	12.05	**0.0001**
	Highway	2	14.38	**0.0004**
	Type	1	26.59	**0.0001**
	Highway x Type	2	1.26	0.3153
	Residuals	14		

Table 3. Pre-planned contrasts between restored and control roadside sites for several plant community diversity metrics. For each metric, the estimates represent the restored-control site contrasts for each individual highway. Diversity metrics were calculated from species cover data collected during two vegetation surveys conducted during the 2019 growing season, and the effects of highway and site type on the diversity metrics were first investigated using analyses of variance. Native richness, proportion native cover and FQI were natural log-transformed to meet model assumptions, so estimates and standard errors for these contrasts are reported on the log scale. df = 14 for each contrast, and a Sidak adjustment for 3 tests was performed for each model. P values less than 0.05 are bolded. 40 = Highway 40, HG = Herb Gray Parkway, 407 = Highway 407 East Extension.

Metric	Highway	Estimate	SE	t ratio	P value
Total richness	40	20.25	10.94	1.852	0.2346
	HG	37.00	10.94	3.384	**0.0133**
	407	2.13	9.22	0.231	0.9942
ln(Native richness)	40	0.82	0.26	3.102	**0.0232**
	HG	1.11	0.26	4.236	**0.0025**
	407	0.23	0.22	1.028	0.6876
$H_{all\ species}$	40	0.19	0.31	0.620	0.9058
	HG	0.72	0.31	2.333	0.1016
	407	-0.06	0.26	-0.236	0.9939
$H_{native\ species}$	40	0.44	0.45	0.985	0.7144
	HG	1.22	0.45	2.750	**0.0462**
	407	0.52	0.38	1.374	0.4708
$S_{all\ species}$	40	0.02	0.06	0.420	0.9675
	HG	0.09	0.06	1.503	0.3968
	407	-0.04	0.05	-0.763	0.8407
$S_{native\ species}$	40	0.06	0.14	0.452	0.9600
	HG	0.30	0.14	2.147	0.1422
	407	0.15	0.12	1.311	0.5089
ln(Proportion native cover)	40	0.59	0.33	1.807	0.2521
	HG	0.54	0.33	1.650	0.3212
	407	-0.03	0.28	-0.117	0.9992
ln(FQI)	40	1.60	0.59	2.726	**0.0484**
	HG	2.36	0.59	4.032	**0.0037**
	407	1.15	0.49	2.327	0.1028

Table 4. Ten most abundant species at restored, control and reference sites associated with three Southern Ontario highways. Cover refers to species' percent cover in 1x1m quadrats, assessed during two vegetation surveys in 2019. Species' cover values were averaged across all sites of the same type at each highway. CC = Coefficient of Conservatism, as assigned by Oldham et al. (1995). Species native to Ontario are bolded.

Highway 40								
Control			Restored			Reference		
Species	Cover	CC	Species	Cover	CC	Species	Cover	CC
Lolium arundinaceum	31.7	0	*Festuca rubra*	40.4	0	***Symphyotrichum praealtum***	32.9	8
Carex atherodes	31.0	6	***Solidago canadensis***	23.3	1	*Lotus corniculatus*	23.3	0
Festuca rubra	20.9	0	***Andropogon gerardii***	13.6	7	*Dipsacus fullonum*	17.1	0
Lathyrus tuberosus	8.9	0	*Lathyrus tuberosus*	12.7	0	*Poa pratensis*	13.1	0
Bromus inermis	7.3	0	*Bromus inermis*	8.7	0	***Solidago canadensis***	9.3	1
Dipsacus fullonum	6.9	0	***Solidago rigida***	8.3	7	***Solidago rigida***	7.7	7
Solidago canadensis	6.1	1	***Ratibida pinnata***	7.5	9	***Symphyotrichum ericoides***	6.8	4
Cirsium arvense	4.9	0	***Sorghastrum nutans***	5.3	8	***Andropogon gerardii***	6.5	7
Elymus repens	3.8	0	***Penstemon digitalis***	5.2	6	***Penstemon digitalis***	6.2	6
Parthenocissus vitacea	3.6	4	*Lotus corniculatus*	5.1	0	***Elymus canadensis***	5.4	8

Herb Gray Parkway								
Control			Restored			Reference		
Species	Cover	CC	Species	Cover	CC	Species	Cover	CC
Lolium arundinaceum	38.1	0	*Lotus corniculatus*	11.8	0	***Solidago canadensis***	32.4	1
Poa pratensis	19.7	0	*Poa pratensis*	11.7	0	***Rubus allegheniensis***	10.3	2
Elymus repens	18.3	0	*Cirsium arvense*	10	0	***Schizachyrium scoparium***	7.7	7
Apocynum cannabinum	10.8	3	*Securigera varia*	9.5	0	***Solidago rugosa***	6.6	4
Dipsacus fullonum	8.8	0	***Solidago canadensis***	6.0	1	***Pycnanthemum virginianum***	5.8	6
Lathyrus latifolius	6.4	0	***Sorghastrum nutans***	5.5	8	***Euthamia graminifolia***	5.1	2
Vicia cracca	5.9	0	***Rhus aromatica***	4.5	8	***Desmodium canadense***	3.6	5
Cornus racemosa	5.0	2	*Trifolium pratense*	3.6	0	***Fragaria virginiana***	3.1	2
Eleagnus angustifolia	4.2	0	***Elymus canadensis***	3.3	8	***Monarda fistulosa***	2.5	6
Bromus inermis	2.9	0	***Solidago rigida***	2.8	7	***Onoclea sensibilis***	2.5	4

Highway 407

Control			Restored		
Dominant Species	Cover	CC	Dominant Species	Cover	CC
Festuca rubra	40.8	0	*Festuca rubra*	44.0	0
Vicia cracca	17.9	0	*Agrostis gigantea*	14.3	0
Poa compressa	10.7	0	***Solidago canadensis***	11.0	1
Solidago canadensis	9.8	1	*Phalaris arundinacea*	10.5	0
Trifolium pratense	7.6	0	*Lotus corniculatus*	6.2	0
Poa pratensis	6.6	0	*Poa pratensis*	5.7	0
Cirsium arvense	6.1	0	*Vicia cracca*	5.1	0
Melilotus albus	4.9	0	*Trifolium pratense*	4.8	0
Dactylis glomerata	4.5	0	*Puccinellia distance*	4.2	0
Medicago lupulina	3.5	0	*Elymus repens*	4.2	0

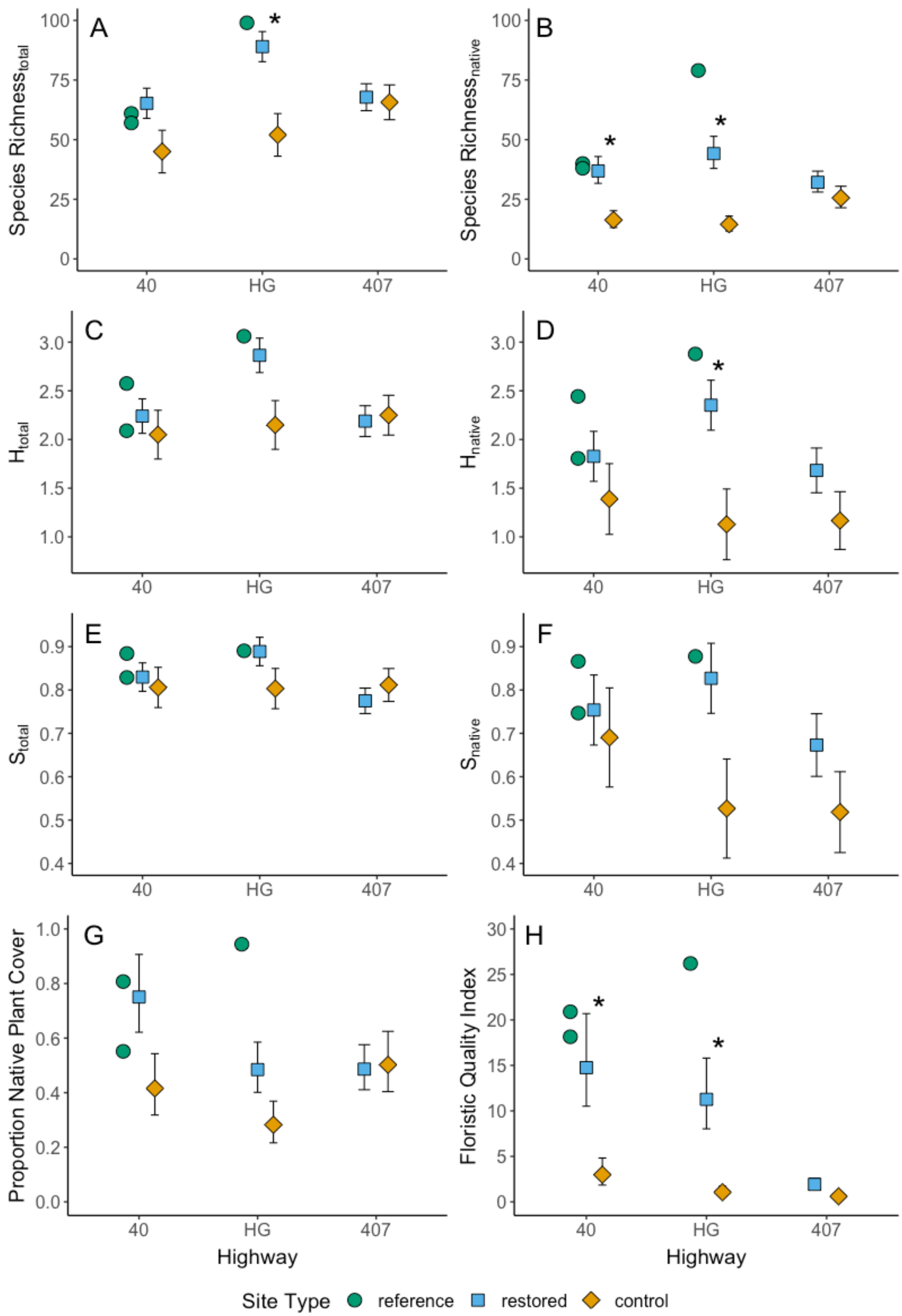
A
B
C
D
E
F
G
H
Species Richness$_{total}$
Species Richness$_{native}$
H_{total}
H_{native}
S_{total}
S_{native}
Proportion Native Plant Cover
Floristic Quality Index
40
HG
407
Highway
Site Type
reference
restored
control

Figure 5. Plant diversity metrics of restored and control roadsides along three Southern Ontario highways (40 = Highway 40, HG = Herb Gray Parkway, 407 = Highway 407 East Extension). Data is shown as the mean (±SE) for replicate sites within each highway × site type combination. Reference diversity estimates from nearby high-quality prairie sites are provided for comparison. A) total plant species richness, B) native plant species richness, C) Shannon-Wiener diversity, D) Shannon-Wiener diversity of native species, E) Simpson's diversity, F) Simpson's diversity of native species, G) proportion native plant cover and H) Floristic Quality Index. Diversity metrics were calculated from species cover data collected during two vegetation surveys conducted during the 2019 growing season. Means are from estimated marginal means calculated for each model, $n = 20$. Stars indicate that restored and control sites associated with a particular highway differed significantly from each other ($p < 0.05$) in a pre-planned contrast analysis. Herb Gray Parkway and Highway 40 restorations generally had higher native plant diversity than control sites, while Highway 407 restorations were similar in diversity to control sites.

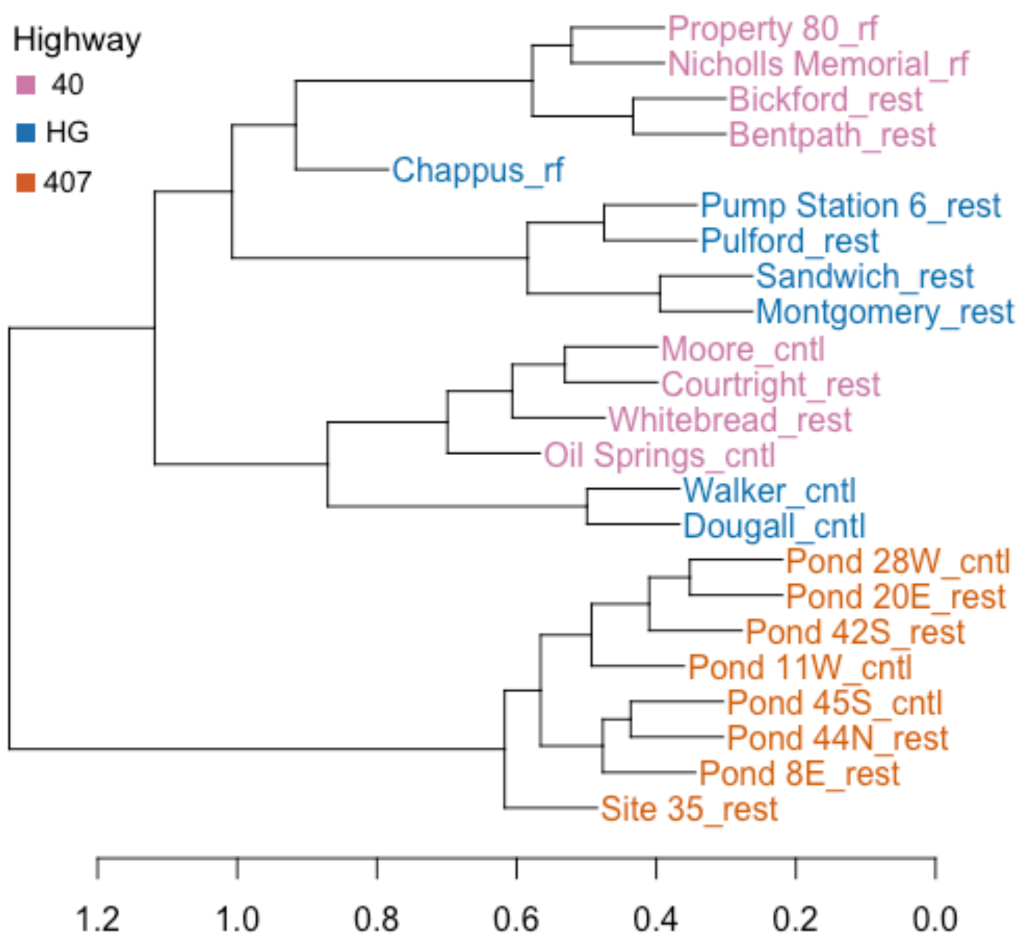

Figure 6. Clustering of restored, control and reference sites at three Southern Ontario highways based on plant community composition. The cluster tree was produced from a hierarchical cluster analysis using Ward's agglomerative method. Sites were clustered based on a Bray-Curtis dissimilarity matrix calculated using natural log-transformed plant species abundance data. Abundance was measured as species cover, which was assessed during two vegetation surveys conducted over the 2019 growing season. Herb Gray Parkway restorations and some Highway 40 restorations clustered more closely with reference sites than control sites, while Highway 407 sites formed a distinct cluster and did not separate out by site type. The scale indicates the distance between clusters. Site names ending in 'rf' are reference sites, while the suffix 'rest' indicates restored sites and 'cntl' indicates control sites. 40 = Highway 40, HG = Herb Gray Parkway, 407 = Highway 407 East Extension.

Bee Community Response to Restoration and Highway Differences

Overall response of bee diversity and abundance to restoration

Bee genus richness and bee abundance varied more among highways than among site types (Table 5; Figure 7). Highway 407 sites had significantly higher bee genus richness and abundance than Highway 40 and Herb Gray Parkway sites (Tables 5 & 6; Figure 7). The bee genus richness of Highway 407 sites was 1.8x that of Highway 40 roadside sites and 1.5x that of Herb Gray Parkway roadside sites. The bee abundance of Highway 407 sites was 2.7x that of Highway 40 roadside sites and 3.1x that of Herb Gray Parkway roadside sites (Figure 7). Overall, restored sites had greater bee abundance than control sites, but the difference in bee abundance between restored and control sites depended on the highway (Table 5; Figure 7). Restoration did not have a significant effect on bee genus richness.

Response of bee abundance and diversity to restoration at Highway 40

Restored sites along Highway 40 had similar bee genus richness and abundance compared to one of the nearby reference sites, but had lower bee genus richness and abundance than the other nearby reference site. As expected, restored sites had greater average bee genus richness and bee abundance than control sites, but differences between restored and control sites were not significant (Table 6; Figure 7).

Response of bee abundance and diversity to restoration at the Herb Gray Parkway

Restored sites along the Herb Gray Parkway did not contain as many bee genera as the Chappus reference site, having an average of 11 genera compared to the 15 genera found at Chappus (Figure 7A). However, restored sites had greater average bee abundance than Chappus (Figure 7B). As expected, restored sites had greater bee genus richness and abundance than control sites, though only the difference in abundance was significant (Table 6; Figure 7). Bee abundance was over five times higher in restored sites compared to control sites (Figure 7B).

Response of bee diversity and abundance to restoration at Highway 407

While there were no reference sites near the Highway 407, bee diversity and abundance at Highway 407 sites was similar to or higher than that of the reference sites located near Highway 40 and the Herb Gray Parkway (Figure 7). However, Highway 407 restored sites did not differ significantly from control sites in their bee genus richness or abundance (Table 6). In fact, bee genus richness and abundance in restored sites were, on average, lower than in control sites (Figure 7).

Response of bee community composition to restoration

In the cluster analysis, sites appeared to cluster mainly by highway, as most sites from the same highway were found in the same cluster (Figure 8). However, as expected, Herb Gray Parkway restored sites clustered more closely with the Chappus reference site than with the control sites. Three out of the four Highway 40 restorations also clustered most closely with a reference site. Most Highway 407 sites formed a single cluster regardless of site type. However, two of the Highway 407 sites clustered with the Herb Gray Parkway restored and reference sites.

Effects of plant diversity and bare ground on bee genus diversity and abundance

The diversity of bee genera sampled at each site was not correlated with the site's total (both native and non-native) plant species richness but was positively correlated (slope = 0.06752) with the site's native plant species richness, though this correlation was only marginally significant (Table 7; Figure 9A,B). The abundance of bees caught over the sampling season was significantly positively correlated with both total and native plant species richness (slope = 0.0231 and 0.0181 on the log scale for total and native plant richness, respectively;

Figure 9C,D). The percentage of bare ground available at a site was significantly positively correlated with the abundance of ground-nesting bees (slope = 0.08349 on the log scale) but not the genus richness of ground-nesting bees (Table 7; Figure 10).

Table 5. Analyses of variance for the effects of site type, highway and their interaction on the total bee genus richness and bee abundance. F and p values are reported for type II sums of squares. Bee abundance was natural log-transformed to meet model assumptions. Although bees were sampled up to ten times at each site from May to September, data from May (two samples) and late June (one sample) were not included in the calculations of richness and abundance because sampling was not completed for all highways during those periods. Reference sites were not included in the analysis. n = 20. P values less than 0.05 are bolded. The adjusted R^2 for the model containing bee genus richness is 0.53 while the adjusted R^2 for the model containing bee abundance is 0.70.

Dependent variable	Factor	df	F	P value
Bee genus richness	Model	5,14	5.26	**0.0063**
	Highway	2	12.75	**0.0007**
	Type	1	0.16	0.6993
	Highway x Type	2	0.39	0.6810
	Residuals	14		
ln(Bee abundance)	Model	5,14	9.83	**0.0003**
	Highway	2	12.20	**0.0009**
	Type	1	5.05	**0.0412**
	Highway x Type	2	10.27	**0.0018**
	Residuals	14		

Table 6. Pre-planned contrasts of bee genus richness and bee abundance between restored and control roadside sites along three Southern Ontario highways (40 = Highway 40, HG = Herb Gray Parkway, 407 = Highway 407 East Extensions). For each metric, estimates represent the restored-control site contrasts for each individual highway. Bee abundance was natural log-transformed to meet model assumptions, so estimates and standard errors for bee abundance contrasts are reported on the log scale. df = 14 for each contrast, and a Sidak adjustment for 3 tests was performed for each model. P values less than 0.05 are bolded.

Dependent variable	Highway	Estimate	SE	t ratio	P value
Bee genus richness	40	1.50	2.23	0.672	0.8842
	HG	1.25	2.23	0.560	0.9282
	407	-0.80	1.88	-0.425	0.9664
ln(Bee abundance)	40	0.60	0.38	1.581	0.3557
	HG	1.71	0.38	4.517	**0.0015**
	407	-0.52	0.32	-1.643	0.3247

Table 7. Analyses of covariance for the effects of highway, plant diversity and bare ground on bee genus richness and abundance. F and p values are reported for type II sums of squares. Interaction terms were dropped from the models because none were significant. Bee abundance was natural log-transformed to improve homoscedasticity of residuals. Although bees were sampled up to ten times at each site from May to September, data from May (two samples) and late June (one sample) were not included in the calculations of richness and abundance because sampling was not completed for all highways during those periods. For analyses that included bare ground as an independent variable, only genera that are known to at least sometimes nest in the ground were included in calculations of abundance and richness. N=23. P values less than 0.05 are bolded. The adjusted R^2 values for the models, in the order that they are presented below, are: 0.57, 0.60, 0.54, 0.29, 0.40, 0.59.

Dependent variable	Independent variables	Factor	df	F	P value
Bee genus richness	Highway + Plant richness	Model	3,19	10.83	**0.0002**
		Highway	2	14.36	**0.0002**
		Plant richness	1	2.80	0.1106
		Residuals	19		
	Highway + Native plant richness	Model	3,19	12.03	**0.0001**
		Highway	2	17.04	**0.0001**
		Native plant richness	1	4.32	0.0514
		Residuals	19		
	Highway + Type + mean bare ground cover	Model	5,17	7.93	**0.0005**
		Highway	2	14.61	**<0.0001**
		Type	2	1.35	0.2915
		Bare ground cover	1	1.49	0.0940
		Residuals	17		
ln(Bee abundance)	Highway + Plant richness	Model	3,19	8.20	**0.0011**
		Highway	2	10.52	**0.0008**
		Plant richness	1	9.19	**0.0069**
		Residuals	19		
	Highway + Native plant richness	Model	3,19	5.83	**0.0053**
		Highway	2	7.96	**0.0031**
		Native plant richness	1	4.60	**0.0450**
		Residuals	19		
	Highway + Type + mean bare ground cover	Model	5,17	6.69	**0.0013**
		Highway	2	11.63	**0.0007**

	Type	2	0.49	0.6184
	Bare ground cover	1	5.91	**0.0265**
	Residuals	17		

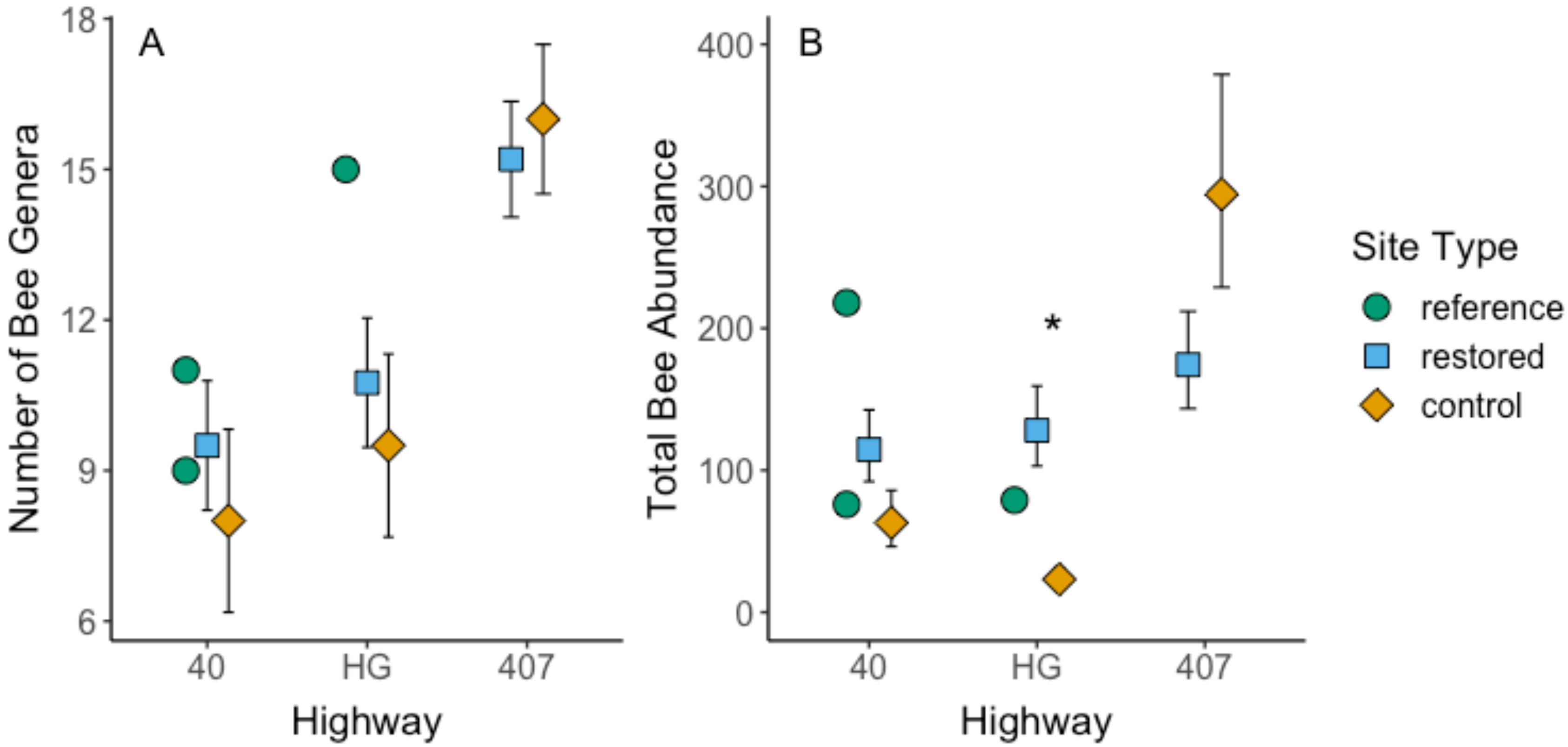

Figure 7. Bee genus richness (A) and abundance (B) in restored and control roadsides along three Southern Ontario highways (40 = Highway 40, HG = Herb Gray Parkway, 407 = Highway 407 East Extension). Data is shown as the mean (±SE) for replicate sites within each highway × site type combination. Reference bee genus richness and abundance estimates from nearby high-quality prairie sites are provided for comparison. Bees were collected across seven sampling periods from June to September 2019. Means are from estimated marginal means calculated for each model, n=20. Stars indicate that restored and control sites associated with a particular highway differed significantly from each other ($p < 0.05$) in a pre-planned contrast analysis. Highway 407 sites had greater bee genus richness and abundance compared to sites along the other two highways. However, restoration only increased bee abundance and, to a lesser extent, bee genus richness, at Highway 40 and the Herb Gray Parkway.

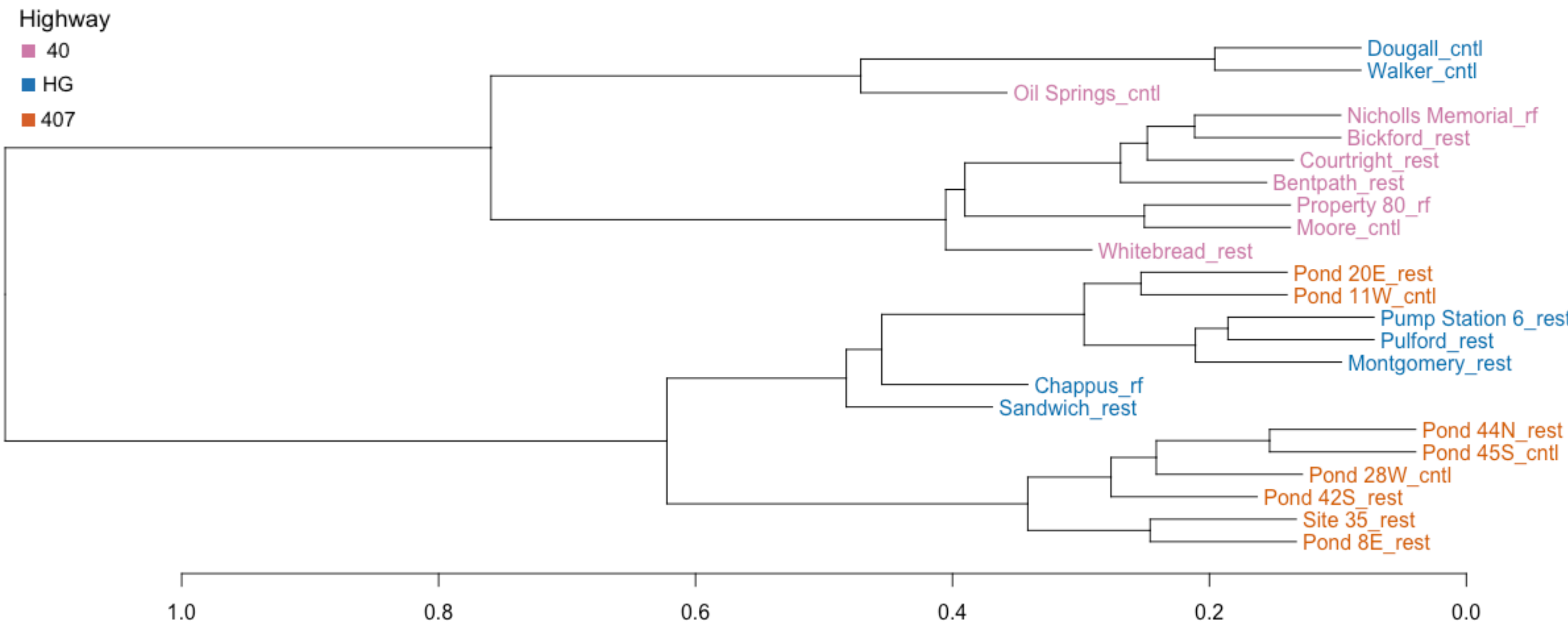

Figure 8. Clustering of restored, control and reference sites at three Southern Ontario highways based on bee community composition. The cluster tree was produced from a hierarchical cluster analysis using Ward's agglomerative method. Sites were clustered based on a Bray-Curtis dissimilarity matrix calculated using natural log-transformed bee genus abundance data. Bee abundance data was summarized from 7 samples collected from June to September 2019. Site names ending in 'rf' are reference sites, while the suffix 'rest' indicates restored sites and 'cntl' indicates control sites. 40 = Highway 40, HG = Herb Gray Parkway, 407 = Highway 407 East Extension. Sites clustered mainly according to highway, with some separation of site types as well (e.g. Herb Gray Parkway sites).

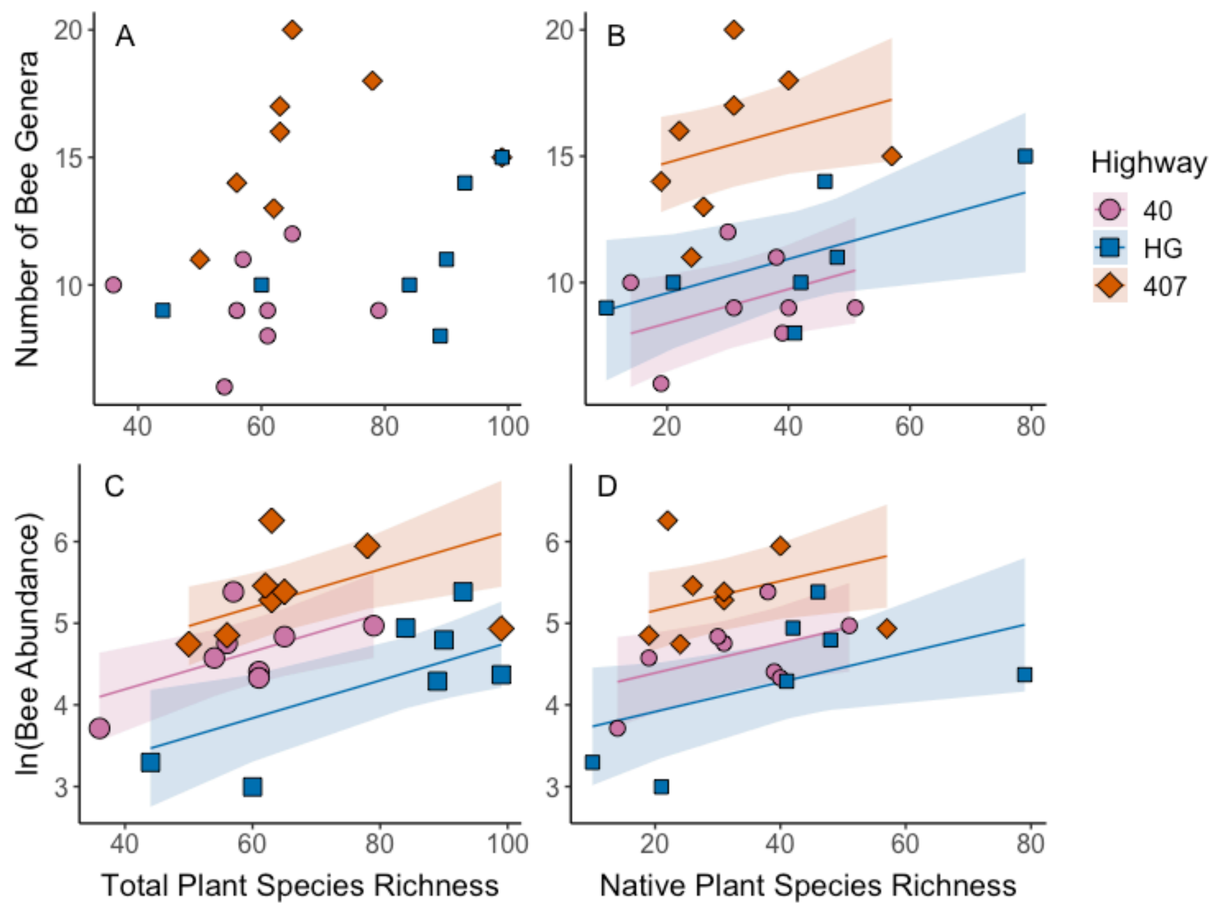

Figure 9. Bee genus richness (A,B) and bee abundance (C,D) vs. total (A,C) and native (B,D) plant species richness in study sites along three Southern Ontario highways (40 = Highway 40, HG = Herb Gray Parkway, 407 = Highway 407 East Extension). Bee genus richness and abundance were totalled across 7 samples collected from June to September 2019 in 13 restored roadside sites, 7 control roadside sites and 3 reference sites. Plant species richness was assessed during two vegetation surveys conducted in June/July and September 2019. Regression lines are included for models in which P ≤ 0.05 for the effect of plant species richness. Same slopes models were used because the interaction between highway and plant species richness was not significant for any model. Shaded areas represent 95% confidence intervals around each slope. Native plant species richness was positively correlated with both bee genus richness and abundance, while total plant species richness was only positively correlated with bee abundance.

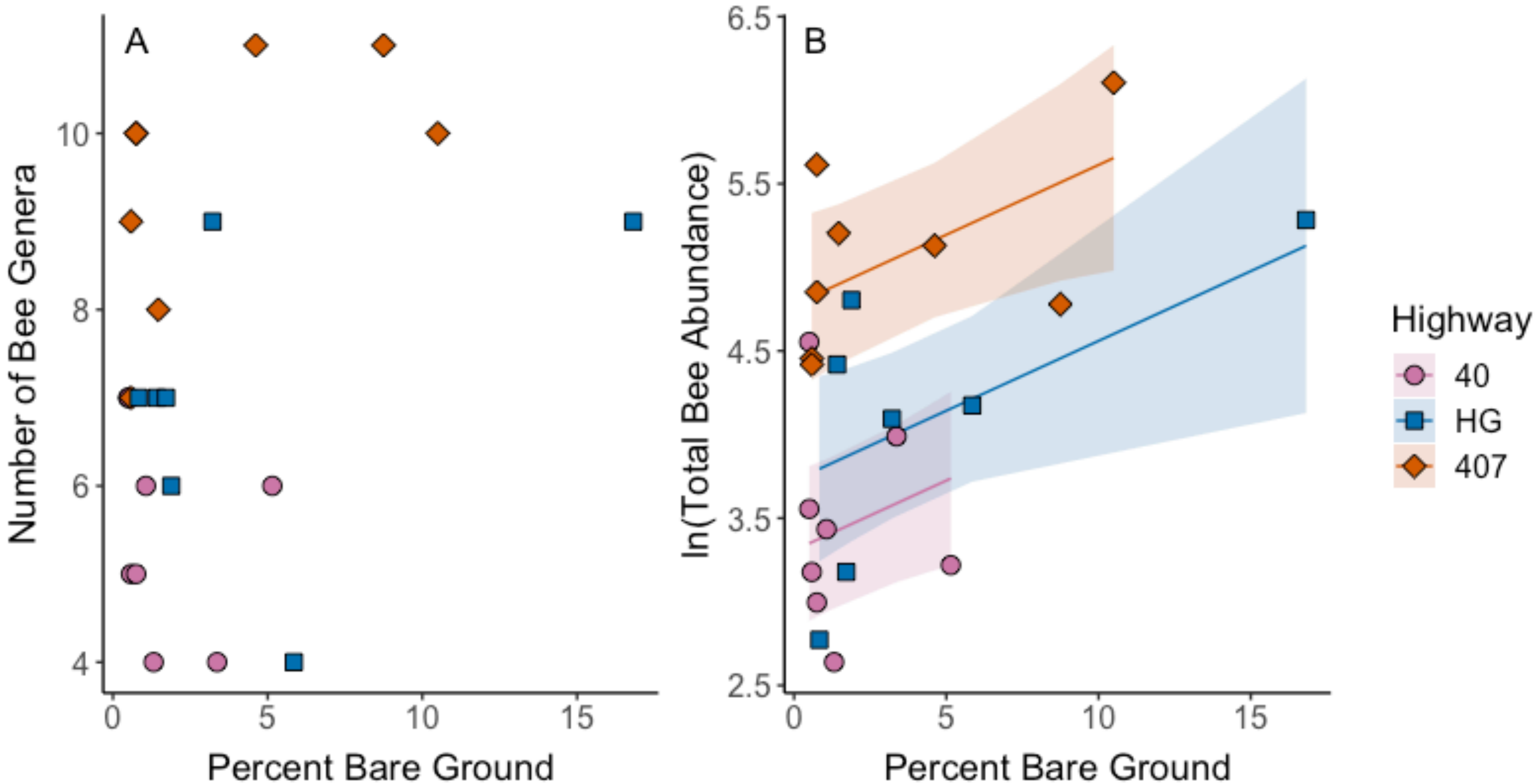

Figure 10. Bee genus richness (A) and abundance (B) vs. average percentage of bare ground in study sites along three Southern Ontario highways (40 = Highway 40, HG = Herb Gray Parkway, 407 = Highway 407 East Extension). Bee genus richness and abundance were totalled across 7 samples collected from June to September 2019 in 13 restored roadsides, 7 control roadsides and 3 reference sites. Bare ground was assessed during two vegetation surveys conducted in June/July and September 2019. Regression lines are not included for the model containing bee genus richness because the effect of bare ground was not significant. Same slopes models were used because the interaction between highway and bare ground was not significant for either model. Shaded areas represent 95% confidence intervals around each slope. Bare ground was positively correlated with bee abundance but was not correlated with bee genus richness.

Soil Carbon Storage

Effects of site and site type on soil carbon

Most sites and site types had similar amounts of soil carbon regardless of the depth (Figure 6), and similar or higher soil carbon levels compared to the Chappus reference site. While site, site type and their interaction had highly significant effects on soil carbon at all depths except the 0-20cm depth (Table 8), these effects were mostly driven by the high amounts of soil carbon present at the Courtright control site (Figure 11). Courtright was the only site in which the paired restored and control sites differed from one another, and the Courtright control site contained an average of 2.6 times more carbon in the top 60cm of soil than the other roadside sites (Figure 11D). In the 40-60cm depth, the Bentpath restored site (established in 2010) also contained significantly more carbon than the Whitebread and Courtright restored sites (established in 2002 and 2004, respectively) (Figure 11C). Notably, when the Courtright control site was dropped from the analysis, the direction of the restoration effect changed for the 40-60cm depth, and restored sites contained more soil carbon on average than the remaining control sites (not shown).

Relationships between soil carbon and plant functional groups

Soil carbon in the 0-20cm depth was significantly positively correlated with the cover of nitrogen fixing forbs and woody plants (Table 9), with woody plants showing a slightly more positive correlation (slope = 0.009389 on log scale for N-fixing plants and 0.013408 on log scale for woody plants; Figure 12A,B). Correlations between soil carbon and plant functional group cover decreased with soil depth (Table 9). In the 20-40cm depth, woody plant cover was not significantly correlated with soil carbon and nitrogen-fixing forb cover was only marginally significantly correlated with soil carbon (Table 9; Figure 12C,D). There were no significant relationships between soil carbon and the cover of any plant functional groups in the 40-60cm depth. When soil carbon amounts were combined for all three soil depths, only the cover of nitrogen-fixing forbs was significantly and positively correlated with soil carbon (slope = 0.011130 on the log scale, Table 7). However, when one data point with high leverage was removed from the analyses, the effect of woody species increased in significance while the effect of nitrogen-fixing species decreased.

Table 8. Analyses of variance for the effects of site, site type (restored or control) and their interaction on total soil carbon (g/m^2) in different soil depths. F and p values are reported for type II sums of squares. Soil samples were collected from restored and control roadside sites along Highway 40 in Lambton County, Ontario in September 2019. Carbon levels were adjusted for the soil bulk density of each site. Samples from the reference site were not included in the analyses. n = 48 for the analyses on the 0-20cm and 20-40cm depths and n = 46 for the analyses on the 40-60cm and combined depths. P values less than 0.05 are bolded. The adjusted R^2 values for the models, in the order that they are presented below, are: 0.15, 0.79, 0.77, 0.83.

Depth	Factor	df	F	P value
0-20cm	Model	7, 40	2.23	0.0518
	Type	1	0.08	0.7724
	Site	3	2.67	0.0608
	Type x Site	3	2.51	0.0725
	Residuals	40		
20-40cm	Model	7, 40	26.51	**<0.0001**
	Type	1	24.66	**<0.0001**
	Site	3	22.64	**<0.0001**
	Type x Site	3	30.99	**<0.0001**
	Residuals	40		
40-60cm	Model	7, 38	22.34	**<0.0001**
	Type	1	5.32	**0.0267**
	Site	3	17.38	**<0.0001**
	Type x Site	3	32.12	**<0.0001**
	Residuals	38		
Total (0-60cm)	Model	7, 38	33.39	**<0.0001**
	Type	1	12.60	**0.0010**
	Site	3	28.63	**<0.0001**
	Type x Site	3	43.11	**<0.0001**
	Residuals	38		

Table 9. Multiple regression analysis of the effects of plant functional group abundance on total soil carbon in different soil depths. The functional groups included in the models were C_3 graminoids, C_4 graminoids, nitrogen fixing forbs, other forbs and woody plant species. F and p values are reported for type II sums of squares. No interactions among independent variables were included and soil carbon was natural log-transformed to meet model assumptions. Soil samples were collected from restored and control roadside sites along Highway 40 plus a remnant prairie in September 2019. Carbon levels were adjusted for the soil bulk density of each site. The cover of species in each functional group was assessed in a 1x1m quadrat centered around each soil coring location. N = 54 for the analyses on the 0-20cm and 20-40cm depths and n = 52 for the analyses on the 40-60cm and combined depths. P values less than 0.05 are bolded. The adjusted R^2 values for the models, in the order that they are presented below, are: 0.08, 0.18, 0.04, 0.15.

Depth	Factor	df	F	P value
0-20cm	Model	5,48	1.94	0.1043
	C3 graminoids	1	1.47	0.2306
	C4 graminoids	1	1.34	0.2525
	Forbs	1	1.26	0.2680
	N fixing forbs	1	5.81	**0.0198**
	Woody	1	4.41	**0.0409**
	Residuals	48		
20-40cm	Model	5,48	3.32	**0.0117**
	C3 graminoids	1	< 0.01	0.9653
	C4 graminoids	1	2.13	0.1505
	Forbs	1	0.31	0.5827
	N fixing forbs	1	3.25	0.0777
	Woody	1	2.50	0.1207
	Residuals	48		
40-60cm	Model	5,46	1.46	0.2224
	C3 graminoids	1	0.71	0.4042
	C4 graminoids	1	0.83	0.3676
	Forbs	1	1.88	0.1766
	N fixing forbs	1	2.16	0.1487
	Woody	1	0.57	0.4546
	Residuals	46		
All Depths	Model	5,46	2.78	**0.0280**
	C3 graminoids	1	0.29	0.5954
	C4 graminoids	1	1.44	0.2370
	Forbs	1	0.94	0.3373
	N fixing forbs	1	4.65	**0.0363**
	Woody	1	0.79	0.3775
	Residuals	46		

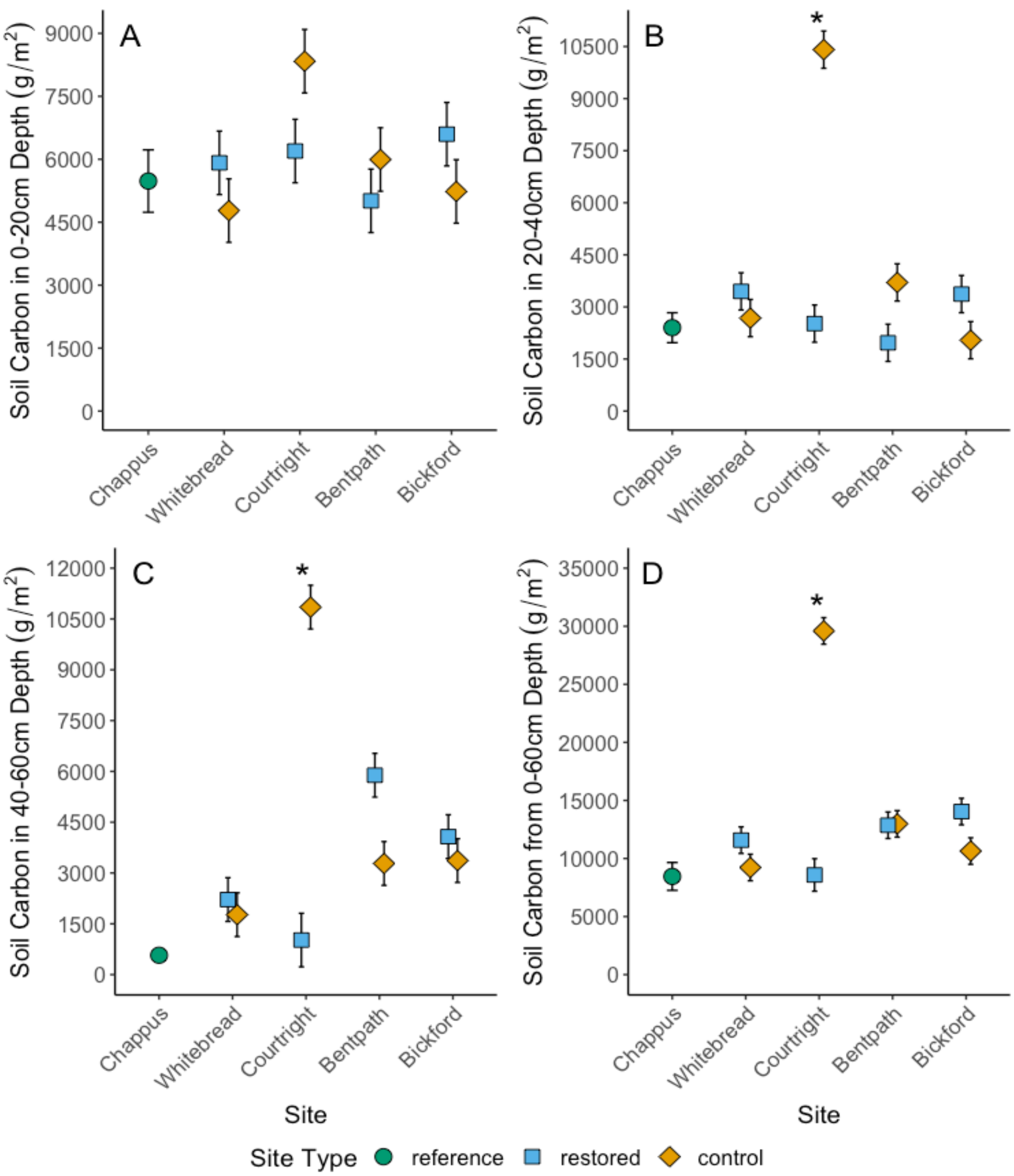

Figure 11. Total soil carbon in different soil depths at restored and control roadsides along Highway 40 in Lambton County, Ontario. Data shown as mean (±SE) for replicate samples within each site × site type combination. Soil carbon estimates from Chappus, a remnant prairie in Essex County, are provided for comparison. A) 0-20cm soil depth, B) 20-40cm depth, C) 40-60cm depth and D) all depths combined (0-60cm). Soil samples were collected from each site in September 2019 and carbon levels were adjusted for soil bulk density. Means are from estimated marginal means calculated for each model. n=6 for each site x type combination except for the Courtright restored site, for which n = 4 for the 40-60cm and combined depths. A star indicates that the paired restored and control site differed significantly from each other ($p < 0.05$). Sites are ordered by restoration age (Chappus = remnant, Whitebread = 17 years, Courtright = 14 years, Bentpath and Bickford = 10 years). Most sites contained similar levels of soil carbon, with the exception of the Courtright control site, which had significantly higher carbon levels compared to all other site × site type combinations.

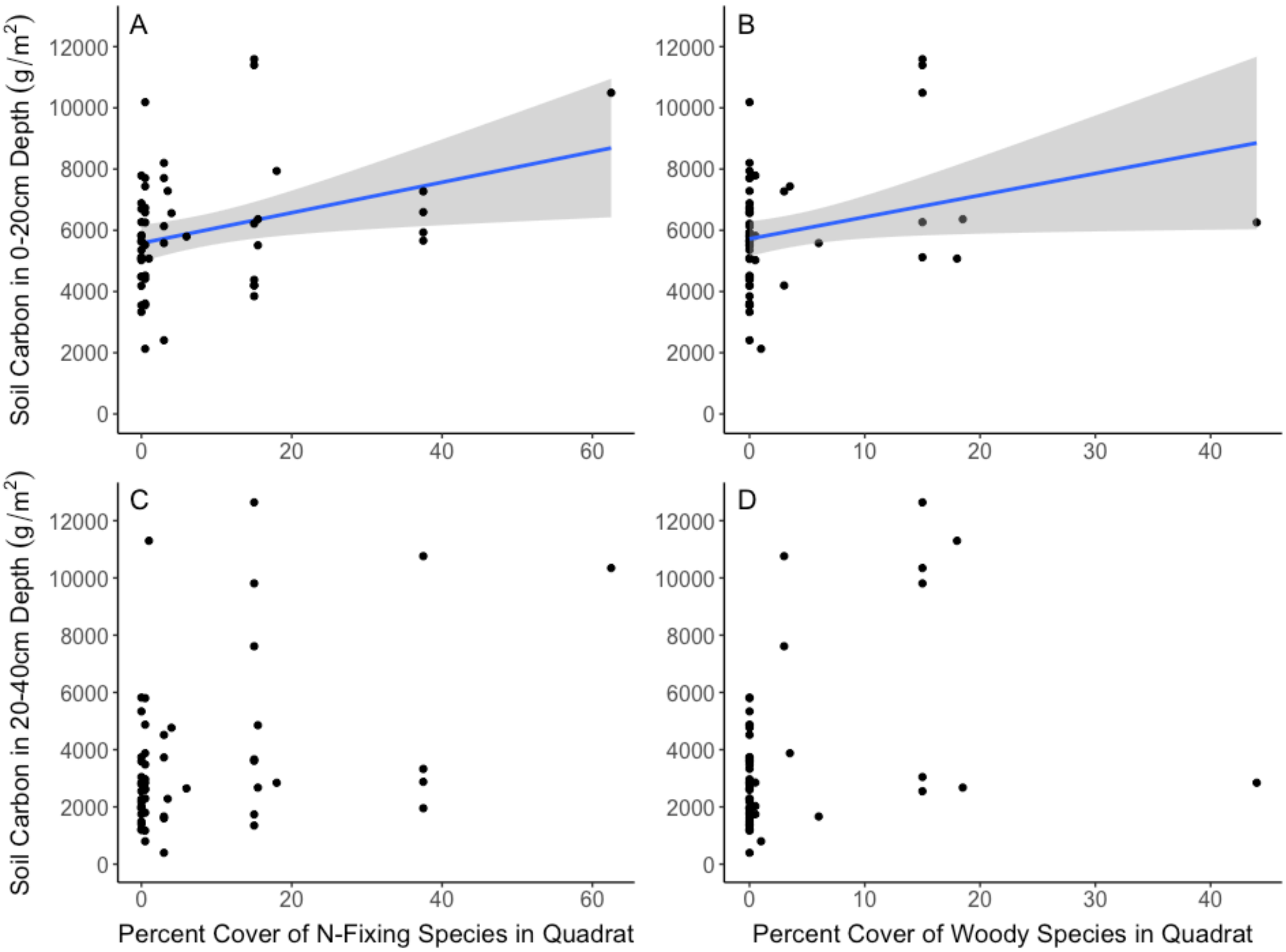

Figure 12. Total soil carbon vs. the cover of nitrogen-fixing forbs and woody species in restored and control roadside sites and a remnant prairie in Southern Ontario. A, B) 0-20cm soil depth; C, D) 20-40cm soil depth; A, C) nitrogen-fixing species; B, D) woody species. Soil samples were collected from sites in September 2019 and carbon levels were adjusted for soil bulk density. Plant functional group cover was assessed in 1x1m quadrats centered around the soil sampling locations. n=54. Regression lines are shown for models in which the effect of the plant functional group was significant. Shaded areas represent 95% confidence intervals around each slope. Nitrogen-fixing and woody species were positively correlated with soil carbon in the top 20cm of soil but were not significantly correlated with soil carbon in the 20-40cm soil depth.

AM Fungi

Effects of soil treatment and inoculum source on A. gerardii *biomass*

Mycorrhizal treatment (‘AM fungal’ or ‘control’), inoculum source and their interaction had highly significant effects on the aboveground biomass of *A. gerardii* plants after twelve weeks of growth (Table 10). Although there was a small but significant main effect of mycorrhizal treatment, the magnitude and direction of the effect of soil treatment varied significantly depending on the inoculum source (Tables & 11; Figure 13).

Effects of site age and restoration status on mycorrhizal growth response

The mycorrhizal growth response of *A. gerardii* plants was not affected by whether or not the inoculum had come from a restored site or a control site (Table 12). However, site age had a marginally significant effect on mycorrhizal growth response. Plants grown in soil collected from older sites (measured as time since restoration or major disturbance) tended to respond more negatively to the AM fungal treatment than plants grown in soil collected from newer sites (slope = -0.009259; Figure 14).

Table 10. Analyses of variance for the effects of mycorrhizal treatment (control or AM fungal), inoculum source (one of 18 different study sites) and their interaction on *A. gerardii* biomass. *A. gerardii* plants were grown from seed in sterilized background soil with either live (AM fungal treatment) or sterilized (control treatment) soil inoculum collected from 17 different roadside sites plus one remnant prairie. A microbial wash containing non-AM fungal microbes from the soil inoculum was added to control pots. F and p values are reported for type III sums of squares. n=285 and the adjusted R^2 = 0.16. P values less than 0.05 are bolded.

Factor	df	F	P value
Model	35,249	2.52	**<0.0001**
Inoculum source	17	1.65	**0.0018**
Treatment	1	2.95	**0.0002**
Source x Treatment	17	3.36	**<0.0001**
Residuals	249		

Table 11. Pre-planned contrasts of *A. gerardii* biomass between AM fungal and control treatments derived from different inoculum sources. Estimates represent the AM fungal-control treatment contrasts for each inoculum source. *A. gerardii* plants were grown from seed in sterilized background soil with either live (AM fungal treatment) or sterilized (control treatment) soil inoculum collected from 17 different roadside sites plus one remnant prairie. A microbial wash containing non-AM fungal microbes from the soil inoculum was added to control pots. df = 249 for each contrast, and p values were adjusted using a Sidak adjustment for 18 tests. P values less than 0.05 are bolded.

Inoculum source	Estimate	SE	t ratio	P value
Liberty stockpile	-0.0041	0.0064	-0.641	1.0000
DC 2019	0.0029	0.0064	0.456	1.0000
Site 79	0.0046	0.0066	0.691	1.0000
DC 2018	0.0011	0.0064	0.170	1.0000
Site 75	-0.0119	0.0064	-1.853	0.7021
35/115	0.0246	0.0064	3.837	**0.0028**
Pond 58E	-0.0093	0.0064	-1.442	0.9470
Pond 28W	-0.0002	0.0064	-0.027	1.0000
Pond 44N	0.0097	0.0064	1.508	0.9230
Site 35	-0.0060	0.0064	-0.941	0.9995
Montgomery	0.0162	0.0064	2.523	0.1990
Pulford	0.0046	0.0064	0.709	1.0000
Bentpath	-0.0149	0.0064	-2.315	0.3230
Courtright	-0.0201	0.0069	-2.894	0.0721
Whitebread	-0.0119	0.0064	-1.861	0.6956
Walker	-0.0052	0.0064	-0.814	0.9999
Moore	-0.0111	0.0064	-1.726	0.7999
Chappus	-0.0181	0.0064	-2.814	0.0910

Table 12. Analysis of covariance for the effects of the age (since construction or restoration) and type (restored or control) of site from which inoculum was sourced on the mycorrhizal growth response of *A. gerardii* seedlings. Plants were grown from seed in a greenhouse for twelve weeks with sterilized or live inoculum collected from one of 18 study sites. F and p values are reported for type II sums of squares. The interaction between site age and site type was dropped from the model because it was not significant. Seedlings grown in inoculum from the remnant prairie site were not included in this analysis since site age could not be assigned. N=17, adjusted $R^2 = 0.12$.

Factor	df	F	P value
Model	2,14	2.07	0.1637
Site Age	1	3.11	0.0997
Site Type	1	2.07	0.1725
Residuals	14		

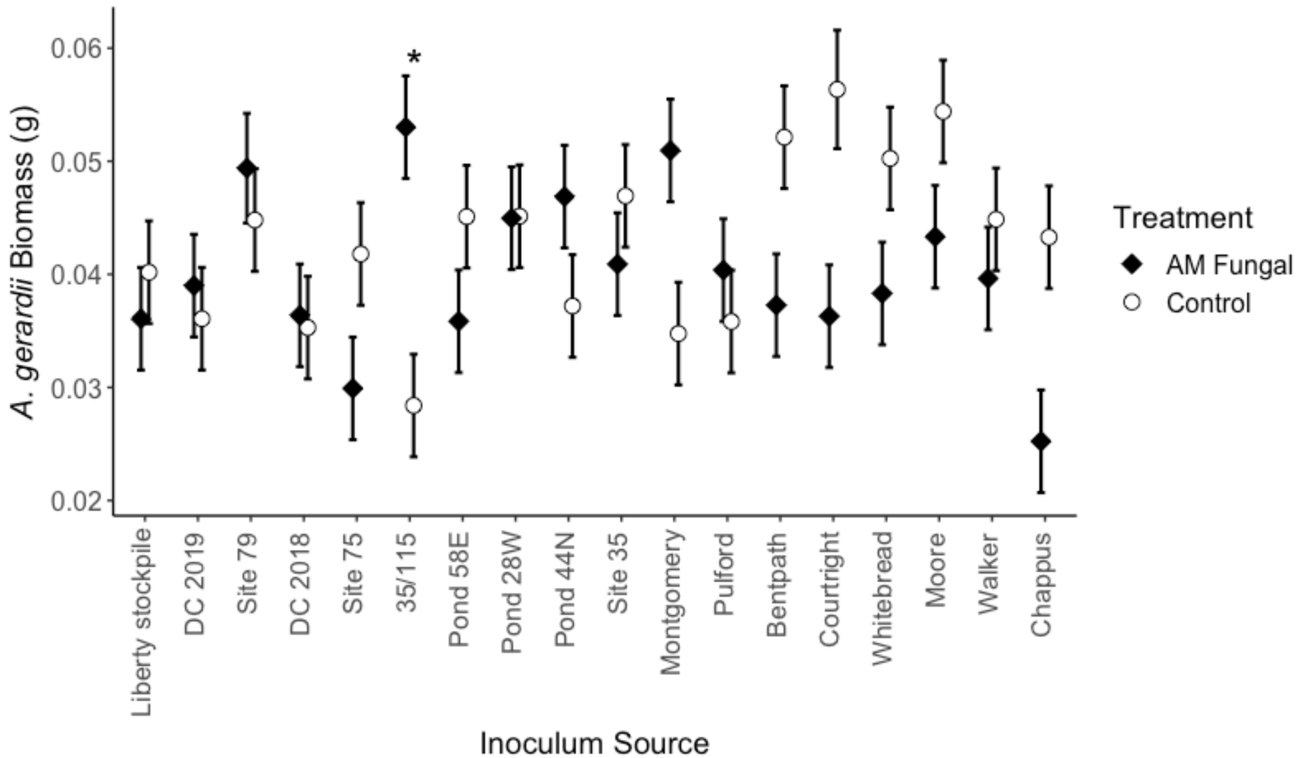

Figure 13. Biomass of *A. gerardii* plants grown in either an AM fungal treatment or control treatment with inoculum sourced from 18 different study sites. Data shown as mean (±SE) for replicate pots within each treatment × inoculum source combination. *A. gerardii* plants were grown for twelve weeks in pots containing live soil inoculum (AM Fungal treatment) or sterilized soil inoculum plus a microbial wash (Control treatment), with inoculum sourced from seventeen different roadside sites plus a remnant prairie. n=8 for most treatment x inoculum source combinations, with the exception of three combinations for which one individual was removed from the analysis because the individual died prior to the end of the experiment. A star indicates that *A. gerardii* biomass differed significantly ($p < 0.05$) between the two mycorrhizal treatments for a particular inoculum source in a pre-planned contrast analysis. Sites are ordered according to their age (time since disturbance/restoration), with the newest sites on the left (Liberty stockpile = 0 years; DC 2019 and Site 79 = 1 year; DC 2018 and Site 75 = 2 years; 35/115 and Pond 58E = 3 years; Pond 28W, Pond 44N and Site 35 = 4 years; Montgomery and Pulford = 5 years; Bentpath = 10 years; Courtright = 14 years; Whitebread = 17 years; Walker = 37 years; Moore = 45 years; Chappus = prairie remnant). The difference between treatments varied significantly depending on the inoculum source.

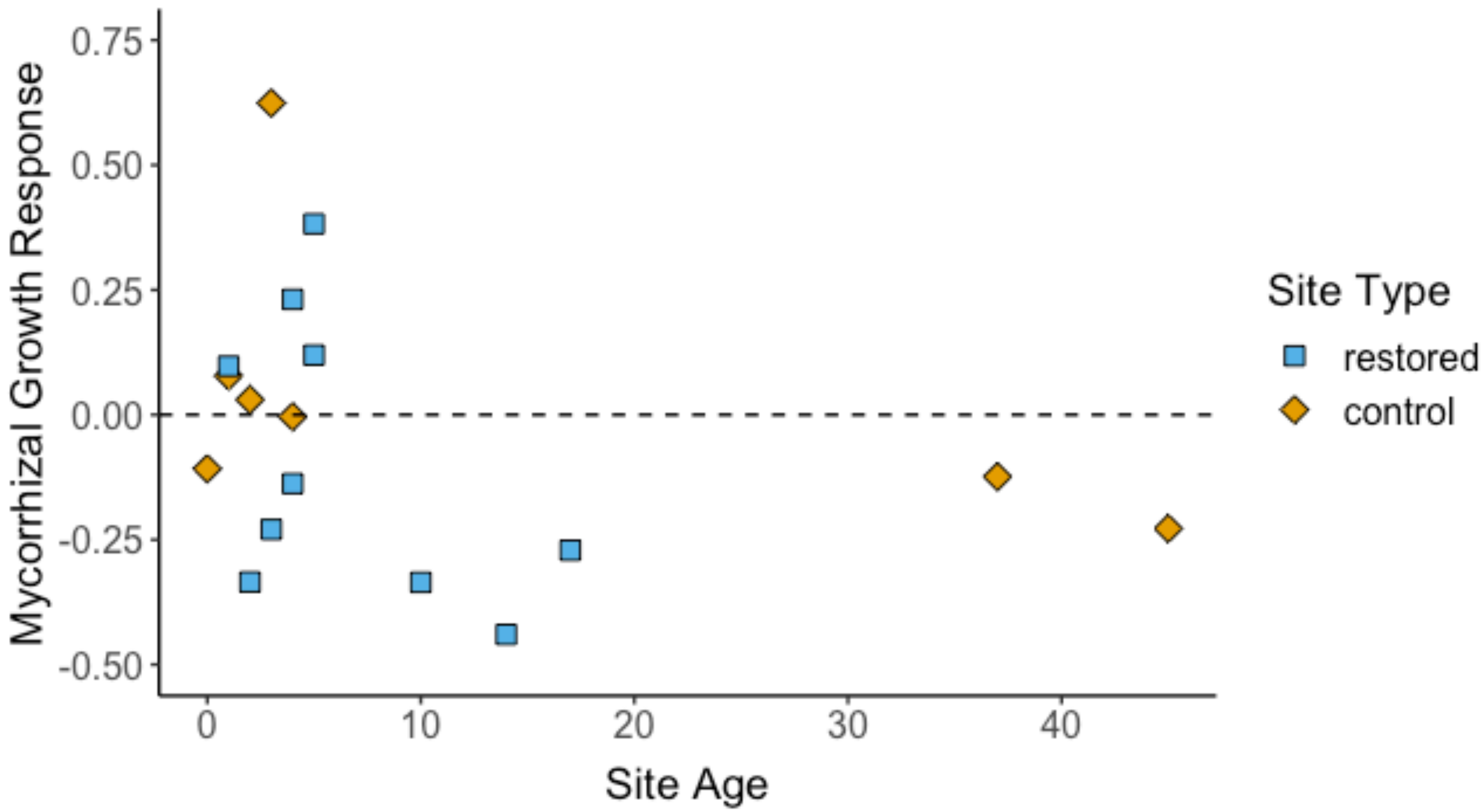

Figure 14. Mycorrhizal growth response of *A. gerardii* seedlings to soil inoculum sourced from 17 roadside sites. Sites included ten restored sites and five control sites of various ages since restoration or construction, plus one stockpile (age = 0). *A. gerardii* plants were grown for twelve weeks in pots containing live soil inoculum (AM Fungal treatment) or sterilized soil inoculum plus a microbial wash (Control treatment). n = 17. The dashed line indicates a growth response of zero, or equal biomass in the AM fungal and control treatments. Points above this line indicate that plants in the AM fungal treatment had greater average biomass than plants in the control treatment, while points below this line indicate that plants in the AM fungal treatment had lower average biomass than plants in the control treatment. Mycorrhizal growth response varied from positive to neutral to negative, but generally decreased with site age.

DISCUSSION

In this study, I aimed to investigate whether seeding roadsides with native plants restored not only the vegetation community, but also other components of the ecosystem, including the bee community, soil carbon and the effect of AM fungi on native plant growth. The effect of restoration on these ecosystem components varied among sites, indicating that roadside restoration success is context-dependent and may be influenced by implementation and management decisions as well as landscape factors. The landscape context in particular appears to have a strong influence on at least some aspects of restoration, which is unsurprising since roadside restorations exist as small, narrow parcels of land within highly variable landscapes. Comprehensive monitoring will therefore be necessary to identify unique challenges and solutions for each roadside restoration project. I also found that different ecosystem components responded to restoration in different ways. While native plant diversity and bee abundance increased with restoration as expected, the responses of other ecosystem components were more surprising.

Vegetation community

Overall, restored sites had greater native plant richness and native Shannon-Wiener diversity than control sites and, while they contained some of the same common non-native species as control sites, most restored sites also contained a number of dominant, high-quality native species that were not present in control sites. The lack of native grassland species in control sites suggests that many native grassland specialists are unable to establish themselves in roadsides independently. Establishment of uncommon or specialist grassland species may be hindered by underrepresentation in the seed bank or dispersal limitation, which is particularly likely in fragmented landscapes like Southern Ontario where remnant populations are scarce

(Bakker & Berendse 1999; Donath et al. 2003; Waldén et al. 2017). However, the successful establishment of thriving populations of these native grassland species in restored roadsides indicates that roadside conditions may be suitable for threatened grassland plant species as long as human intervention is used to overcome dispersal and seed bank limitations. The establishment of new populations along roadsides then increases the regional abundance of these species, thus increasing the chances for future dispersal and population expansion (Waldén et al. 2017). In this way, roadside restorations can act as corridors that promote the persistence of threatened plant communities in fragmented landscapes.

There were clear differences among highways in the habitat quality of restorations, which were strongly expressed by FQI scores. Restorations along Highway 40 and the Herb Gray Parkway had much higher FQI scores compared to their respective control sites, while Highway 407 restorations had low FQI scores that were similar to Highway 407 control sites. Thus, restorations along Highway 40 and the Herb Gray Parkway successfully improved habitat quality and established populations of more sensitive native species that were not present in control sites, while Highway 407 sites did not. Furthermore, the Highway 40 sites, which were the oldest, had the highest FQI scores and the newest sites along Highway 407 sites had the lowest FQI scores, suggesting that habitat quality may increase over time since restoration. This is encouraging since other studies have found decreases in plant diversity with time in grassland restorations, sometimes after peaks in diversity at intermediate ages (Baer et al. 2002; McLachlan & Knispel 2005; Middleton et al. 2010; Hansen & Gibson 2014). It is notable that even the abundance-weighted FQI, which is likely to be more conservative than the traditional FQI, was able to detect clear differences among site types and highways. The sensitivity of abundance-weighted FQI to differences in plant community composition and its incorporation of plant abundance data makes it an excellent metric for evaluating restoration success.

While restorations along Highway 40 and the Herb Gray Parkway achieved similar levels of native plant diversity as the Highway 40 reference sites, they did not achieve the diversity or of the Chappus remnant prairie site. The similar levels of diversity found in restored roadsides compared to the Highway 40 references, which were restorations outside of the right-of-way, indicate that restoration can be as successful along disturbed roadsides as it is in larger, less disturbed areas of land. However, the fact that none of the restored sites matched the native diversity of the remnant prairie, a result supported by many similar studies (Kindscher & Tieszen 1998; Sluis 2002; Martin et al. 2005; Polley et al. 2005; Middleton et al. 2010), suggests that restorations are unlikely to achieve remnant-level diversity, at least in the first few decades. Nevertheless, the cluster analysis revealed that some of the restored sites, especially the newer Highway 40 restorations, were similar in plant species composition to the references. The fact that intermediate-aged restorations, rather than the oldest restoration, were most similar in composition to reference sites highlights the need for disturbance such as burning and mowing for maintaining high-diversity grasslands over time (Bowles & Jones 2013; Koyama et al. 2017) and may also reflect improvements in restoration practices and seed mixes. Monitoring these projects periodically over time will help to optimize management to maintain plant diversity and determine whether restored sites are progressing towards the reference state.

The Herb Gray Parkway restoration sites were seeded only one year earlier than the Highway 407 sites (Table 1), and yet they contain much greater native plant diversity and a composition that is more similar to the reference sites than the Highway 407 sites. Both spatial and year-to-year weather variation can influence plant establishment in grassland restorations

(Bakker et al. 2003; Seabloom 2011; Stuble et al. 2017) and may have led to more successful restorations along the Herb Gray Parkway. However, restoration practices can also have a strong influence on outcomes (Bakker et al. 2003; Dickson & Busby 2009; Middleton et al. 2010). Restoration along the Herb Gray Parkway was strictly supervised by restoration specialists, which likely ensured that decisions such as the timing of seeding were made to optimize restoration success. Highway 407 restorations were not subject to the same level of expert supervision. Furthermore, while Herb Gray Parkway sites were continually managed to eliminate invasive species, several Highway 407 restorations failed to meet erosion control standards and so were actually reseeded with a seed mix that contained invasive species. Competition with introduced or non-target species is known to reduce the establishment of native species in restoration sites (Bakker et al. 2003; Skousen & Venable 2008), and so this difference in approaches to introduced species may help to explain the variation in restoration success. More controlled, replicated experiments on management factors influencing vegetation establishment would help to disentangle the effects of environmental variation and management practices on restoration success.

Bee Diversity and Abundance

While bee abundance and genus richness varied mostly among highways rather than among site types, bee abundance, and to a lesser extent, bee genus richness, did respond positively to plant community restoration as well as site-level plant diversity and available bare ground. These results are consistent with other studies that have found that, compared to control areas, restored roadsides and farm margins support a greater abundance and diversity of bees (Hopwood 2008; Paterson et al. 2019) and habitat-sensitive butterflies (Ries et al. 2001). However, the response of the bee community to restoration varied among highways in a pattern that mirrored variation in the success of plant community restoration. At Highway 40 and the Herb Gray Parkway, plant species richness was greater in restored than control sites, and bee abundance and diversity were, on average, higher in restored compared to control sites as well. However, at Highway 407, both plant and bee diversity were similar between control and restored sites. Furthermore, similar to other pollinator studies, I found positive correlations between bee abundance and diversity and the native plant diversity of study sites (Munguira & Thomas 1992; Ries et al. 2001; Croxton et al. 2005; Saarinen et al. 2005; Hopwood 2008; Sjödin et al. 2008; Noordijk et al. 2009). Overall, these results indicate that the effect of restoration on the bee community is dependent on the success of the plant community restoration and highlight the importance of successfully restoring the plant community if benefits for the bee community are desired. However, while bee abundance responded readily to increases in floral resource diversity and nesting habitat (bare ground), the response of bee genus richness was not as pronounced. This indicates that the immigration of new genera to restored habitat may be limited when the restored habitat is small and isolated within a largely inhospitable landscape (Losos et al. 2010; Rösch et al. 2013), which is likely to be the case for many roadside restorations.

Bee diversity and abundance varied significantly among highways, with Highway 407 sites containing a greater abundance and diversity of bees. This suggests that the bee communities of roadside sites may be influenced by factors that were unique to each location, including geography, climate and landscape factors. Bee abundance and diversity often respond positively to increased habitat connectivity and natural land cover (Steffan-Dewenter et al. 2002; Steffan-Dewenter 2003; Carvell et al. 2017; Öckinger et al. 2018; Ballare et al. 2019; Ponisio et al. 2019; Hopfenmüller et al. 2020), high habitat heterogeneity (Boscolo et al. 2017) and reduced arable land use and intensive agricultural practices (Kremen et al. 2002, 2004; Holzschuh et al.

2007; Rundlöf et al. 2008; Sjödin et al. 2008). Increased connectivity and nearby natural habitat may allow bee communities to recolonize new habitats after disturbance (Sivakoff et al. 2018), while a reduction in intensive agricultural practices can benefit bees by reducing harmful pesticide use and increasing floral resource availability (Kremen et al. 2004; Holzschuh et al. 2007; Rundlöf et al. 2008). It is notable that Highway 40 sites were located in a heavily agricultural area and most Herb Gray Parkway sites were located in urban areas, while Highway 407 sites were located in a mosaic of agricultural and natural land. Thus, Highway 407 sites may have been more easily recolonized after construction by diverse bee communities located in nearby natural areas. Furthermore, the Chappus reference site's high bee diversity compared to other Windsor sites may be due to its size and connection to a network of natural areas. Thus, when selecting sites for the restoration of pollinator habitat, land managers should prioritize sites that are located within a landscape of connected, heterogenous natural areas and that are not subject to intensive agricultural practices such as heavy pesticide use.

Soil Carbon Storage

Surprisingly, all roadside sites contained similar or greater levels of soil carbon compared to the remnant grassland, and soil carbon did not differ between restored and control site types in three out of the four roadside sites. Grassland restorations often contain higher amounts of soil carbon compared to unrestored lands but usually do not achieve soil carbon levels similar to remnant prairies even after decades of restoration (Kindscher & Tieszen 1998; Baer et al. 2000, 2002; Fuhlendorf et al. 2002; Matamala et al. 2008; Hansen & Gibson 2014). However, most investigations on soil carbon in restored grasslands have occurred on land previously used for agriculture, (Kindscher & Tieszen 1998; Baer et al. 2000, 2002; Fuhlendorf et al. 2002; Matamala et al. 2008; Hansen & Gibson 2014), which significantly deplete soil carbon

(Davidson & Ackerman 1993; Burke et al. 1995; Knops & Tilman 2000). In contrast, only two of the roadside sites I studied had been cultivated prior to restoration, and these sites likely contained fill soil that may have been transported there from a different location. Thus, the roadside sites may have had relatively high levels of soil carbon prior to restoration, resulting in more similarity in soil carbon among site types. The roadside sites had carbon levels that were comparable to not only the reference site that I assessed but also many other reported reference values for similar soil depths (Kindscher & Tieszen 1998; Baer et al. 2000; Fuhlendorf et al. 2002; Hansen & Gibson 2014). Therefore, restoration of soil carbon levels may not be necessary on soils that have not been depleted of carbon due to agricultural practices, and restoration of native vegetation may not result in additional soil carbon storage beyond baseline levels, at least in the first two decades after restoration.

My results support the hypothesis of a positive relationship between the abundance of nitrogen-fixing and woody species and soil carbon, but do not provide evidence for a relationship between the abundance of C_4 grasses and soil carbon. The positive correlation between the cover of nitrogen-fixing species and soil carbon in the upper soil depths corroborates other findings that soil carbon accumulation is correlated with soil nitrogen accumulation and nitrogen-fixing plant abundance (Knops & Tilman 2000; De Deyn et al. 2011; Yang et al. 2019). While Yang et al. (2019) also found a positive relationship between C_4 grasses and carbon storage, especially when combined with the effect of legumes, in my study sites there was no correlation between C_4 grass cover and soil carbon. However, the effect of C_4 grasses on soil carbon storage is less consistent, as studies have shown that the relationship between C_4 grasses and soil carbon ranges from positive (Knops & Tilman 2000; Yang et al. 2019) to neutral (Cahill et al. 2009) to negative (Ampleman et al. 2014). The variation in the effect of C_4 grasses on soil carbon accumulation may be mediated by the abundance of other functional groups, since interactions among functional groups (e.g. legumes and C_4 grasses) as well as overall plant diversity can influence soil carbon storage (Ampleman et al. 2014; Lange et al. 2015; Yang et al. 2019). More research into the mechanisms by which certain plant functional groups can alter soil carbon storage will produce insight into how land managers might manage vegetation communities to optimize carbon sequestration and thereby mitigate climate change.

AM Fungi

Interestingly, *A. gerardii* seedlings in my study showed an overall negative response to the AM fungal treatment. This was unexpected since most evidence suggests that *A. gerardii* is positively responsive to and highly dependent on AM fungi (Hetrick et al. 1989, 1990; Wilson & Hartnett 1997, 1998). However, though the response of *A. gerardii* to AM fungi is generally positive, the mycorrhizal response of individual plants can vary from negative to positive depending on environmental factors, especially soil phosphorus levels (reviewed in Johnson et al. 1997; Weremijewicz & Seto 2016). At high soil phosphorus levels, AM fungi can reduce the growth of *A. gerardii* individuals compared to plants grown without AM fungi (Weremijewicz & Seto 2016). Thus, although I added sand to dilute the nutrient levels of the potting soil used in this experiment, it is possible that phosphorus levels in my study were still above the threshold required to produce a positive response to AM fungi. It is unlikely that the soil inoculum had a significant effect on the overall nutrient levels of each pot due to the low ratio of inoculum soil to background soil. Follow-up studies using background soil collected from roadside sites rather than a potting soil and sand mixture would be beneficial in characterizing the response of *A. gerardii* to AM fungi in the nutrient levels characteristic of roadside conditions.

While the growth response of *A. gerardii* to AM fungi varied depending on the site from which the inoculum was sourced, there was no effect of restoration on growth response and a marginally significant negative relationship between growth response and site age. AM fungal colonization is often greatly reduced in disturbed soils (Moorman & Reeves 1979; Jasper et al. 1989) and there is evidence that colonization recovers over time since disturbance (White et al. 2008). Thus, inoculum from recently disturbed sites likely produced minimal AM fungal colonization, with inoculum from older sites producing high levels of colonization. If AM fungal colonization caused a negative effect on plant growth in my experiment due to high phosphorus levels (as observed in Weremijewicz & Seto 2016), then high levels of colonization with inoculum from older sites may have resulted in a stronger parasitic effect on plant growth, leading to the observed negative correlation between growth response and site age. However, evidence suggests that in some cases, disturbance may actually increase the abundance of AM fungi (Farrell et al. 2020) or alter the fungal community composition (Hart & Reader 2004). Therefore, it is possible that the more positive growth response to inoculum from recently disturbed sites was due to higher, rather than lower, AM fungal colonization or a more favourable fungal community composition. Future measurement of AM fungal colonization of *A. gerardii* roots from my experiment will help to clarify the relationship between AM fungal colonization and site age.

An alternative explanation for the negative relationship between site age and plant response to AM fungi is the potential for changes in the microbial community over time that reduce the benefits of AM fungal colonization for their plant hosts. Changes in the microbial community over time that reduce plant benefit can be driven by negative feedback between the plant and AM fungal communities (Bever 2003; Bauer et al. 2015). Negative feedback can occur if one partner benefits more from the mutualism than the other so that, for example, a particular plant species provides great benefit to a fungal partner that is not particularly beneficial to the plant, leading to an increase in the population of this suboptimal fungal partner (Bever 2002, 2003). This mechanism may help to explain why the initial benefits of AM fungal inoculation for plant establishment (Smith et al. 1998; Wubs et al. 2016; Koziol et al. 2020) may diminish over time (White et al. 2008). Consistent with this hypothesis, the inoculum source in my study that produced the most negative growth response was the inoculum from the remnant prairie, where the plant and microbial communities have been interacting for the longest amount of time. Thus, when deciding whether to alter or add to the soil microbial community of restoration sites, land managers should consider both short-term and long-term impacts of the microbial community on the plant community trajectory.

While my results were unexpected in terms of the overall direction of the plant response to AM fungi, they did indicate that inoculum source can have a significant effect on the magnitude and direction of this response. This supports greenhouse and field studies showing that inoculum source and composition can affect root colonization, plant growth response and the plant community trajectory (Sylvia et al. 1993; Oliveira et al. 2005; Cavender & Knee 2006; Rowe et al. 2007; Maltz & Treseder 2015; Middleton et al. 2015; Wubs et al. 2016; Koziol & Bever 2017). Evidence from these studies suggests that inoculum from a local reference ecosystem is most beneficial for plant growth (Sylvia et al. 1993; Oliveira et al. 2005; Cavender & Knee 2006; Rowe et al. 2007; Middleton et al. 2015), which contrasts with my results, which showed that inoculum from the reference ecosystem produced the most negative response out of all sources. However, most studies used a single source of field-collected inoculum and compared this with commercially produced inoculum (Sylvia et al. 1993; Cavender & Knee

2006; Rowe et al. 2007; Middleton et al. 2015), and so the benefit of the locally sourced inoculum may not be attributable to the specific ecology of the collection site and may simply suggest that field-collected inoculum outperforms commercial inoculum. Thus, my study was valuable in demonstrating that native soil inoculum is not always beneficial for native plant growth and, in some cases, a more positive plant response can be achieved with inoculum collected from recently disturbed, low diversity field sites. Thus, if local, high quality reference sites do not exist near restoration sites where inoculum is needed, land managers may achieve similar or more positive results by collecting field inoculum from less diverse sites.

Conclusions and Recommendations

Overall, my results highlight the need for a comprehensive evaluation of success of ecosystem restoration projects, as each ecosystem component responded differently to restoration. This is consistent with other studies that have found diverging responses of different taxa or ecosystem components to restoration activities (Mutch 2007; Hansen & Gibson 2014). Since measurement of many ecosystem components is extremely resource-intensive, restoration practitioners should aim to monitor a selection of key components that capture the diversity, structure and functioning of the ecosystem (Ruiz-Jaen & Aide 2005), while prioritizing the measurement of components that are aligned with their specific restoration objectives. The diverging responses of different ecosystem components to restoration also suggests that a specific set of restoration activities may benefit some taxa but harm others. Thus, taking a landscape-scale approach to restoration and aiming to create a heterogenous landscape through the restoration of different communities may be necessary ensure that the needs of all taxa are met within the landscape (Lengyel et al. 2020). Both comprehensive monitoring and integration within the landscape are key components of restoration projects that will help restoration practitioners to achieve the overall goal of restoring healthy ecosystems (Hobbs & Norton 1996; SER 2004).

Selecting appropriate measures of restoration success requires that projects have clear goals and objectives (SER 2004). However, for the projects that I studied, the specific restoration objectives or chosen indicators of success were not always clear. For example, the Highway 407 restoration plan stated that a goal of the restoration was to 'offset impacts to vegetation communities/habitat features that would adequately reflect the ecological functions that will be lost' (Arthur 2015). Yet, the restoration plan does not mention specific ecological functions that are to be restored or suggest measurable indicators for any ecosystem functions. Similarly, while the Herb Gray Parkway restoration had a goal of restoring habitat for endangered species and polliantors, the proposed indicators of restoration success were vegetation-focused. These plans appear to assume that restoration of the vegetation community will lead to the restoration of other ecosystem components, which my results and others have shown is not always the case (Hobbs & Norton 1996). Clear, practical and measurable objectives allow restoration practitioners to not only measure success, but also to prioritize ecosystem components and improve outcomes via adaptive management, which requires monitoring data as a first step in making management decisions (SER 2004; Westgate et al. 2013). Thus, future projects should ensure that goals are clear, prioritized and attached to measurable objectives that can be assessed to inform management decisions.

Common goals of roadside and prairie restoration including establishing and conserving native plant communities, supporting pollinator populations and sequestering soil carbon, and my results provide insight into practices and considerations that will help to achieve these goals. Comparison of the Herb Gray Parkway and Highway 407 sites showed that initial investment in reducing competition from non-native plants was important for successful restoration of the vegetation community. Since native seed mixes do not always provide cover as quickly as non-native seed mixes (Skousen & Venable 2008), practitioners should use appropriate nurse crops and select sites where erosion is less likely to avoid jeopardizing restoration success. The high plant diversity of restoration sites of intermediate ages suggests that management practices including mowing and burning may help to maintain plant diversity. If restoring the pollinator community is a priority, land managers should focus on increasing native plant diversity, providing adequate bare ground for nesting, and selecting sites that are connected to a network of diverse natural habitats. Land managers should consider whether increasing soil carbon is feasible given the history of the restoration site and, if so, include native nitrogen-fixing species in seed mixes to promote this process. Finally, my results show that adding AM fungal inoculum to sites is not always beneficial, so practitioners should consider soil nutrients levels and available inoculum sources when deciding whether to apply inoculum. Overall, monitoring of diverse metrics and adjusting restoration practices accordingly will likely lead to the best results.

This study also demonstrated the importance of an appropriate healthy reference ecosystem when evaluating restoration. References are important because successful restoration should aim to restore an ecosystem to a healthy state (the reference) rather than simply move an ecosystem away from an unhealthy state (SER 2004; Wortley et al. 2013). While several vegetation metrics showed that restoration had improved the diversity of ecosystems compared to the unrestored state, none of these metrics suggested that restored ecosystems were equivalent to remnant, undisturbed ecosystems, which echoes the results of numerous other studies (Sluis 2002; Wilkins et al. 2003; Hansen & Gibson 2014). Thus, references serve as an important reminder that restoration should not be used as a justification for further ecosystem destruction or degradation (Young 2000; McDonald et al. 2016). Furthermore, my study exemplifies the limitations of using only a single remnant community as a reference, as it made statistical comparison difficult and restored ecosystems could never be expected to exactly match a specific reference site (SER 2004; McDonald et al. 2016). While I was limited by the availability of additional reference sites and thus attempted to use older restorations as references in lieu of truly intact communities, these proved to be of insufficient quality to serve as true references. Thus, future prairie restoration monitoring in Southern Ontario, where remnant prairies are rare, may benefit from using historical information and ecological descriptions (as suggested by SER 2004) of the tallgrass prairie community to construct appropriate reference models that encompass a range of reference states to be compared to restored ecosystems.

My results indicate that while roadsides can be restored in a way that benefits biodiversity, they also present some challenges that may constrain restoration success. Restoration along the Herb Gray Parkway and Highway 40 both successfully increased native plant richness and established populations of sensitive plant species. However, Highway 407 restorations generally failed to increase native plant diversity, possibly due to a lack of expertise in the implementation phase as well as erosion concerns. Since erosion control is a high priority along roadsides, many roadsides may not be suitable for restoration if native vegetation cannot establish quickly enough to prevent erosion. Furthermore, sustaining restored plant communities over time may require management such as mowing and controlled burns (Koyama et al. 2017; Jakobsson et al. 2018), which may be logistically difficult to perform along roadsides (J. Lozon 2018, Ontario NativeScape, Wallaceburg, ON, personal communication). Additionally, though my study confirmed that increasing native plant diversity along roadsides can increase bee abundance, it also showed that roadsides may not always be situated within a hospitable landscape due to a lack of connectivity with surrounding habitats. Other studies have also suggested that constructing pollinator habitat along the roadside may lead to increased insect mortality and a net negative impact on insect populations (Baxter-Gilbert et al. 2015; Keilsohn et al. 2018), though few studies have measured both benefits and costs of different roadside habitats for insects (but see Munguira & Thomas 1992; Skórka et al. 2013). Finally, restoring roadsides did not have the positive impact on soil carbon that restoration appears to provide on other land use types. Thus, the suitability of roadsides for grassland restoration appears to be context-dependent, and land managers should consider potential constraints and project objectives when deciding on the most suitable sites for restoration.

In conclusion, my study demonstrated that even in challenging roadside environments, restoration can be successful in improving native plant community diversity and composition. Furthermore, this is the first study on roadside restoration that used a comprehensive ecosystem approach to evaluating success, and it revealed that successful restoration of ecosystem components beyond the vegetation community can be difficult and context-dependent. Future restoration projects should aim to include multiple ecosystem components in their goals, objectives and monitoring programs so that we can better understand how different restoration practices influence more than just the plant community. My hope is that this data will be used by project managers to inform their restoration practices and improve restoration outcomes for these restorations and new restorations in the future.

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APPENDIX A: SEED MIXES

The following table describes the species included in seed mixes applied to the study sites included in the vegetation surveys and bee sampling. An ‘x’ indicates that the species was included in the mix. Seed mix information was provided by land managers involved in the restoration of the study sites. Seed Mix A was applied to Whitebread, Courtright, Property 80 and Nicholls Memorial at Highway 40. Seed Mix B was applied to Bickford and Bentpath at Highway 40. Seed Mix C was applied to Pulford at the Herb Gray Parkway. Seed Mix D was applied to Sandwich and Montgomery at the Herb Gray Parkway. Seed Mix E was applied to Pump Station 6 at the Herb Gray Parkway. Seed Mix F was applied to Pond 8E at Highway 407. Seed Mix G was applied to Pond 20E and Pond 42S at Highway 407. Seed Mix H was applied to Site 35 at Highway 407. Seed Mix I was applied to Pond 44N at Highway 407. The MTO mix was applied to all control sites, possibly with some variations. Woody species were planted at some sites but are not included in this table.

	Seed Mix									
Species	A	B	C	D	E	F	G	H	I	MTO
Achillea millefolium	x									
Agastache nepetoides	x		x		x					
Agrimonia gryposepala							x		x	
Agrostis perennans			x	x	x					
Allium cemuum	x	x								
Anaphalis margaritacea							x		x	
Andropogon gerardii	x	x	x	x	x		x	x		
Anemone canadensis	x									
Anemone cylindrica	x		x		x	x	x		x	
Antennaria neglecta			x		x	x				
Apocynum androsaemifolium							x		x	
Apocynum cannabinum							x		x	
Aquilegia canadensis			x		x					
Arabis glabra							x		x	
Asclepias sullivantii	x		x		x					
Asclepias syriaca						x	x		x	
Asclepias tuberosa	x		x	x	x					
Asclepias verticillata	x									
Blephilia ciliata	x									
Calamagrostis canadensis								x		
Campanulastrum americanum			x		x					
Carex bebbii			x	x	x					
Carex blanda								x		

Species	Seed Mix									
	A	B	C	D	E	F	G	H	I	MTO
Carex comosa			x	x	x					
Carex gracillima								x		
Carex granularis								x		
Carex hystericina			x	x	x			x		
Carex lacustris								x		
Carex lupulina			x	x	x			x		
Carex pennsylvanica								x		
Carex praticola			x	x	x					
Carex retrorsa			x	x	x			x		
Carex stipata								x		
Carex stricta								x		
Carex vulpinoidea			x	x	x			x		
Ceanothus americanus	x									
Cephalanthus occidentalis	x									
Cirsium discolor							x		x	
Coreopsis lanceolata			x		x					
Coreopsis tripteris		x	x	x	x					
Danthonia spicata						x	x			
Desmodium canadense	x		x	x	x		x	x	x	
Dicanthelium acuminatum						x				
Doellingeria umbellata	x									
Echinacea pallida		x								
Elymus canadensis	x		x	x	x	x	x	x		
Elymus riparius								x		
Elymus trachycaulus								x		
Elymus virginicus							x	x		
Eupatorium perfoliatum	x							x		
Euphorbia corollata	x		x		x					
Euthamia graminifolia							x		x	
Eutrochium maculatum								x		
Festuca rubra										x
Festuca subverticillata						x				
Fragaria virginiana						x	x		x	
Gentiana crinata	x									
Glyceria grandis								x		
Glyceria striata								x		
Gnaphalium obtusifolium							x		x	

Species	Seed Mix									
	A	B	C	D	E	F	G	H	I	MTO
Helenium autumnale	x									
Helianthus divaricatus			x	x	x					
Helianthus giganteus	x	x	x		x					
Heliopsis helianthoides		x	x		x			x		
Hibiscus moscheutus		x								
Juncus tenuis			x				x	x	x	
Koeleria macrantha						x				
Lespedeza capitata	x		x	x	x	x	x	x	x	
Liatris aspera			x		x					
Liatris spicata	x									
Lobelia inflata							x		x	
Lobelia siphilitca		x						x		
Lolium multiflorum								x		
Lolium perenne										x
Lythrum alatum	x									
Monarda fistulosa	x		x	x	x	x	x	x	x	
Muhlenbergia schreberi						x				
Oenothera biennis			x	x	x	x	x	x	x	
Oryzopsis asperifolia						x				
Panicum capillare						x				
Panicum virgatum	x	x	x	x	x		x	x		
Penstemon digitalis	x	x	x	x	x		x		x	
Penstemon hirsutus	x	x	x		x	x	x		x	
Poa compressa			x							
Poa pratensis										x
Potentilla arguta	x						x	x	x	
Prenanthes alba	x									
Pycnanthemum tenuifolium							x		x	
Pycnanthemum verticillatum var. pilosum		x								
Pycnanthemum virginianum	x	x	x	x	x	x	x		x	
Ratibida pinnata	x	x	x	x	x					
Rudbeckia hirta	x	x	x	x	x	x	x	x	x	
Schizachne purpurascens ssp. purpurascens						x				
Schizachyrium scoparium	x	x	x	x	x	x	x	x		
Scirpus atrovirens								x		
Scirpus cyperinus								x		

	Seed Mix									
Species	A	B	C	D	E	F	G	H	I	MTO
Silene antirrhina							x		x	
Silphium lacinatum	x									
Silphium perfoliatum			x	x	x					
Silphium terebinthinaceum	x									
Solidago flexicaulis								x		
Solidago juncea							x	x	x	
Solidago nemoralis	x		x		x	x	x		x	
Solidago riddellii		x	x		x					
Solidago rigida	x	x	x	x	x					
Sorghastrum nutans	x	x	x	x	x	x	x	x		
Spartina pectinata	x									
Sporobolus asper	x									
Sporobolus cryptandrus	x		x	x	x	x	x	x		
Sporobolus neglectus						x	x			
Symphyotrichum cordifolium								x		
Symphyotrichum ericoides	x		x		x		x	x	x	
Symphyotrichum laeve	x					x				
Symphyotrichum lateriflorum			x		x					
Symphyotrichum novae-angliae			x		x		x	x	x	
Symphyotrichum oolentangiense	x		x		x	x	x	x	x	
Symphyotrichum pilosum							x		x	
Symphyotrichum puniceum								x		
Symphyotrichum urophyllum							x		x	
Thalictrum dasycrapum	x									
Tradescantia ohiensis	x	x								
Trifolium repens										x
Verbena hastata	x	x						x		
Verbena stricta		x	x		x	x	x	x		
Verbena urticifolia							x			
Vernonia fasciculata			x	x	x					
Vernonia missourica	x	x								
Veronicastrum virginicum	x	x								
Yellow Coneflower' (unknown sp)			x		x					

APPENDIX B: PLANT SPECIES LISTS

The following table lists all of the plant species found at each of the 23 study sites used for vegetation surveys and bee sampling. An 'x' indicates that the species was found at the site. The pale blue colour indicates restored sites, while pale yellow indicates control sites and pale green indicates reference sites.

	Highway 40								Herb Gray Parkway							Highway 407							
Species	Whitebread	Courtright	Bentpath	Bickford	Moore	Oil Springs	Property 80	Nicholls Memorial	Pulford	Sandwich	Pump Station 6	Montgomery	Dougall	Walker	Chappus	Pond 8E	Pond 20E	Site 35	Pond 44N	Pond 42S	Pond 11W	Pond 45S	Pond 28W
Abutilon theophrasti												x	x					x					
Acer negundo									x							x	x	x	x		x	x	x
Acer rubrum							x				x												
Acer sp												x				x	x					x	x
Acer x freemanii									x														
Achillea millefolium	x	x			x		x	x					x		x	x		x		x			
Agalinis purpurea															x								
Ageratina altissima																		x					
Agrostis gigantea		x	x		x	x				x	x			x	x	x		x	x	x	x		x
Aletris farinosa															x								
Alisma sp					x																		
Alliaria petiolata													x										
Althaea officinalis			x																				
Ambrosia artemisiifolia	x	x	x	x	x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Ambrosia trifida						x	x		x		x		x										

	Highway 40								Herb Gray Parkway							Highway 407							
Species	Whitebread	Courtright	Bentpath	Bickford	Moore	Oil Springs	Property 80	Nicholls Memorial	Pulford	Sandwich	Pump Station 6	Montgomery	Dougall	Walker	Chappus	Pond 8E	Pond 20E	Site 35	Pond 44N	Pond 42S	Pond 11W	Pond 45S	Pond 28W
Amphicarpaea bracteata															x								
Andropogon gerardii		x	x	x			x	x	x	x					x			x	x				
Anemone sp															x								
Antennaria neglecta															x								
Apocynum cannabinum	x											x	x		x								
Aquilegia canadensis												x											
Arctium minus		x				x							x				x				x	x	x
Aronia melanocarpa										x													
Artemisia vulgaris									x			x	x	x									
Asclepias incarnata			x			x	x	x															
Asclepias sullivantii			x	x			x																
Asclepias syriaca	x	x	x	x	x	x	x	x	x		x	x	x	x	x			x	x	x	x	x	x
Asclepias tuberosa	x																						
Asparagus officinalis	x														x								
Asteraceae sp															x								
Atriplex patula											x							x					
Atriplex prostrata		x																x			x		
Barbarea vulgaris									x														
Betula sp																x							
Bidens frondosa			x															x		x			
Brassica sp																						x	

	Highway 40								Herb Gray Parkway							Highway 407							
Species	Whitebread	Courtright	Bentpath	Bickford	Moore	Oil Springs	Property 80	Nicholls Memorial	Pulford	Sandwich	Pump Station 6	Montgomery	Dougall	Walker	Chappus	Pond 8E	Pond 20E	Site 35	Pond 44N	Pond 42S	Pond 11W	Pond 45S	Pond 28W
Brassicaceae sp													x										
Bromus commutatus	x	x	x	x					x	x	x	x	x			x			x		x	x	
Bromus inermis		x			x				x	x			x	x		x					x		x
Bromus sp															x								
Calystegia sepium	x								x		x		x	x									
Campsis radicans											x												
Capsella bursa-pastoris																							x
Carex atherodes						x																	
Carex aurea																						x	
Carex granularis												x			x	x						x	
Carex muehlenbergii?																				x			
Carex ovales sp	x		x			x		x	x		x												
Carex ovalis			x			x																	
Carex pellita																		x					
Carex sp A			x				x		x							x				x			
Carex sp B															x								
Carex sp C															x								
Carex sp D										x													
Carex sp E															x								
Carex sp F			x																				
Carex swanii?															x								

	Highway 40								Herb Gray Parkway							Highway 407							
Species	Whitebread	Courtright	Bentpath	Bickford	Moore	Oil Springs	Property 80	Nicholls Memorial	Pulford	Sandwich	Pump Station 6	Montgomery	Dougall	Walker	Chappus	Pond 8E	Pond 20E	Site 35	Pond 44N	Pond 42S	Pond 11W	Pond 45S	Pond 28W
Carex vulpinoidea			x			x		x			x	x				x		x	x				
Ceanothus americanus															x								
Celtis occidentalis									x														
Cerastium fontanum																x		x					x
Cerastium sp										x													
Cercis canadensis									x		x												
Chenopodium album									x		x												
Cichorium intybus		x			x				x	x	x	x		x					x	x		x	x
Cirsium arvense	x	x		x	x	x			x	x	x	x	x	x		x	x	x	x	x	x	x	x
Cirsium discolor															x								
Cirsium vulgare	x	x	x	x	x			x	x		x	x		x			x		x	x	x		x
Comandra umbellata															x								
Convallaria majalis															x								
Convolvulus arvensis											x		x	x			x					x	
Coreopsis tripteris	x		x	x				x		x		x			x								
Cornus racemosa		x	x	x	x		x						x	x	x	x						x	x
Crataegus sp		x	x	x			x	x							x								
Crepis tectorum																x			x		x	x	x
Cyperus esculentus											x												
Dactylis glomerata								x			x	x	x	x		x					x	x	x
Dasiphora fruticosa									x		x							x					

	Highway 40								Herb Gray Parkway							Highway 407							
Species	Whitebread	Courtright	Bentpath	Bickford	Moore	Oil Springs	Property 80	Nicholls Memorial	Pulford	Sandwich	Pump Station 6	Montgomery	Dougall	Walker	Chappus	Pond 8E	Pond 20E	Site 35	Pond 44N	Pond 42S	Pond 11W	Pond 45S	Pond 28W
Daucus carota	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Descurainia sophia																							x
Desmodium canadense		x	x	x				x	x		x				x	x	x	x					
Dichanthelium acuminatum																						x	
Dichanthelium sp											x				x								
Diervilla lonicera											x												
Digitaria ischaemum																							x
Diplotaxis muralis											x	x											
Dipsacus fullonum	x	x	x	x	x	x	x	x	x			x	x	x									
Doellingeria umbellata															x								
Dyssodia papposa																			x	x			
Echinochloa crus-galli			x					x		x		x		x						x	x		
Elaeagnus angustifolia													x										
Elaeagnus umbellata															x				x	x			
Eleocharis erythropoda															x								
Elymus canadensis								x	x	x	x	x				x	x	x	x		x		
Elymus repens	x	x	x		x	x		x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
Elymus virginicus				x							x						x	x				x	
Epilobium ciliatum			x	x					x	x	x	x			x	x							
Epilobium coloratum							x	x										x					
Epilobium hirsutum			x																				

Species	Highway 40								Herb Gray Parkway							Highway 407							
	Whitebread	Courtright	Bentpath	Bickford	Moore	Oil Springs	Property 80	Nicholls Memorial	Pulford	Sandwich	Pump Station 6	Montgomery	Dougall	Walker	Chappus	Pond 8E	Pond 20E	Site 35	Pond 44N	Pond 42S	Pond 11W	Pond 45S	Pond 28W
Equisetum arvense		x													x			x	x	x	x	x	x
Equisetum hyemale															x								
Erigeron annuus	x	x	x	x	x		x	x	x	x		x			x	x	x	x	x	x	x	x	x
Erigeron canadensis									x	x	x							x	x	x		x	x
Erigeron philadelphicus		x		x	x		x			x	x				x	x		x				x	x
Erucastrum gallicum																	x			x			
Erysimum cheiranthoides				x																			
Eupatorium altissimum										x			x		x								
Eupatorium perfoliatum																		x	x				
Euphorbia corollata															x								
Euthamia graminifolia			x	x			x	x	x	x		x			x	x	x	x	x	x	x	x	x
Eutrochium purpureum															x								
Festuca rubra	x	x			x	x									x	x	x		x	x	x	x	x
Fragaria virginiana			x				x	x							x			x				x	
Frangula alnus					x																		
Fraxinus sp		x	x										x		x								
Galium aparine			x																				
Galium mollugo					x											x	x	x		x	x	x	
Geranium maculatum		x													x								
Geum aleppicum			x	x																			
Geum canadense			x	x	x		x	x															

	Highway 40								Herb Gray Parkway							Highway 407							
Species	Whitebread	Courtright	Bentpath	Bickford	Moore	Oil Springs	Property 80	Nicholls Memorial	Pulford	Sandwich	Pump Station 6	Montgomery	Dougall	Walker	Chappus	Pond 8E	Pond 20E	Site 35	Pond 44N	Pond 42S	Pond 11W	Pond 45S	Pond 28W
Geum laciniatum				x																			
Geum sp			x																				
Glechoma hederacea										x		x	x										
Hamamelis virginiana											x												
Helenium autumnale								x															
Helianthus giganteus	x	x	x	x				x											x				
Heliopsis helianthoides		x	x	x												x			x				
Hesperis matronalis		x				x																	
Hibiscus moscheutos			x	x																			
Hieracium sp															x								
Hieracium umbellatum		x																					
Hordeum jubatum	x	x	x		x	x	x	x	x	x	x	x		x					x	x	x		
Hypericum perforatum		x	x	x												x			x	x		x	
Hypoxis hirsuta															x								
Impatiens sp					x																		
Inula helenium																x				x			
Juglans nigra		x									x												
Juncus articulatus																						x	
Juncus bufonius																		x					
Juncus compressus					x														x				
Juncus dudleyi			x														x	x	x	x		x	x

	Highway 40								Herb Gray Parkway							Highway 407							
Species	Whitebread	Courtright	Bentpath	Bickford	Moore	Oil Springs	Property 80	Nicholls Memorial	Pulford	Sandwich	Pump Station 6	Montgomery	Dougall	Walker	Chappus	Pond 8E	Pond 20E	Site 35	Pond 44N	Pond 42S	Pond 11W	Pond 45S	Pond 28W
Juncus effusus			x					x															
Juncus pendulatus															x								
Juncus pylaei			x																				
Juncus round fruits												x											
Juncus secundus									x														
Juncus sp										x					x	x		x					
Juncus tenuis				x												x		x					
Juncus torreyi										x		x			x				x				
Juniperus communis	x			x									x	x				x					
Krigia biflora															x								
Lactuca serriola		x			x		x		x	x	x	x						x		x	x		x
Lathyrus latifolius	x							x					x	x									
Lathyrus tuberosus		x		x	x	x																	
Leonurus cardiaca																							x
Lepidium campestre		x			x		x	x	x	x	x												
Lespedeza capitata		x						x							x								
Leucanthemum vulgare		x	x		x		x		x	x			x	x		x	x	x	x	x	x	x	x
Liatris spicata	x						x	x							x								
Linaria vulgaris									x	x	x		x						x	x			
Lobelia siphilitica																		x					
Lolium arundinaceum	x				x	x	x	x		x	x	x	x	x		x	x					x	

	Highway 40								Herb Gray Parkway							Highway 407							
Species	Whitebread	Courtright	Bentpath	Bickford	Moore	Oil Springs	Property 80	Nicholls Memorial	Pulford	Sandwich	Pump Station 6	Montgomery	Dougall	Walker	Chappus	Pond 8E	Pond 20E	Site 35	Pond 44N	Pond 42S	Pond 11W	Pond 45S	Pond 28W
Lolium perenne									x		x												
Lotus corniculatus	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
Lycopus americanus						x			x						x								
Lysimachia arvensis												x		x						x			
Lythrum salicaria				x					x	x		x										x	
Maianthemum stellatum																		x					
Medicago lupulina	x	x	x		x	x	x		x	x	x	x	x	x		x	x	x	x	x	x	x	x
Medicago sativa					x								x				x	x	x	x		x	x
Melilotus albus	x				x				x	x	x	x				x	x	x	x	x	x	x	x
Melilotus officinalis	x	x	x		x	x	x	x	x	x	x	x	x	x					x	x			
Melissa officinalis												x											
Mentha canadensis												x									x		
Monarda fistulosa	x	x	x					x	x	x	x	x			x								
Myosotis arvensis										x													
Nepeta cataria						x							x	x									
Oenothera biennis	x		x		x				x	x	x	x					x	x	x	x		x	
Oenothera perennis																						x	
Onoclea sensibilis															x								
Osmunda regalis															x								
Oxalis stricta											x	x	x		x	x	x		x			x	
Oxybasis glauca																		x					

	Highway 40								Herb Gray Parkway							Highway 407							
Species	Whitebread	Courtright	Bentpath	Bickford	Moore	Oil Springs	Property 80	Nicholls Memorial	Pulford	Sandwich	Pump Station 6	Montgomery	Dougall	Walker	Chappus	Pond 8E	Pond 20E	Site 35	Pond 44N	Pond 42S	Pond 11W	Pond 45S	Pond 28W
Panicum capillare									x		x							x		x			
Panicum virgatum	x	x	x	x			x		x	x	x	x			x		x	x					
Parthenocissus quinquefolia											x		x					x					
Parthenocissus sp																x							x
Parthenocissus vitacea						x								x				x					
Pastinaca sativa														x							x		
Penstemon digitalis	x		x	x			x	x	x	x	x	x											
Penstemon hirsutus			x																				
Persicaria maculosa				x					x	x	x	x	x					x			x		
Phalaris arundinacea								x									x	x		x	x	x	x
Phleum pratense	x						x	x	x	x	x					x	x	x		x	x	x	
Phragmites australis ssp. australis	x	x	x	x	x	x			x	x		x	x	x	x	x	x		x	x	x	x	x
Physocarpus opulifolius																	x						
Picea glauca																		x					
Pilosella caespitosa					x			x							x								x
Plantago lanceolata	x	x							x	x	x	x		x	x								
Plantago major	x	x	x	x		x					x							x			x	x	x
Plantago rugelii										x	x					x		x		x			
Poa compressa		x	x	x	x		x	x	x	x		x	x	x	x	x		x	x		x	x	
Poa pratensis	x	x		x			x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x
Poaceae sp 1															x								

	Highway 40								Herb Gray Parkway							Highway 407							
Species	Whitebread	Courtright	Bentpath	Bickford	Moore	Oil Springs	Property 80	Nicholls Memorial	Pulford	Sandwich	Pump Station 6	Montgomery	Dougall	Walker	Chappus	Pond 8E	Pond 20E	Site 35	Pond 44N	Pond 42S	Pond 11W	Pond 45S	Pond 28W
Poaceae sp 2													x										
Poaceae sp 3							x																
Polygala sanguinea															x								
Polygala verticillata															x								
Polygonum aviculare									x														
Polygonum sp																		x			x		
Populus alba													x									x	
Populus grandidentata									x													x	
Populus sp									x		x	x	x		x		x	x					
Populus tremuloides																			x		x		
Potentilla anserina															x								
Potentilla indica											x												
Potentilla norvegica					x		x												x				x
Potentilla recta										x		x			x	x			x	x		x	
Potentilla simplex															x								
Potentilla sp																		x					x
Prunella vulgaris									x	x	x	x			x								
Prunus sp															x							x	
Prunus virginiana																		x					
Prunus x cistena	x																						
Puccinellia distans																		x			x		

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Pycnanthemum verticillatum var. pilosum			x																				
Pycnanthemum virginianum	x		x	x			x	x		x		x			x	x		x					
Pyrus calleryana?								x															
Quercus alba									x		x												
Quercus bicolor		x																					
Quercus macrocarpa							x																
Quercus rubra							x																
Ranunculus acris																		x		x			
Ranunculus repens																		x					
Ratibida pinnata	x		x	x			x	x	x	x		x	x										
Rhamnus cathartica											x			x	x	x						x	x
Rhus aromatica											x												
Rhus copallinum											x												
Rhus typhina	x								x	x	x		x				x	x		x		x	
Robinia pseudoacacia									x	x	x												
Rosa sp			x							x			x					x					
Rosaceae shrub																	x						
Rubus allegheniensis															x							x	
Rubus idaeus										x											x		
Rubus occidentalis		x								x					x							x	

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Rubus odoratus																		x					
Rudbeckia hirta		x	x	x	x			x	x	x	x	x			x	x	x	x	x			x	
Rumex crispus	x		x	x	x	x	x	x	x	x	x	x		x				x	x	x	x	x	x
Salix bebbiana?																		x					
Salix eriocephala																	x					x	
Salix exigua?																						x	
Salix petiolaris															x								
Salix sericea?																						x	
Salix sp									x							x		x			x	x	
Sambucus sp																	x						
Schizachyrium scoparium											x				x								
Schoenoplectus acutus										x		x											
Scirpus atrovirens									x									x	x			x	
Scirpus pendulus			x																	x			
Scleria triglomerata															x								
Securigera varia	x	x								x	x								x				
Setaria faberi							x	x		x	x	x	x										
Setaria pumila	x	x	x		x	x	x	x	x	x	x	x				x	x	x	x		x		x
Setaria viridis			x											x						x		x	
Silene latifolia									x	x								x					
Silene noctiflora																						x	

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Silene vulgaris																x							
Silphium laciniatum	x																						
Silphium perfoliatum	x		x						x	x		x											
Silphium terebinthinaceum				x			x																
Sinapis arvensis			x	x	x				x		x		x					x					
Sisyrinchium montanum															x	x				x		x	x
Solanum carolinense											x				x								
Solanum dulcamara						x					x			x			x						
Solidago canadensis/altissima	x	x	x	x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x	x	x	x
Solidago gigantea																			x				
Solidago juncea															x								
Solidago nemoralis								x							x								
Solidago riddellii			x	x			x	x							x								
Solidago rigida	x			x			x	x	x	x	x	x	x										
Solidago rugosa									x						x								
Solidago sempervirens										x	x	x	x	x									
Sonchus arvensis	x	x	x	x	x	x			x	x	x	x	x	x		x	x	x	x	x	x	x	x
Sonchus asper																		x					
Sorghastrum nutans	x	x	x	x			x	x	x	x	x	x			x	x		x	x				
Spergularia media												x		x									
Symphyotrichum ciliatum																							x

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Symphyotrichum cordifolium																		x					
Symphyotrichum ericoides	x	x	x	x	x	x	x	x	x	x	x	x	x	x		x		x	x	x		x	x
Symphyotrichum laeve															x			x					
Symphyotrichum lanceolatum	x	x	x	x	x	x	x	x	x	x		x				x	x	x	x	x	x	x	x
Symphyotrichum novae-angliae	x	x	x	x	x		x	x		x		x	x				x	x	x	x	x	x	x
Symphyotrichum pilosum	x		x	x	x		x	x	x	x	x	x			x								
Symphyotrichum praealtum							x		x						x								
Symphyotrichum urophyllum															x			x					
Taraxacum erythrospermum				x	x	x		x	x	x	x	x				x		x	x		x	x	x
Taraxacum officinale	x	x	x	x	x	x			x	x	x	x	x			x	x	x	x	x	x	x	x
Thlaspi arvense		x		x					x		x		x					x					x
Thuja occidentalis																		x					
Tilia americana												x											
Tradescantia ohiensis			x																				
Tragopogon dubius											x												
Tragopogon sp																							x
Trifolium campestre										x									x				
Trifolium pratense	x	x		x			x	x	x	x	x	x		x		x	x	x	x	x	x	x	x
Trifolium repens									x	x	x	x				x	x	x	x		x	x	x
Tripleurospermum inodorum																		x					x
Tussilago farfara																x	x	x		x	x	x	x

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Typha angustifolia		x			x	x											x	x	x		x	x	x
Ulmus sp		x	x							x	x	x											
Unknown dicot 1														x									
Unknown dicot 2															x								
Unknown dicot 3															x								
Unknown dicot 4																		x					
Unknown dicot 5										x													
Unknown dicot 6												x											
Unknown woody sp 1				x								x											
Unknown woody sp 2			x																				
Unknown woody sp 3					x																		
Verbascum blattaria									x	x	x	x											
Verbascum thapsus		x			x				x			x							x	x	x	x	x
Verbena hastata					x					x	x	x						x					
Verbena stricta												x											
Verbena urticifolia										x		x	x		x								
Vernonia missurica			x	x			x	x		x		x			x								
Veronica officinalis									x	x													
Veronicastrum virginicum	x			x				x							x								
Viburnum opulus ssp. trilobum									x														
Vicia cracca		x			x		x		x	x	x			x		x	x	x	x	x	x	x	x

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Vicia sativa			x							x			x										
Vicia tetrasperma										x													
Vicia villosa							x		x														
Vincetoxicum rossicum													x							x			
Viola sagittata															x								
Vitis riparia		x	x	x			x	x	x	x	x	x	x	x	x	x		x	x	x	x	x	x
Xanthium strumarium																				x			