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## The Influence of Adjacent Forest and Agriculture on Restored Grassland Diversity and Composition

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Graduate Program in Biology

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## Abstract

Little native grassland remains in North America due to land-use changes. Conversion to agriculture is a common means of loss. This fragmentation creates edges in the landscape and associated edge effects. Grassland plant communities are susceptible to edge effects, directly via dispersal and indirectly through environment. This study took place in Norfolk County, Ontario, Canada, in a landscape of agriculture, forest, rural development, and patches of grassland restored by Nature Conservancy Canada. I examined restored grassland edges bordering forest and crops. An intensive study at a single site identified spatial and soil environmental influences on plant diversity and composition. I also sampled vegetation at six replicate restored grassland sites bordering both forest and crops to find patterns. Environment and space explained plant composition, but plant traits did not. Site attributes can likely explain edge effects case by case. Distinguishing between site and temporal effects will be important for future studies.

## Keywords

Edge effects, grassland, plant composition, restoration, core habitat, agriculture, aboveground-belowground linkages, plant functional traits, forest succession

## Co-Authorship Statement

Dr. Zoë Lindo contributed substantially to study design, thesis editing, and to the structure of this document. A manuscript may be submitted for future publication.

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## Chapter 1

### 1 General Introduction

#### 1.1 Grassland restoration in fragmented landscapes

Climatic changes marking the end of the Pleistocene Era caused glaciers to recede and migrations of plants and animals in North America (Sherow 2007). Grasslands developed at this time as novel ecosystems on newly deposited soils (Sherow 2007). Over time, indigenous human management of grasslands (e.g. fire) influenced certain plant and animal populations, and in turn human hunters (Sherow 2007). This human influence occurred during a time of climatic variability, and both factors greatly determined the communities existing today (Sherow 2007). However, over the last 200 years (post-European settlement), approximately 99% of North American grassland has been lost through land conversion, largely to agriculture, and also to other human development (Samson and Knopf 1994). Therefore, grasslands now exist either as remnant patches, where changes in plant composition are accelerating (Alstad et al. 2016), or as restored habitat, re-vegetated with varied outcomes (Millikin et al. 2016). In North America, conservation efforts seek to preserve grassland as a rare habitat, and meanwhile, restoration initiatives seek to reverse the loss of prairie and restore biodiversity and ecosystem function (Tilman et al. 2014).

This fragmentation and loss of grassland habitat negatively affect the vascular plant species (and seed) pool, as well as seed dispersal ability among grassland fragments (Poschlod et al. 1998, Bakker and Berendse 1999), because dispersal depends highly on proximity to, and pathway from, source plant communities. Thus, grassland restoration often involves the application of a seed mixture, the species diversity of which will hinge on restoration goals (e.g. weed suppression, high diversity), as well as time and budget constraints (Török et al. 2011). Limited seed availability is a top constraint in grassland restoration (Rowe 2010), and ideal seeding rates and composition do not often align with restoration project budgets. Goldblum et al. (2013) found the optimal seeding rate of

bulk seed mixes for tallgrass prairie restoration to be 56.0 kg/ha, which maximized diversity and increased the presence of high conservation value plant species, with higher seeding rates showing few advantages. The species diversity of a seed mix, another factor in obtaining propagules for restoration, may vary due to land parcel size and budget constraints, as high diversity seeding can be very costly, especially over large areas (Török et al. 2011).

Soil composition and fertility also play into restoration planning. High co-existence of vascular plant species in semi-natural grasslands is associated with low soil fertility (Janssens et al. 1998). However, when restoration occurs on formerly agricultural land, soils are often highly fertile due to residual fertilizers applied for crop cultivation, thus slowing grassland plant community re-assembly, unless nutrients are removed (Bakker and Berendse 1999, Walker et al. 2004). Both the establishment of restored plant communities and their maintenance after establishment depend on multiple factors. Bakker et al. (2003) identified that the effectiveness of restoration management can vary with year (e.g. precipitation), which will interact with the competitive abilities of introduced species. Herbicide application, cutting, burning, mechanical removal, grazing, and seeding are all methods used to reduce unwanted vegetation and increase diversity of desired species (Bakker and Berendse 1999, Kettenring and Adams 2011). Control over what species establish at a restoration site is limited, in part because unwanted species may already be present in the seed bank, dispersed there by former populations, populations within dispersal range, or otherwise transported there (actively or passively) (Bakker et al. 1996).

Due to the aforementioned land conversion, grassland restoration often occurs in fragmented landscapes (Bakker and Berendse 1999), on sometimes isolated, relatively small patches that share borders with a variety of land cover types. The shape and size of the patch (perimeter : surface area) will largely determine the extent of edge effects within these patches (Ries et al. 2004). Associated with both biotic and abiotic factors, and most commonly studied within forests, edge effects are also known to occur at grassland edges (Bogaert et al. 2001, Gieselmann et al. 2013, Lee and Power 2013, Rowe

et al. 2013, Winsa et al. 2015, Taft 2016). The severity and nature of edge effects in grasslands can range from damaging, such as species invasion at roadsides (Lee and Power 2013, Rowe et al. 2013), to beneficial, preventing exotic species invasion and maintaining species richness in native prairie when adjacent to restored grassland (Rowe et al. 2013). Grasslands commonly border agricultural fields grown in monoculture (Samson and Knopf 1994), where chemicals are applied to crops and 'weeds' (whether ecologically beneficial or problematic) are common within crop fields and at their perimeters, acting as source populations for surrounding plant communities (Marshall and Moonen 2002). Therefore, knowledge of how a particular land cover type can affect adjacent grassland vegetation within a landscape can lend to restoration success.

Identifying and quantifying edge effects (e.g. the distance from an edge that effects are observed), and a 'breakpoint' in edge effects (the distance at which edge effects are no longer observed), can help determine the overall grassland area effectively restored, depending on grassland parcel size and shape (Bogaert et al. 2001). As breakpoint distance can differ depending on the adjacent land cover type, bordering land covers will be an important consideration in restoration planning, whether in choosing a restoration site, or in an effort to protect existing restored grassland or remnant prairie (Gieselman et al. 2013, Rowe et al. 2013).

## 1.2 Determining restoration success as influenced by edges

Defining restoration success will depend on many factors, and will, of course, depend on restoration goals. Ehrenfeld (2000) discusses the complexity of restoration ecology, and suggests that setting restoration goals should be flexible and appropriate to the unique conditions, and recognize the project scope and limitations at the outset. Mimicking natural conditions serves as a standard of restoration success for some, with the primary focus on reviving communities of associated organisms (Ehrenfeld 2000). Often partnering with this approach is species-centered restoration, which has led to the successful delisting of endangered species (Ehrenfeld 2000). While delisting endangered species is clearly a marker of success, a complication is that while methods may succeed for some taxa, the same approaches may not be beneficial for other taxa (Ehrenfeld

2000). Other goals in grassland restoration may focus primarily on vegetative establishment. Kimball et al. (2015), by comparing native versus non-native plant germination, growth, cover, and density, found that re-vegetating the greatest area per dollar spent did not equate to optimal successful native plant establishment. Additionally, plant species that are often selected and planted for restoration (e.g. food plants of invertebrates targeted for conservation) can perform poorly in the fertile, formerly agricultural land that is often available for restoration (Pywell et al. 2003). Still, restoration success of desirable species is a priority, and restoring these species in high diversity is also a priority, as recent research shows a relationship between diversity and ecosystem productivity, stability, invasibility, and nutrient dynamics (Tilman et al. 2014). These diversity-stability relationships have been suggested as important considerations during the planning phase of restorations for their increased success (Seabloom 2007). Ultimately, the practicality of a restoration endeavor (feasibility, costs), site conditions, site history, and the source and availability of propagules will largely determine restoration success (Török et al. 2011). What is truly restorable for a grassland ecosystem is not fully known, but many believe that attempts should be made to mimic the high diversity found in remnant prairie (Martin et al. 2005). In addition, grassland restoration is complicated by the fact that its ecological properties will change over time. For instance, Camill et al. (2004) found rapid changes in functional diversity of restored grassland to greatly affect ecosystem-level properties, such as the dominant plant species, litter mass, and carbon and nitrogen mineralization rates. Understanding the mechanisms behind such ecosystem changes will then be important for grassland restoration.

Grassland edges, and associated edge effects from neighboring land cover types, may be common and in high density in certain fragmented landscapes (Ma et al. 2013).

Therefore, the effects of these landscape fixtures should be part of the equation when determining restoration success. However, the elements of successful restoration, some of which are presented above, do not necessarily include the successful establishment of vegetation at habitat edges, even though edges have been shown to be major portals for exotic species invasion (Gieselman et al. 2013). Gradients in abiotic and biotic factors will naturally occur at boundaries between two dissimilar land cover types, such as the

temperature change moving from a shaded deciduous forest, through a partially shaded ecotone, and into an open, unshaded meadow. Because environmental gradients will encourage species sorting and the realization of niches, we should expect that edges in the landscape will encourage competition among plant species, and this competition will likely involve unwanted, weedy plant species, which may have high dispersal rates and establish quickly in disturbed locations (Gelbard and Belnap 2003). Therefore, understanding the ties between environment and plant composition will be important for achieving successful vegetative restoration of grassland, including their perimeters, which if diminished, will decrease the total area of grassland effectively restored.

### 1.3 Understanding plant composition using plant-soil relationships

The importance of understanding the interactions between aboveground (plants) and belowground (soil) components of an ecosystem is growing with global change, including land use change (Wardle et al. 2004). Wardle et al. (2004) stress that aboveground and belowground communities can be mutual drivers of ecological processes and elicit both positive and negative feedbacks. As an example, Kardol et al. (2006) found that a negative feedback between a plant community and its associated soil pathogens can encourage grassland succession, and a positive feedback between plants and mycorrhizal fungi can slow succession and promote community evenness. In a different study of plant-soil interactions, Wilsey and Potvin (2000) found total and belowground biomass to increase linearly with increasing evenness using experimental plantings in an old field, while aboveground biomass-evenness relationships depended on the species. Thus, the authors suggested a decrease in evenness would indirectly reduce total primary productivity.

The concepts of aboveground-belowground linkages can be applied to both the natural processes present and human interventions used in restoration ecology. Kardol and Wardle (2010) suggested that integration of aboveground-belowground linkages into restoration ecology would aid in the success of this applied field of study. For example, Kardol and Wardle (2010) examined aboveground-belowground linkages from the



species to the ecosystem level, and found, as examples, that the effects of grazing can slow processes from plant growth rates to overall net primary production; meanwhile top soil removal could lead to lower rates of decomposition. In another study, Maron and Jeffries (2001) found that mowing and biomass removal in coastal grassland caused a shift from exotic annual grasses to mixed exotic and native perennial forbs, and increased species richness, while aboveground biomass decreased and belowground biomass increased. This, in turn, increased vegetative nitrogen retention, which is associated with decreased plant species richness, increased plant biomass, and increased exotic plant invasion (Maron and Jeffries 2001).

Although the principles of aboveground-belowground linkages are beginning their integration into restoration ecology as a discipline (Kardol and Wardle 2010), information is lacking with regard to aboveground-belowground linkages at grassland edges. However, one study by Steinaker and Wilson (2005), within a grassland-forest boundary of the Great Plains, found no difference in nitrogen contribution from litters between forest and grassland, but found differing root distributions as a potential cause for the higher nitrogen in forest soils. Also looking at grassland-forest edges in the Great Plains, Pinno and Wilson (2011) found a strong relationship between total root biomass and total aboveground biomass, with rapid shifts in carbon storage at edges due to woody encroachment. These examples show the importance of aboveground-belowground interactions to ecosystems in general, and also highlight habitat edges as important ecological features in the landscape.

Edges are therefore a form of ecological transition and environmental change that can impact both biotic and abiotic factors, which includes plant community composition. Changes in plant composition at grassland edges can arise from two sources: species interactions with environmental conditions (e.g. soil chemistry), or through dispersal patterns. Influences from both environment and dispersal often result in a predictable general pattern: community compositions at sample locations close together have greater similarity, but communities become more dissimilar with increasing distance between them (Legendre 1993, Wagner and Fortin 2005). Therefore, one might then expect that

land cover adjacent to restored grassland will have a reach of influence, up to a certain measurable distance from the grassland edge, or its breakpoint. Such influence from adjacent land cover will likely affect both aboveground (i.e. species establishment) and belowground factors (i.e. soil moisture). The role of space will be in the nature of dispersal (i.e. dispersal distances) and other factors associated with particular neighboring land cover types (i.e. shading extent, litter fall distance). The manner in which organisms sort themselves will depend on their ecological niche (Hutchinson 1991). This concept of species sorting may then be applied to species sorting of vascular plant species along abiotic and biotic gradients in grassland (including grassland edges), and this sorting will occur according to plant traits (Weiher 2011).

## 1.4 Trait based approaches

The traits of plant species within a community lend to the functional diversity of an ecosystem. Functional diversity is recognized as being beneficial for ecosystem functions, including productivity and resistance to invasion (Mason et al. 2005). Because humans depend on ecosystems for the services that they provide (ecosystem services), priority should be given to understanding ecosystem function and the effects of land use change (Díaz et al., 2013). Garnier et al. (2004) suggested that ecosystem properties depend on plant traits as well as the proportion of species' biomass within an ecosystem (biomass ratio hypothesis). It has also been suggested that functional diversity (the value and range of species traits) is more influential than species richness in determining ecosystem function (Díaz and Cabido 2001). Cornwell and Ackerly (2009) suggested that the distribution of traits and the assembly of plant communities are determined by both habitat filters and a limit to the similarity in species that can coexist.

Trending in community ecology is the use of species traits that generally reflect trade-offs in growth / competitive ability and dispersal / colonization abilities to predict community compositional changes under environmental gradients (Díaz and Cabido 2001, Lavorel and Garnier 2002). These trait-based approaches are pioneered by plant ecologists who have long demonstrated that plant functional traits are strongly correlated with large-scale environmental gradients, such as plant height with mean annual

temperature (Mokany et al. 2006). Less well known is how patterns of plant functional traits change across smaller spatial scales (Liu et al. 2012), particularly in a restoration context (Piqueray et al. 2015). Plant traits can explain both how plants will respond to changes in abiotic and biotic factors, and also the effects plants have on ecosystem processes, such as primary productivity and nutrient retention (Loreau 2000, Lavorel and Garnier 2002, Hooper et al. 2005). Examples of functional response traits include regenerative (dispersal) traits (e.g. dispersal mode, seed mass) that are highly associated with plant response to disturbance, and leaf traits and growth form, associated with response to light and soil resources (Cornelissen et al. 2003). With regard to the ecological concept of the niche, Cornwell and Ackerly (2009) showed a tight relationship between specific leaf area and soil water content and shading. At the same time, traits associated with dispersal ability influence distribution of plant species (Dirnbock and Dullinger 2004), and plant dispersal capacity is positively correlated with spatial patterns independent of environmental structuring (Nekola and White 1999). Therefore, functional trait diversity is expected to be spatially structured, and gradients in plant diversity from either abiotic or biotic factors can be explained by plant functional traits (Siefert et al. 2014). Recently, Biswas et al. (2015) demonstrated that functional trait diversity indices could be spatially explored along environmental gradients using methods similar to species abundance data.

Functional traits can inform restoration ecology as plant traits related to dispersal, establishment, and persistence incorporate the main challenges of plants to re-establish and survive in restored areas (Sandel et al. 2011). Funk et al. (2008) demonstrated how restoration success can be enhanced using a trait-based approach that ensures invasion resistance by maximizing functional diversity, while Camill et al. (2004) demonstrated that trait diversity changes rapidly within the first years following restoration initiatives in tallgrass prairie. More recently Piqueray et al. (2015) showed that trait diversity is influenced by different restoration phases mostly through modifications of environmental conditions. This observation suggests that adjacent land cover types will influence trait diversity along spatial scales associated directly with the dispersal abilities of adjacent habitat plant types, or indirectly through environmental factors on soil properties

associated with land covers. For example, trait-based responses of invasive species to changes in community and ecosystem properties can be used to understand how invasive plants and their impacts will respond to environmental change (Drenovsky et al. 2012). In their study of post-fire grassland restoration, Kulpa and Leger (2012) found that establishment of vegetation in restored areas can be predictable according to natural selection, yet mismatched with current restoration practices. Conservation and restoration activities often focus only on the presence and abundance of species for the measurement of species diversity, despite the dependence of ecosystem function (influenced by diversity) on the traits and niches filled by species (Cadotte et al. 2011).

## 1.5 History of the study region

The present study took place in Norfolk County, in southern Ontario. Indigenous use of natural resources in this area was practiced sustainably prior to European settlement (Weis 2007). However, the region has since become an area of conflict between current European agricultural/urban sprawl and Six Nations people dwelling within a diminishing First Nations reserve (Weis 2007). Beginning with European settlement, urban and agricultural development has extensively diminished forest cover in southern Ontario (Suffling et al. 2003). This region is part of what is known as Carolinian Canada, an ecozone highly impacted by human presence, currently with the highest human population density in Canada and the largest percentage of plant taxa prioritized for conservation (Catling and Porebski 1998).

The current study was conducted on the Norfolk Sand Plain, consisting of glacially deposited sands and silts, which were deemed ideal for farming by European settlers (Chapman and Putnam 1984). European settlement on the Norfolk Sand Plain began in the late 1700s, and the land underwent periods of cropping, soil exhaustion, reforestation, idleness, a tobacco boom, and then a switch to cropping, livestock, and feedstock production (Chapman and Putnam 1984, Weis 2007). Human development here has therefore created a patchwork of urban centers, agricultural fields, and protected lands. Habitat loss and fragmentation endangers species of this region, including many of the pollinators essential for agricultural production within southern Ontario (Kerr and Cihlar

2004, Taki et al. 2007). Nature Conservancy Canada (NCC) manages an interconnected network of protected lands in the Norfolk Sand Plain in Norfolk County, southern Ontario, within which existing forest fragments are conserved, and adjacent lands are actively restored as grassland by seeding with Carolinian species. These restored grasslands have potential for longer-term forest restoration through vegetative succession, while others may be maintained as tallgrass prairie or oak savannah (J. Crosthwaite, NCC, pers. comm., 2015).

In this habitat matrix, two land cover types commonly adjacent to restored grasslands are forest stands and active crop fields. Effects of bordering forest and agriculture on grassland success are important to understand, since they are common neighbors, and also to understand the reach of influence each land cover type has for the determination of 'edge' and 'core' habitat, and the resulting restoration strategies by managers.

Examples of potential abiotic and biotic inputs from forest and crop fields include nutrients from crop fields and the dispersal and/or establishment of forest plant species. These grassland sites share a common environment within Carolinian Canada on the Norfolk Sand Plain (Chapman and Putnam 1984), with similar management in place (herbicide spraying, etc.), and were seeded with many of the same species sourced from within the region. Within such a network of sites, with fairly similar history and maintenance, it is reasonable to look, at least as a starting point, for common patterns in plant composition and diversity at grassland edges. In order to determine the effect of land cover types adjacent to restored grasslands, I examine how plant composition, plant diversity, the belowground environment (measured as soil variables), and measured plant traits differ among grassland boundaries adjacent to differing land cover types.

## 1.6 Objectives

The overall goal of this research is to determine how different land cover types (crop fields — subsequently referred to as agriculture, and forest cover) commonly bordering restored grasslands in Norfolk County influence the spatial patterns of plant diversity and composition, and the causes of these patterns. My research objective has four parts: (1) find patterns in plant diversity and composition with distance from adjacent forest and

crop fields at a single restoration site of intensive study; (2) identify environmental and spatial variables influencing these patterns; (3) attempt to explain patterns using plant functional traits; (4) look for patterns in vegetation at forest and crop field boundaries across six replicate sites, all within close proximity and under similar management.

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## Chapter 2

# 2 Aboveground vegetation along restored grassland boundaries is explained by belowground variables not species traits

## 2.1 Introduction

Interactions between aboveground plants and belowground (soil) physical properties, chemistry, and biota have been shown to influence plant community structure and plant diversity (Hooper et al. 2000, Bardgett et al. 2005, De Deyn and Van der Putten 2005). For example, Harrison and Bardgett (2010) found negative biotic feedbacks to greatly regulate plant performance in mixed grassland communities in a range of soil conditions, in addition to significant abiotic factors. Aboveground and belowground components can be mutual drivers of this structure and diversity, exhibit both positive and negative feedbacks, and are suggested as key to understanding the role of biodiversity (both aboveground and belowground) in terms of global changes, including land use change (Wardle et al. 2004). Aboveground factors, such as plant litter inputs and plant traits, can influence belowground factors, such as soil chemistry and soil microbial communities (Orwin et al. 2010), which can in turn affect aboveground communities in terms of plant diversity and productivity (Van der Heijden et al. 2008, Eisenhauer et al. 2012). Critical changes in plant-soil feedbacks can lead to plant community compositional change (Inderjit and Cahill 2015), and thus understanding these feedbacks can help us predict changes in plant community composition (van der Putten et al. 2013).

Another growing area of research uses plant functional traits to understand plant community composition, how plant communities might respond to various global change factors (Tilman 1999, Kimball et al. 2016), and how plant communities relate to ecosystem-level processes such as nutrient cycling (Lavorel 2013). The use of plant functional traits has been suggested as a tool to guide restoration initiatives for rare and

endangered ecosystems, such as grasslands (Sandel et al. 2011). Because the main goal of vegetative restoration is to recreate the previous plant community structure following cessation of land use change, the selection of species, their proportions, and where they are seeded are key aspects of restoration ecology. However, another key aspect of restoration ecology is the re-initiation of ecosystem functioning of the habitat to ensure maintenance of vegetation composition and possibly to increase resistance against invasion by non-native species from surrounding land cover areas (Prober et al. 2005). Grassland restoration, through the seeding of a diverse mix of native grassland species, and the establishment of a high diversity of those species with minimal invasion of non-natives or otherwise weedy species, could therefore benefit from a greater understanding of the functional traits of successfully restored vegetation communities. In addition, this approach may ultimately link aboveground and belowground processes to further provide a greater understanding of restoration success (Kardol and Wardle 2010).

One significant influence on restoration considerations may also be the bordering land cover types adjacent to a site. Many restoration initiatives involve small areas of habitat, opportunistically acquired by local and regional land conservancies and stewardships (Ando et al. 1998, Groeneveld 2005). For instance, restored grasslands in southern Ontario, Canada commonly have heterogeneous boundary types, including native and non-native, highly managed land cover, such as natural forests, wood lots, and agriculture (pers. obs.). Ultimately, the plant composition of restored grasslands, and the ‘success’ of a restoration initiative might be affected by the bordering land cover type, and the distance to which that land cover type has influence on both the aboveground and belowground elements of the grassland. These ‘edge effects’ may arise from spatial processes such as the dispersal and establishment of non-planted seeds from adjacent land cover, or through indirect environmental factors such as alterations in soil moisture through shading (Breshears 2006), both that could affect species establishment in the restoration initiative. Absolute distance from adjacent land cover boundaries can be a strong factor in determining both aboveground and belowground factors (Gieselman et al. 2013, Rowe et al. 2013, Taft 2016), because abiotic and biotic factors arising from proximity to boundaries produce these edge effects (Duncan and Duncan 2000, Cilliers et

al. 2008). Further to this, the distance that these edge effects penetrate (referred to here as the ‘breakpoint’) will depend on the adjacent land cover type (Gieselman et al. 2013, Lee and Power 2013, Rowe et al. 2013), but may also be predictable among similar adjacent land cover types, depending on the similarity of the seed banks and propagule availability; this similarity may be in species composition or in plant functional traits. For instance, Garnier and Navas (2012) demonstrated that how plant species sort themselves along environmental gradients was highly dependent on plant traits through habitat filtering and limiting similarity. Breakpoints between edge habitat and interior habitat may be identified through patterns of compositional change; plant composition continually changes moving away from an edge and then becomes homogenous indicating the grassland interior (Gieselman et al. 2013). Effectively determining the dimensions of edge habitat through locating the breakpoint in grassland plant composition may provide insight into the minimum size of restoration initiatives (Gieselman et al. 2013), or an indication of cost-benefit trade-offs for maintenance of restored grasslands (Kimball et al. 2015).

The overall goal of this study was to use a trait-based approach for plant community composition to understand spatial and environmental factors for a single restored grassland site. By quantifying aboveground plant community composition, plant functional traits, and the belowground (soil) physical, chemical and biological properties, I aimed to further our understanding of restoration challenges for grasslands in southern Ontario. The approach of this study (Chapter 2) was exploratory, and not designed to test a specific hypothesis, although I used parametric statistics to elucidate trends in my variables measured acknowledging pseudo-replication and therefore low statistical inference. The specific objectives of this chapter were to:

- 1) characterize a ‘successful’ restored grassland site in Norfolk County, Ontario in terms of plant composition, soil variables, and a subset of plant functional traits associated with the plant species at the site,
- 2) apply a plant functional trait-based approach to understanding aboveground (plant) and belowground (soil) linkages,

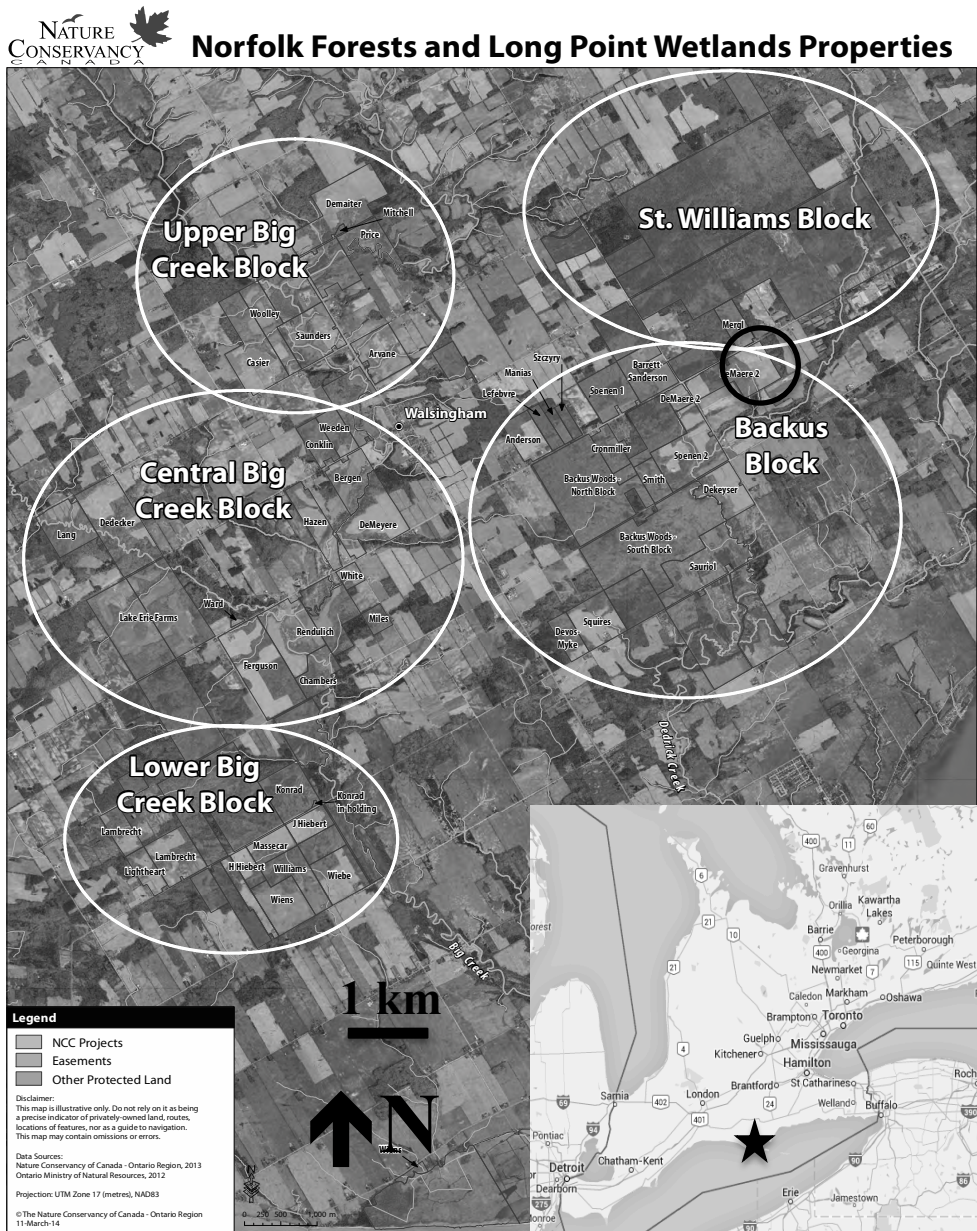
- 3) explore the spatial and environmental contributions to structuring plant community composition in this grassland as they pertain to adjacent land cover borders, and in doing so,
- 4) establish hypotheses regarding the influence of adjacent land cover on plant diversity and composition, which I test and discuss in Chapter 3.

## 2.2 Methods

### 2.2.1 Study area

The study site (DeMaere2 –Home Field, hereafter referred to as DeMaere2 in this chapter, and DeMaere2 (HF) in Chapter 3) is a former tobacco field (followed by rotations of soybeans used for weed control and corn) purchased by Nature Conservancy Canada (NCC) and subsequently seeded with native southern Ontario grassland species in 2010 (Appendix A). The DeMaere2 site (approximately 0.2 km<sup>2</sup> in size) is located approximately 17.5 km north of Long Point, Ontario, and lies within a larger matrix of agricultural, forested, and restored grassland blocks in Norfolk County, southwestern Ontario, Canada (42°41'13.52"N, 80°27'58.47"W) (Figure 2.1). This region is located on the Norfolk Sand Plain and thus soils at DeMaere2 are characterized by coarse sand and silt (Chapman and Putnam 1984). Seeding rates for the site at the time of planting (2010) were as follows: 135.95 kg/km<sup>2</sup> graminoids, 87.32 kg/km<sup>2</sup> forbs, 500 kg/km<sup>2</sup> large woody species, 19.90 kg/km<sup>2</sup> small woody species, and 952.83 kg/km<sup>2</sup> millet (as cover). Herbaceous seed was spread in early April and others planted in early May 2010 (NCC seeding records).





**Figure 2.1** Map of the Norfolk County, Ontario, Canada region showing the location of Nature Conservancy Canada properties. Designated blocks are indicated by white circles. The DeMaere2 Home field site sampled for this study is circled in black. Map provided by and reproduced with permission from NCC. Scale is approximate. The black star on the inset map in lower right shows the location of Norfolk County within southwestern Ontario.

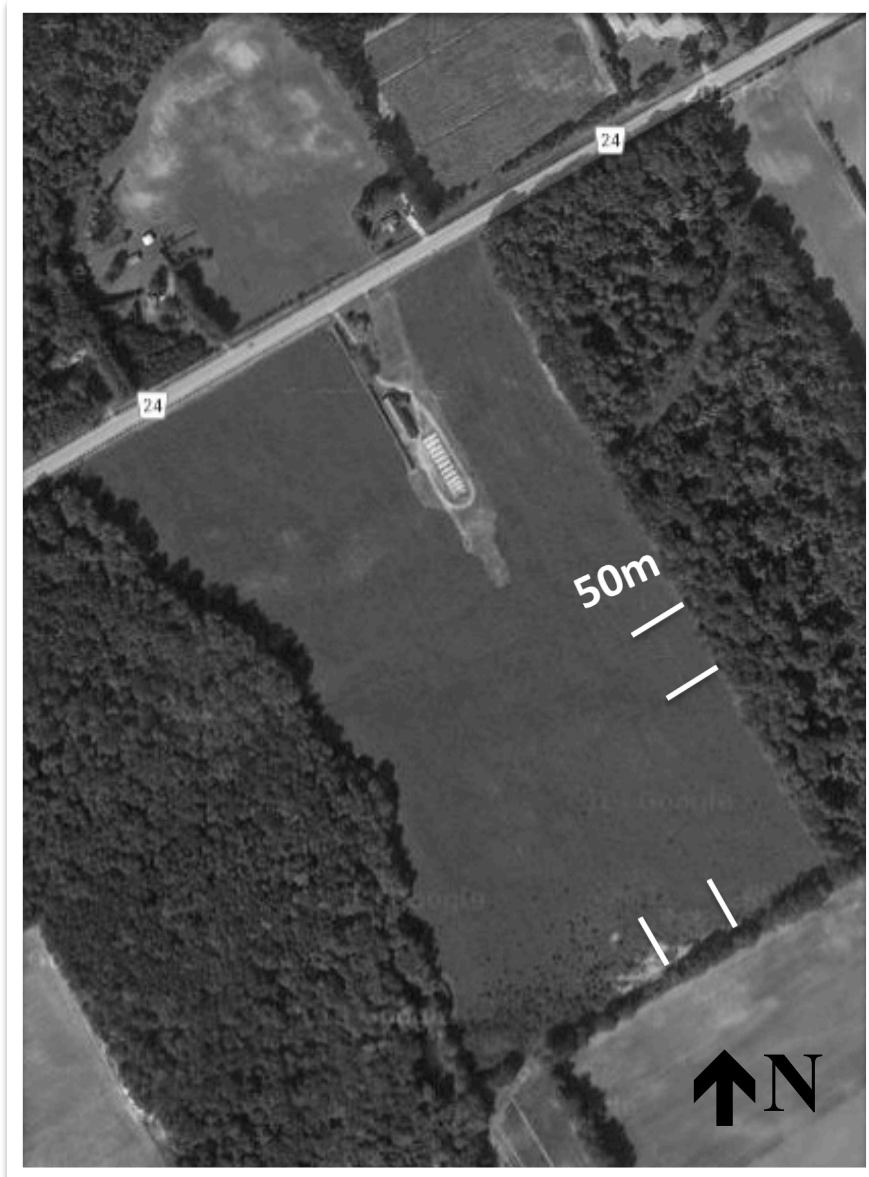
The DeMaere2 site was chosen for the current study due to its purported restoration success (pers. comm. with NCC) and shared borders with both forest to the east (Carolinian deciduous, 30-40 m canopy height) and a crop field to the south (winter wheat in 2014-2015). The bordering forest was predominately composed of oak and maple (*Quercus velutina* Lam., *Quercus rubra* L., *Acer* sp.) with lesser amounts of beech (*Fagus grandiflora* (Ehrh.) Little), alder (*Alnus incana* (L.) Moench), mulberry (*Morus* sp.), hickory (*Carya glabra* (Mill.) Sweet), and sassafras trees (*Sassafras albidum* (Nutt.) Nees), with a fairly open understory. The agriculture-grassland boundary was lined by deciduous trees (*Quercus rubra* L., *Populus deltoides* W. Bartram ex Marshall, *Acer* sp., *Prunus serotina* Ehrh., *Rhus typhina* L.) that were 30-40 m in height on a raised berm of soil that gently sloped downward into the grassland. A rural highway ran along the northern border of the grassland with another adjacent patch of forest along the western border.

### 2.2.2 Sampling design

Two 50 m transects were aligned perpendicular to the forest edge (running east to west) and two 50 m transects perpendicular to the agricultural edge (running south to north) (Figure 2.2). The minimum size requirement for selecting a restored grassland sampling area for this study was 0.01 km<sup>2</sup> of continuous grassland, such that four transects could be positioned at least 50 m apart from one another, as well as at least 50 m from other forested or agricultural boundaries. Transect origins were paced out by foot and placed at the base of the nearest and outermost tree or sapling trunk.

### 2.2.3 Vegetation sampling and trait measurements

In order to compare changes in plant diversity and composition with distance from adjacent land cover (forest and agriculture), a 1 m × 1 m plot was positioned at 0 m, 10 m, 20 m, 30 m, 40 m, and 50 m from each boundary, along each transect. All vegetation sampling (n = 24 plots) took place between June and July of 2015. Each plot was placed on the north (forest-grassland) or east (agriculture-grassland) side of each transect (for consistent sampling orientation for the observer). Within each 1m × 1m plot, all plant



**Figure 2.2 Aerial image of the DeMaere2 Home field site in Norfolk County, Ontario, Canada. This property was actively restored and is under management by Nature Conservancy Canada. White lines indicate 50 m transects used for vegetation and soil sampling in 2015 (two perpendicular to the forest edge to the east and two perpendicular to the crop field the south). Distinct topography, sandy slopes, and tree line are evident at the southern grassland border adjacent to the crop field. Image from Google.**

species were identified and visual cover estimates were recorded. Overlap was allowed in cover percentages, resulting in some total plot cover estimates greater than 100%.

For the measurement of plant functional traits, the greatest plant height of each species within each plot was measured, and three of the uppermost leaves of each species were sampled (from different individuals when possible, and representing the greatest spatial distribution within a plot). Species with less than three individuals in a plot were not sampled in that plot, unless the individuals covered a substantial proportion of the plot (e.g. tree saplings). Sampled leaves were stored and transported in labeled, sealable plastic bags with a moist paper towel and kept at 4 °C at Western University in London, Ontario, Canada until processing for further traits. These measurements took place within 1-3 days of vegetation collection.

The main trait of interest was specific leaf area (SLA), which is a numerical index of leaf size based on surface area and dry weight. The three leaves sampled for each species for each plot (n = 154) during vegetation surveys were attached to a white 8.5" × 11" sheet of paper using transparent tape, with the upper leaf surfaces facing up. Leaves were assigned a number and labeled accordingly (to be visible on scan images). The leaves were then scanned in color along with a metric ruler for later digital measurements, and images were saved as PDFs. Each leaf (with petiole) was then placed in an individual paper coin envelope and dried at 60 °C for 72 hours. Leaves were subsequently weighed to determine individual leaf dry mass.

Digital images were processed using ImageJ® to calculate the one-sided leaf area for each individual leaf. To calculate specific leaf area (SLA), one-sided leaf area (cm<sup>2</sup>) of each leaf was divided by the same leaf's dry mass (g). The SLA values for each of the three leaves (per species per plot) were averaged to represent that species within a particular sampling plot using the equation:

$$SLA = \frac{\left( \frac{\text{area leaf}_1}{\text{dry mass leaf}_1} \right) + \left( \frac{\text{area leaf}_2}{\text{dry mass leaf}_2} \right) + \left( \frac{\text{area leaf}_3}{\text{dry mass leaf}_3} \right)}{3}$$

Leaves showing herbivory or decay were removed from the dataset. Community weighted means (CWM) for SLA and height were calculated for each sampling plot using the equation:

$$CWM_{\text{trait}} = \sum (p_i \times \text{trait}_i)$$

where  $p_i$  is the proportional vegetation cover contribution of species  $i$  within a plot and  $\text{trait}_i$  is the trait value of species  $i$ . In addition to SLA and maximum plant height measurements, supplemental plant trait information was gathered from the following online sources: United States Department of Agriculture Plants Database (USDA, NRCS 2016), Ontario Wildflowers (Muma 2012), Ontario Grasses (Muma 2012), and Ontario Weeds (Ontario Ministry of Agriculture, Food, and Rural Affairs 2016). See Appendix B for a summary of these traits for the top 21 species observed at the DeMaere2 site (as determined by total percent cover:  $\geq 0.25 \text{ m}^2$  over all 24 plots), as well as CWM values calculated for measured traits (SLA and height). Traits other than SLA and height were categorical and therefore CWMs for these traits were not calculated.

#### 2.2.4 Soil sampling and analysis of environmental variables

In order to identify environmental variables causing changes in plant diversity and composition with distance from adjacent land cover, soil samples were taken on August 4, 2015 from the same plots used for vegetation sampling. From each plot, a  $2500 \text{ cm}^3$  (roughly  $16 \text{ cm}$  length  $\times$   $16 \text{ cm}$  width  $\times$   $10 \text{ cm}$  depth) block of soil was taken for soil laboratory analyses. Soil blocks were kept at  $4 \text{ }^\circ\text{C}$  until laboratory analyses were performed. Absolute dimensions ( $l \times w \times h$ ) of each soil block were recorded, and all live vegetation was clipped at the soil surface (shoot-root interface), placed in paper bags, dried in a  $60^\circ\text{C}$  oven for 72 hours, and weighed in order to determine dry weight of plant aboveground biomass for each block. To measure plant litter dry weight, any plant litter present was removed from the surface of each soil block, and also dried at  $60 \text{ }^\circ\text{C}$  for 72 hours and weighed. The remainder of each soil block was passed through a  $2 \text{ mm}$  sieve in order to homogenize soil for biological, physical, and chemical analyses. Plant roots encountered were carefully separated from the soil and washed with tap water in a fine

sieve to remove any remaining sand particles, then dried at 60 °C for 72 hours and weighed. Sieved soil from each soil block was kept in separate containers at 4 °C for further analyses.

#### 2.2.4.1 Soil physical analyses

Soil moisture was determined gravimetrically using 15 g of sieved soil from each soil block, which was weighed and dried at 60 °C for 48 hours, then weighed again in order to determine soil moisture. Soil moisture was calculated as the difference in mass between fresh (wwt) and dry (dwt) soil:

$$\% \text{ Moisture} = \left( \frac{\text{wwt (g)} - \text{dwt(g)}}{\text{dwt (g)}} \right) \times 100$$

Dried soil used for moisture calculations was then used to approximate soil organic matter content using the Loss-on-Ignition (LOI) method (Nelson and Sommers, 1982). Soil samples were further dried at 105 °C for 24 hours. Ceramic crucibles were cleaned with acetone, fired in a muffle furnace for 4 hours at 400 °C, and kept in a desiccator until used for sample measurement. Crucibles were then weighed, and to each crucible was added approximately 5 g (to the nearest 0.001 g) of dried soil from each soil block. Crucibles containing soil were then placed in a 400 °C muffle furnace for 24 hours, and cooled in a desiccator. Crucibles with soil were then weighed again, and mass after ignition was subtracted from mass prior to ignition in order to determine loss on ignition (LOI):

$$\% \text{ organic matter} = \left( \frac{\text{pre - ignition (g)} - \text{post - ignition(g)}}{\text{pre - ignition (g)}} \right) \times 100$$

#### 2.2.4.2 Soil biological analyses

To measure the basal respiration (heterotrophic CO<sub>2</sub> flux), 100 g (dry weight equivalent) fresh soil (sieved to 2 mm) from each soil block was added to a 500 mL glass jar with a vented lid (to maintain optimal moisture conditions) and allowed to acclimate to room

temperature (from 4 °C storage temperature) for 24 hours. Measurements were standardized for the total headspace in each jar. Respiration mesocosms (jars) were attached with surgical tubing to a unique port on a Licor InfraRed Gas Analyzer (IRGA) with a multiplexer unit, which allows each sample to be measured for 1.5 minutes (plus 30 seconds purge time) every 90 minutes. The CO<sub>2</sub> evolution was measured over 5 hours and 30 minutes, and the average respiration rate during this time was reported as  $\mu\text{l CO}_2/\text{g dwt}/\text{hour}$  as in Anderson (1982).

Following basal respiration measurements, soil samples were then evaluated for microbial biomass using the Anderson and Domsch (1978) glucose stimulated soil respiration Substrate Induced Respiration (SIR) method. In order to achieve maximum substrate-induced respiration prior to microbial growth, soil samples were detached from the IRGA and 10.0 mg glucose powder was added to the soil in plastic bags and shaken to mix. Soil samples were then replaced and re-attached to the IRGA machine, and allowed to cycle for 10 hours. Carbon dioxide evolution rates ( $\mu\text{l CO}_2/\text{g dwt}/\text{h}$ ) in the 6<sup>th</sup> hour of SIR measurement were used to calculate microbial biomass following the equation by Anderson and Domsch (1978):

$$\text{Microbial biomass C / g dwt} = 40.4y + 0.37$$

where  $y$  = glucose induced respiration ( $\text{ml CO}_2/\text{g dwt}/\text{h}$ )

### 2.2.4.3 Soil chemical analyses

To assess the available phosphorus ( $\text{PO}_4^{+3}$ ) of each soil sample, 40 mL Bray's solution (dilute ammonium fluoride solution) was added to 5 g (dry weight equivalent) sieved soil from each soil block in an Erlenmeyer flask. Flasks were shaken for 1 hour and filtered through Whatman #42 filter paper, using vacuum filtration. The filtrate was then stored in a -10 °C freezer until analysis. Immediately before analysis, the filtrate was thawed and available phosphate ( $\text{PO}_4^{+3}$ ) was analyzed colourimetrically using the fluoride method on an AA3 autoanalyser using 1:1 dilutions with dH<sub>2</sub>O where necessary (due to high initial phosphate concentrations in filtrates) (Olsen and Summers, 1982).

Available nitrogen ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ) was extracted from soil samples using 40 mL 1N KCl solution added to 5 g (dry weight equivalent) sieved soil from each soil block in an Erlenmeyer flask. Flasks were shaken for 1 hour and filtered through Whatman GF/A filter paper, using vacuum filtration. The filtrate was then stored in a  $-10^\circ\text{C}$  freezer until analysis. Immediately before analysis, the filtrate was thawed and available nitrogen analyses were performed colourimetrically using an autoanalyser, ammonium by the indophenol blue method (Keeney and Nelson, 1982) and nitrate using the hydrazine method.

Soil samples were assessed for pH by combining 2 g (dry weight equivalent) sieved soil from each soil block with 22 mL  $\text{dH}_2\text{O}$ , which were stirred to form a slurry. After 1 hour, a glass  $\text{H}^+$  sensing electrode and digital pH meter were used to measure soil pH. Soil slurries used to measure pH were then vacuum filtered using a Whatman #42 filter, and the filtrate was used to measure electrical conductivity (EC) in deci-Siemans/m ( $\text{dS/m}$ ) using a digital conductivity meter.

### 2.2.5 Field-based decomposition rate

To measure decomposition rates within sampling plots, dried vegetation and litter from all plots (collected as vegetation and litter biomass measures from soil blocks) was used to create two litterbags per sampling plot (vegetation, litter). Dried vegetation from all soil samples was homogenized and 0.85 g was used in each litterbag, similarly, dried plant litter was homogenized across all soil samples and 1.00 g was used in each litterbag. All vegetation and litter amounts were weighted and recorded to the nearest 0.001 g. Litterbags ( $5\text{ cm} \times 4\text{ cm}$ ) were made of 1 mm mesh fly screen and sealed using a heat sealer. Two litterbags (1 containing dried vegetation and 1 containing dried plant litter) were returned to each plot within the field site on September 27, 2015 and secured with a pin flag. Litterbags were retrieved fifty-five weeks later, on October 17, 2016, and oven-dried at  $60^\circ\text{C}$  for 48 hours. Contents were then carefully removed and re-weighed. Decomposition rates were assessed as mass loss using the following equation:



$$\% \text{ mass loss} = \frac{\text{initial dwt (g)} - \text{final dwt (g)}}{\text{initial dwt (g)}} \times 100$$

## 2.2.6 Statistical analyses

All plant and soil related variables were analyzed using a repeated measures Analysis of Variance (RM ANOVA) in Statistica 7 software (StatSoft Inc. 2004) with adjacent land cover as an independent variable, while distances along transects were used as repeating factors to account for spatial non-independence. I acknowledge that transects are not independent of each other, but use the parametric statistics to quantify differences among plots along these two edges bordering forest and agricultural lands. Plant variables analyzed at the plot level include: species richness (#spp/m<sup>2</sup>), total percent cover (%), Shannon's diversity indices (H'), Simpson's diversity indices (D), CWM values of SLA (cm<sup>2</sup>/g), and maximum plant height (cm), vegetation biomass (g dwt), and root biomass (g dwt). Soil variables include: soil moisture (% dwt), LOI (% dwt), available nitrogen, PO<sub>4</sub><sup>+</sup> (mg/L), pH (in dH<sub>2</sub>O), EC (dS/m), microbial basal respiration (ml CO<sub>2</sub>/g dwt/hour), microbial biomass (mg C<sub>micr</sub>/g soil dwt), litter dwt (g), and decomposition via mass loss (%). The RM ANOVA was followed by Tukey *post hoc* tests where necessary, in order to find significant differences in measured variables between pairs of all plots sampled at the DeMaere2 study site.

To further investigate breakpoints within transects (when significant distance effects were found using RM ANOVA), planned comparisons were performed in Statistica 7 software (StatSoft Inc. 2004) using three models: M1) breakpoint at 30 m (0 m, 10 m, and 20 m plots (as equivalents) contrasted with 30 m, 40 m, and 50 m plots (as equivalents)), M2) breakpoint after 30 m (0 m, 10 m, 20 m, and 30 m plots (as equivalents) contrasted with 40 m and 50 m plots (as equivalents)), and finally M3) breakpoint at land cover edge (0 m plots contrasted with 10 m, 20 m, 30 m, 40 m, and 50 m plots (as equivalents)). Adjacent land cover types of forest and agriculture for all three models were treated as contrasting variables to one another. The first two models are based on the breakpoint in species richness observed during vegetation sampling at the DeMaere2 field site, as well

as evidence in the literature for a 30 m breakpoint in plant composition of grasslands bordering agriculture (Gieselmann et al. 2013), while the third model tests a breakpoint hypothesized also by personal visual observation in the field.

A principal component analysis (PCA) ordination was performed using the ‘vegan’ and ‘ade4’ packages in R software (Dray and Dufour 2007, R Foundation for Statistical Computing 2013, Oksanen et al. 2015). I used plant composition data for the most abundant vascular plant species ( $n = 21$ ), defined as all species whose combined percent vegetation cover within all plots sampled at the DeMaere2 site in 2015 equaled  $0.25 \text{ m}^2$  or more across all plots. Main effects ANOVA was used to determine factors driving the first and second PC axes for plant community composition; ANOVA tested whether case score loadings were significantly related to adjacent land cover type or distance (categorical variables). Species factor coordinates relative to the first and second PC axes were used to interpret the PCA.

Nonmetric multidimensional scaling (NMDS) was also performed using the ‘vegan’ package in R software (R Foundation for Statistical Computing 2013, Oksanen et al. 2015) separately for both plant composition (% cover of each species within each plot) and the nine soil variables examined. Subsequent analysis by PERMANOVA (permutation ANOVA) was performed on plant composition and soil variables also using the ‘vegan’ package in R to determine whether plots were significantly different between adjacent land cover types (R Foundation for Statistical Computing 2013, Oksanen et al. 2015). SIMPER (Similarity Percent) analyses of plant species composition was performed to find average similarity of plots adjacent to each land cover type (forest, agriculture) and dissimilarity between plots adjacent to different land cover types using Primer 5 software (PRIMER-E Ltd. 2001). SIMPER also identifies the percent contributions of plant species as main drivers for within land cover edge type similarity, and between land cover edge type dissimilarity. I used a cutoff for contributions at 90%.

To test whether patterns of vegetation within plots were significantly related to their spatial location and/or soil environmental variables, I used Mantel and Partial Mantel tests with the ‘vegan’ package in R (R Foundation for Statistical Computing 2013,

Oksanen et al. 2015). The Mantel and Partial Mantel tests used dissimilarity matrices (Bray-Curtis, square root transformed) for plant composition (% cover data for each of the 67 species observed by plot) and soil environmental variables (all of the nine variables measured) and a distance matrix (Euclidean, no transformations) using latitude and longitude GPS data converted to decimal degrees. The Mantel test used 999 permutations to test for correlations among dissimilarity matrices via the Pearson method. The Partial Mantel tested for correlations between dissimilarity or distance matrices while controlling for the effects of a third covariate matrix (e.g. whether plant community composition is significantly correlated to spatial locations, while controlling for the environment (soil variables) changing over spatial locations). Distance decay plots were made using similarity values (1 – dissimilarity value from the dissimilarity matrices generated for Mantel tests) between pairs of sampling plots. For these distance decay plots, and unlike the Mantel correlations which used dissimilarities amongst all plot pairs, only within-transect pairwise similarities were used, thus the maximum distance examined was 50 m.

Lastly, to test for relationships among plant traits, plant species identifications and soil environmental variables, I used a co-inertia analysis called RLQ (Dolédec et al. 1996). RLQ analysis links three data matrices described here as: R (nine environmental variables across 24 sites), L (21 species across 24 sites), and Q (21 species each with seven traits) (Dolédec et al. 1996). Traits were chosen to represent distinct trait categories associated with differing plant organs, as recommended by Laughlin (2014). As not all plants identified in this study could be assigned species-level trait information, I focused on the top 21 species as determined by greatest percent cover (minimum 0.25 m<sup>2</sup> total cover across all 24 plots at the DeMaere2 site) for my final analysis of trait-environmental relationships using RLQ. The RLQ analysis was used to create an ordination of sample plots based on how they correspond to soil environmental variables and plant trait values. Significant trait – environment correlations were subsequently tested using the fourth-corner approach (Legendre et al. 1997, Dray and Legendre 2008), which finds correlations between specific R and Q variables. RLQ and fourth-corner analyses were

run using the ‘vegan’ and ‘ade4’ packages in R software (Dray and Dufour 2007, R Foundation for Statistical Computing 2013, Oksanen et al. 2015).

## 2.3 Results

### 2.3.1 Plant variables

Across all 24 plots, a total of 67 species were identified as morphologically separate species, with 39 species being identified to the species level, and an additional 13 to genus; 15 morphotypes could not be confidently confirmed to genus or species because they lacked key morphological traits at the time of sampling. The total number of vascular plant species encountered in transects bordering forest (42 species) was similar to that in transects bordering agriculture (43 species). See Appendix A for a list of species observed in this study, both seeded by Nature Conservancy Canada and not seeded.

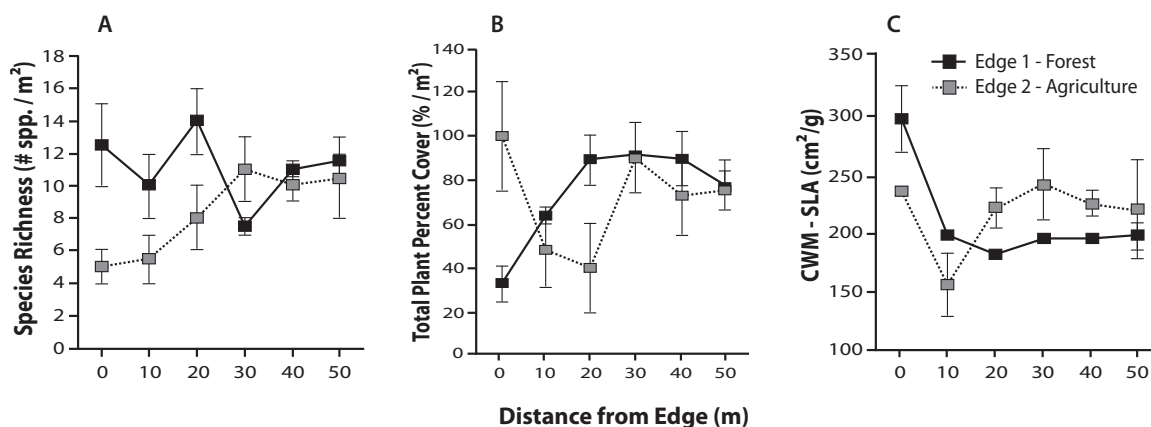
For the analysis of vegetation measures, RM ANOVA results showed no significant differences among plots bordering different land cover types (forest, agriculture), but did show interactive effects between land cover edge type and distance for species richness and percent vegetation cover of plots, as well as distance effects for CWM for SLA (Table 2.1). Transects bordering forest and transects bordering agriculture did demonstrate different patterns in species richness with distance from boundaries. Transects bordering agriculture show an asymptotic relationship with distance, increasing in species richness from 0 m to 30 m, with richness stabilizing between 30 m and 50 m. Transects bordering forest were somewhat variable with 30 m plots being considerably lower in species richness than at 0 – 20 m and 40 – 50 m (Figure 2.1A). Average total percent cover at the plot scale was similar between quadrats bordering forest and bordering agriculture, but highly variable (forest edge average 73% (ranging between 20% and 102%), agriculture edge average 70% (ranging between 17% and 125%)) (Figure 2.1B).

Specific leaf area (SLA) was relatively high at the forest edge (0 m) and relatively low at 10 m from the crop field edge, compared to the remainder of the plots (Figure 2.1C). Tukey *post hoc* tests for CWM SLA showed that significant differences were between 0 m and 20 m for forest transects and 0 m and 10 m for agriculture transects (Figure 2.1C). Planned comparisons using all three models (M1, M2, and M3) for species richness, percent cover, and CWM SLA were only significant for species richness under M1 ( $P = 0.028$ ), which contrasted 0 m, 10 m, and 20 m plots (as equivalents) with 30 m, 40 m, and 50 m plots (as equivalents). Model 2, which contrasted 0 m, 10 m, 20 m, and 30 m plots (as equivalents) with 40 m and 50 m plots (as equivalents), was also nearly significant for species richness ( $P = 0.052$ ). Model 3, contrasting 0 m plots with 10 m, 20 m, 30 m, 40 m, and 50 m plots (as equivalents) was nearly significant for percent cover ( $P = 0.058$ ), where the forest and agricultural plots at 0 m displayed relatively lower and higher percent cover than other plots, respectively (Figure 2.1B).

**Table 2.1 Repeated measures ANOVA results for plant variables (community, biomass, diversity indices, measured trait) for plots placed at 10 m increments from boundaries bordering two land cover types (forest (n = 2) and agriculture (n = 2)).**

Variable	Forest-edge	Agriculture-edge	Land Cover		Distance		Land Cover × Distance	
	Mean (± SE)	Mean (± SE)	F <sub>(1,2)</sub>	P	F <sub>(5,10)</sub>	P	F <sub>(5,10)</sub>	P
Species richness (#spp/m <sup>2</sup> )	11.1 (0.8)	8.3 (0.9)	4.11	0.180	1.61	0.244	3.66	<b>0.038</b>
Percent cover (%)	73.9 (6.9)	70.9 (8.5)	0.079	0.805	1.77	0.208	4.10	<b>0.028</b>
Vegetation dwt (g)	1.04 (0.36)	0.49 (0.18)	1.47	0.350	0.550	0.735	0.909	0.512
Roots dwt (g)	6.84 (1.24)	6.63 (1.86)	0.016	0.911	1.46	0.285	2.68	0.087
Shannon's Diversity (H')	0.728 (0.04)	0.642 (0.05)	1.41	0.357	0.921	0.506	0.534	0.746
Simpson's Diversity (D)	4.513 (0.44)	3.877 (0.44)	0.783	0.470	0.562	0.728	0.739	0.611
CWM SLA (cm <sup>2</sup> /g)	211.1 (12.6)	216.8 (11.5)	0.234	0.677	4.49	<b>0.021</b>	2.70	0.085
CWM Height (cm)	50.7 (3.8)	67.6 (11.3)	1.16	0.395	1.45	0.287	2.28	0.126

All plant measurements were taken at the 1 m × 1 m plot level. Land cover represents one of two vegetation types bordering restored grassland: forest (deciduous) or agriculture (crop field). Distance represents absolute distance of plots along 50 m transects within restored grassland at two edges (two perpendicular to forest and two perpendicular to agriculture). Significant *P* values are bolded.



**Figure 2.3 Plant variables A) Mean vascular plant species richness (number of species/square meter), B) Mean vascular plant species percent cover and C) CWM SLA of 1 m × 1 m vegetation plots, plotted against absolute distance from forest and agriculture boundaries. Values are means (with standard error) of plots (n = 24) within two transects per land cover edge type at each distance from the bordering land cover boundary. Data were collected from plots along 50 m transects within a restored grassland in Norfolk County, Ontario, Canada, with two transects perpendicular to forest (deciduous) and two transects perpendicular to agriculture (crop field). Black points and lines represent transects bordering forest (n = 2) and grey points and lines represent transects bordering agriculture (n = 2). Plant inventories took place during June and July of 2015. The site was seeded by Nature Conservancy Canada in 2010.**

The results of RM ANOVA testing for differences in diversity indices (Shannon's and Simpson's) were not significantly different between land cover edge types or among distances (Table 2.1). Likewise, neither vegetation nor root dry weights differed between land cover edge types or among distances (Table 2.1). Although not significant, plots bordering forest had a lower average CWM height than plots bordering agriculture (Table 2.1). The difference in CWM for plant height is attributed to high numbers of relatively tall *Salix* sp. (willow) saplings at 10 m along agricultural transect 1.

## 2.3.2 Soil variables

### 2.3.2.1 Physical variables

Results from RM ANOVA showed that soil moisture at DeMaere2 was not significantly different between plots bordering forest and plots bordering agriculture, or among plots at differing distances from boundaries (Table 2.2). However, average soil moisture among plots bordering forest was greater than among plots bordering agriculture (Figure 2.2A). Loss on ignition (LOI) was not significantly different between bordering land cover types or among distances (Table 2.2).

### 2.3.2.2 Chemical variables

Available nitrogen (combined results for nitrates and ammonium) was detected in greater amounts in soils bordering agriculture than in soils bordering forest, although available nitrogen was not significantly different between adjacent land cover types or among distances (Table 2.2). Phosphorus was greater in soils bordering forest than in soils bordering agriculture, although this effect was also not significant for land cover edge type or among distances, due to similar phosphorous content in soils 40 m and 50 m from each land cover type (Figure 2.2B). There was no significant difference between the pH of soils bordering forest and soils bordering agriculture, or among distances from boundaries (Table 2.2). Electrical conductivity was greater in agricultural edge soils than in forest edge soils, and while this variable did not show significant differences between land cover edge types or among distances alone, EC did show a significant interaction



between land cover edge and among distance from boundaries (Table 2.2, Figure 2.2C). Electrical conductivity, while variable between 0 m to 30 m from agricultural boundaries, was consistently higher than at 0 m to 30 m from forest boundaries, agricultural edge and forest edge plots at 40 m and 50 m were more similar to each other, with forest soil EC increasing and agricultural soil EC relatively low. *A priori* contrasts did not show significant breakpoints for EC. Tukey *post hoc* tests showed significant differences between two land cover edge/distance pairs: agriculture 10 m with both forest 30 m and agriculture 50 m.

### 2.3.2.3 Biological variables

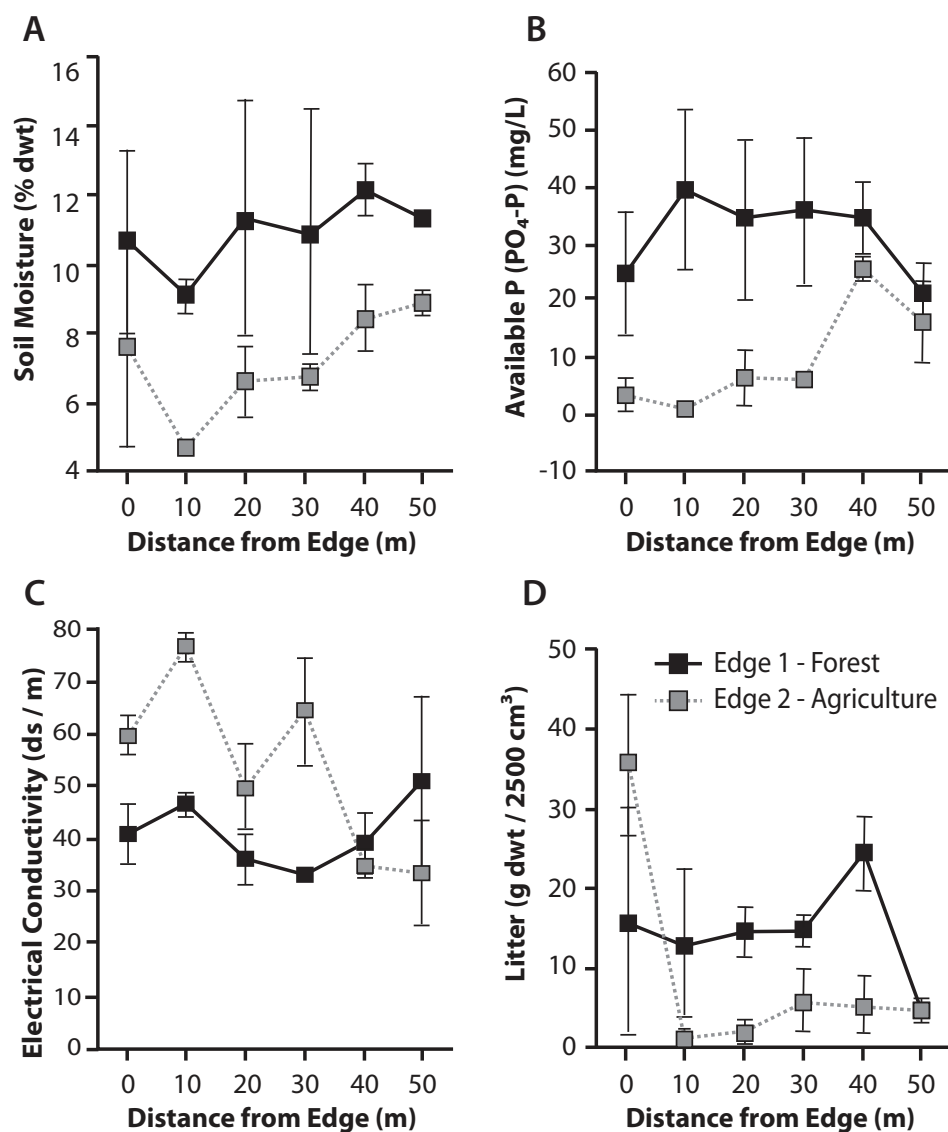
Basal respiration rates were generally low across all sampled soils, yet significant differences were found among distances from boundaries, as well as a significant interaction between land cover edge and distance (Table 2.2). Using *a priori* planned comparisons, basal respiration showed significance for M1 ( $P = 0.045$ ) and M3 ( $P = 0.002$ ). However, microbial biomass did not show any significant differences for land cover edge type or distance (Table 2.2).

Plant litter biomass was significantly greater in forest edge soils than in agricultural edge soils except at 0 m where litter was greater along the agricultural boundaries than the forest boundaries (Table 2.2, Figure 2.2D). Despite this observation, litter biomass did not differ significantly with distances from boundaries. Tukey *post hoc* tests showed the difference to exist between 0 m plots at forest boundaries and 0 m plots at agricultural boundaries ( $P = 0.049$ ).

**Table 2.2 Repeated measures ANOVA results for soil environmental variables (physical, biological, chemical) for plots bordering two land cover types (forest (n = 2) and agriculture (n = 2)) at 10 m increments from boundaries.**

Variable	Forest-edge	Agriculture-edge	Land Cover		Distance		Land Cover × Distance	
	Mean (± SE)	Mean (± SE)	F <sub>(1,2)</sub>	<i>P</i>	F <sub>(5,10)</sub>	<i>P</i>	F <sub>(5,10)</sub>	<i>P</i>
Soil moisture (% dwt)	10.87 (0.77)	7.15 (0.59)	3.29	0.211	1.54	0.263	0.184	0.962
LOI (% dwt)	3.23 (0.30)	2.17 (0.45)	1.08	0.409	2.09	0.149	0.532	0.748
pH (in dH <sub>2</sub> O)	7.28 (0.18)	7.38 (0.10)	0.073	0.813	0.607	0.697	1.78	0.204
EC (dS/m)	40.92 (3.03)	53.04 (5.18)	6.5	0.125	2.72	0.084	3.43	<b>0.046</b>
Available P (mg/L)	31.63 (3.91)	9.69 (2.82)	6.1	0.132	1.45	0.289	2.19	0.136
Available N (mg/L)	0.50 (0.06)	0.67 (0.12)	0.395	0.594	1.3	0.338	0.299	0.902
Basal respiration (μl CO <sub>2</sub> /g dwt/hour)	8.96x10 <sup>-1</sup> (5.1x10 <sup>-5</sup> )	9.96x10 <sup>-1</sup> (8.2x10 <sup>-5</sup> )	0.769	0.473	4.24	<b>0.025</b>	6.3	<b>0.007</b>
Microbial biomass (mg C micro/g dwt)	0.398 (0.003)	0.403 (0.008)	0.379	0.601	2.73	0.083	0.533	0.747
Litter dwt (g)	14.47 (2.79)	9.09 (3.82)	49.9	<b>0.02</b>	2.79	0.079	2.23	0.131

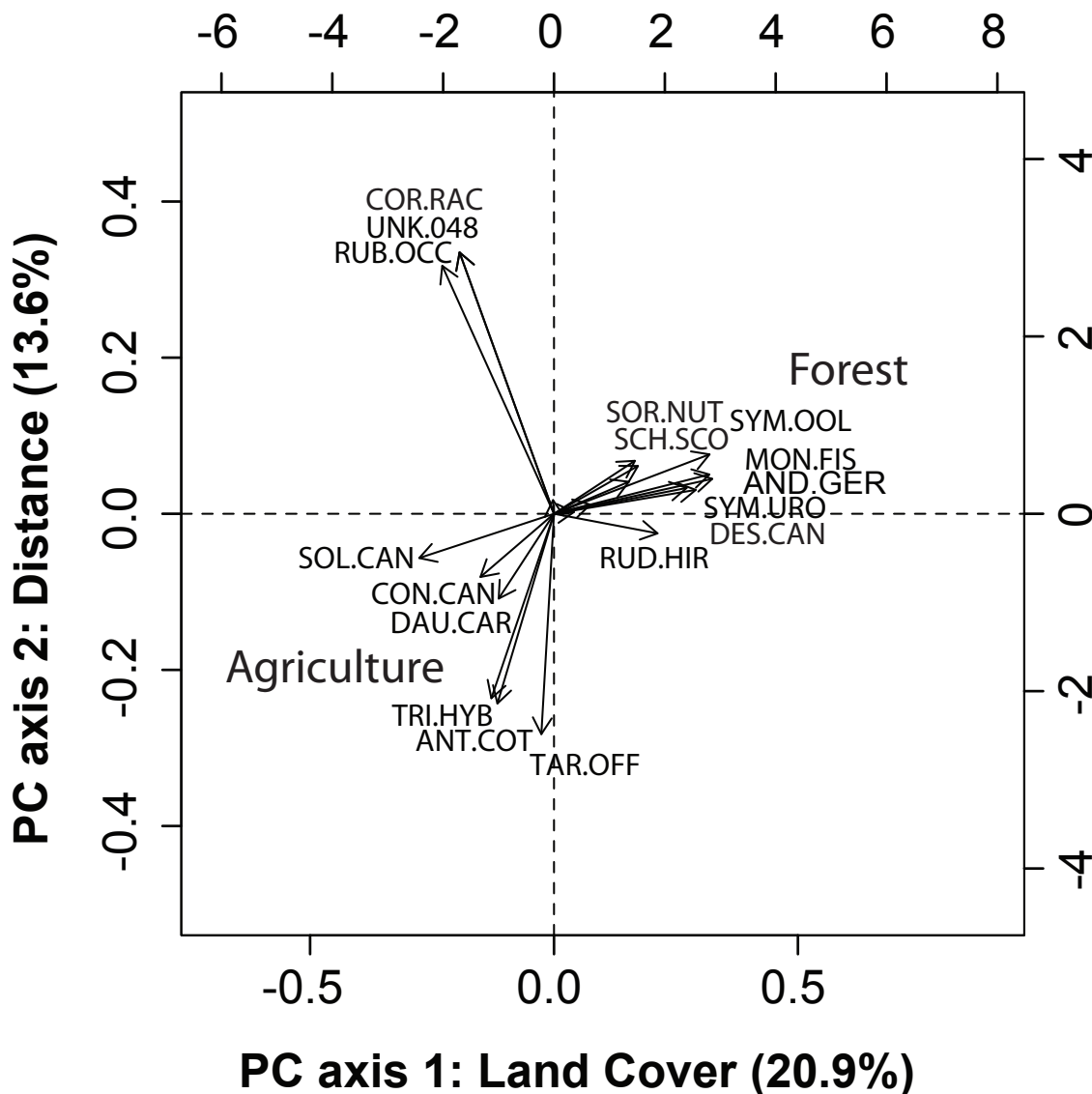
All soil measurements were taken at the 1 m × 1 m plot level. Land cover represents one of two vegetation types bordering restored grassland: forest (deciduous) or agriculture (crop field). Distance represents absolute distance of plots along 50 m transects within restored grassland at two edges (two perpendicular to forest and two perpendicular to agriculture). Significant *P* values are bolded.



**Figure 2.4** Soil variables A) soil moisture (% dwt), B) available phosphorus (PO<sub>4</sub> mg/L), C) electrical conductivity (dS/m), and D) litter dry weight (g) for plots located on transects in restored grassland and perpendicular to boundaries with two land cover types – forest (deciduous) and agriculture (crop field). Values are means (with standard error) of two transects per land cover edge type at each distance from the bordering land cover boundary.

### 2.3.3 Plant community composition

The PCA of plant composition for the 21 most abundant species (as determined by greatest total percent cover across all 24 plots sampled ( $\geq 0.25 \text{ m}^2$ )) suggests that the main driver structuring this composition (PC axis 1 explaining 20.9% of the variation) was land cover edge type ( $F_{1,22} = 29.22$ ,  $P < 0.001$ ) (Figure 2.3). Positive values for axis 1 represent species found primarily in plots bordering forest, while negative values primarily represent species in plots bordering agriculture. Species driving PC axis 1, associated with the forest edge, were *Andropogon gerardii* (species score = 0.35), *Monarda fistulosa* (0.34), while *Symphyotrichum oolentangiense* (0.34) and *Solidago canadensis* (-0.30) were associated with the agriculture edge. The second PC axis, explaining an additional 13.6% variation in plant community composition was found to represent distance from edges of both land covers ( $F_{1,22} = 4.502$ ,  $P = 0.045$ ). Positive values for PC axis 2 represent lesser distances from boundaries, while negative values represent greater distances from boundaries. The axis for PC 2 was driven by *Cornus racemosa* (0.44), unknown grass 48 (0.44), and *Rubus occidentalis* (0.42) (associated with lesser distances from boundaries) and *Taraxacum officinale* (-0.38), *Anthemis cotula* (-0.32), and *Trifolium hybridum* (-0.31) (associated with greater distances from boundaries).

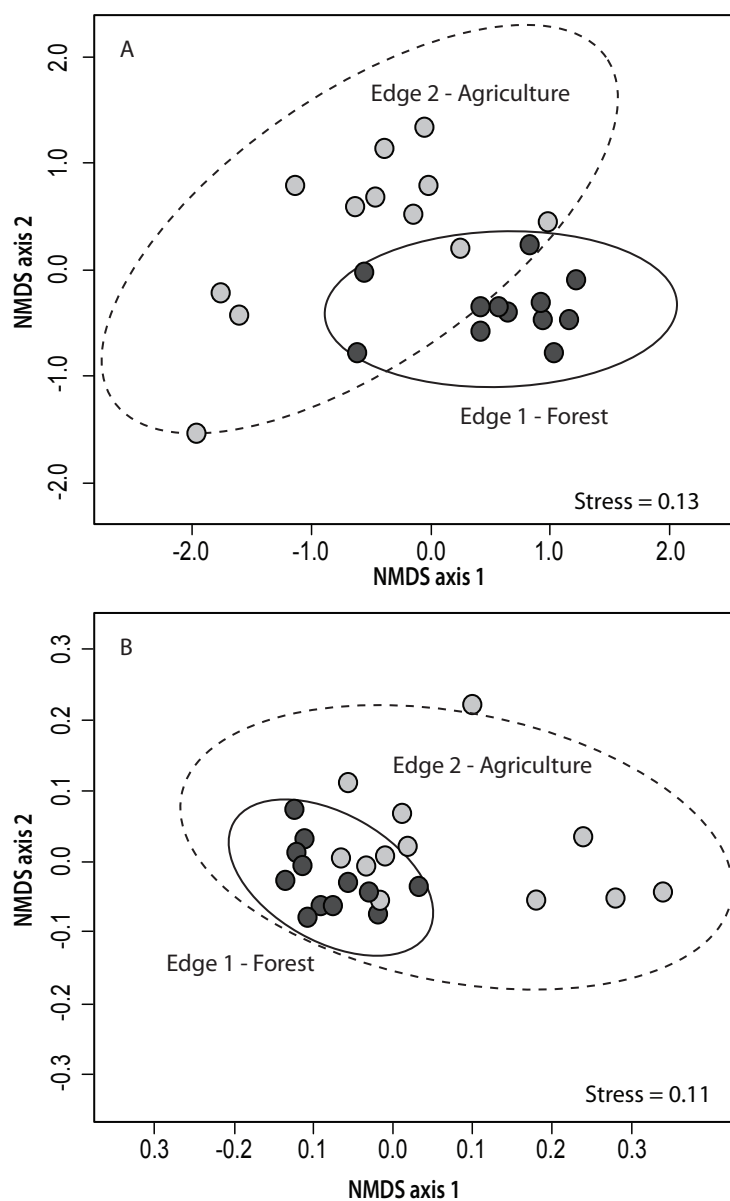


**Figure 2.5** Principal components analysis (PCA) for 21 species observed across 24 plots sampled within a restored grassland in Norfolk County, Ontario, Canada. Vegetation plots were sampled along transects perpendicular to either forest or agriculture boundaries at 0 – 50 m from the land cover edge. The PC axis 1 explained 20.9 % of the variation in plant composition and was related to adjacent land cover type – forest (deciduous) versus agriculture (crop field), while PC axis 2 explained an additional 13.6% of the variation in

plant composition, and was related to absolute distance from the edge of the adjacent land cover. Six-letter codes represent the following species: *Alnus incana* (ALN.INC), *Andropogon gerardii* (AND.GER), *Anthemis cotula* (ANT.COT), *Conyza canadensis* (CON.CAN), *Cornus racemosa* (COR.RAC), *Daucus carota* (DAU.CAR), *Desmodium canadense* (DES.CAN), *Monarda fistulosa* (MON.FIS), *Populus deltoides* (POP.DEL), *Rubus occidentalis* (RUB.OCC), *Rudbeckia hirta* (RUD.HIR), *Schizachyrium scoparium* (SCH.SCO), *Solidago canadensis* (SOL.CAN), *Sorghastrum nutans* (SOR.NUT), *Symphyotrichum oolentangiense* (SYM.OOL), *Symphyotrichum urophyllum* (SYM.URO), *Taraxacum officinale* (TAR.OFF), *Trifolium hybridum* (TRI.HYB), Unknown grass 33 (UNK.033), Unknown grass 48 (UNK.048), and Unknown grass 57 (UNK.057).

The NMDS of plant composition showed higher similarity among plots bordering forest than plots bordering agriculture (stress: 0.13) (Figure 2.4A), and the PERMANOVA test found significant differences between land cover edge types ( $F_{1,23} = 5.194$ ,  $P = 0.001$ ) as well as among distances from land cover edges ( $F_{1,23} = 1.853$ ,  $P = 0.043$ ). The SIMPER analyses of plant species composition further confirmed that plots along forest edge transects and plots along agricultural edge transects had high dissimilarity (92.2%), driven by *Solidago canadensis* (12.9%), *Andropogon gerardii* (11.3%), *Schizachyrium scoparium* (9.8%), *Monarda fistulosa* (7.0%), and *Rudbeckia hirta* (4.7%). The average similarity within the agriculture boundary transect was 14.2%, while the average similarity within the forest boundary transect was 30.0%. Among agriculture edge plots, similarity was driven by high abundances of *Solidago canadensis* (explaining 64.5% of the similarity), *Symphotrichum leave* (7.8%), *Andropogon gerardii* (4.7%), *Taraxacum officinale* (4.5%), and *Trifolium hybridum* (3.0%). Average similarity among plots bordering forest was driven by *Andropogon gerardii* (explaining 39.0% of the similarity), followed by *Schizachyrium scoparium* (16.2%), *Monarda fistulosa* (13.4%), *Rudbeckia hirta* (12.5%), *Symphyotrichum oolentangiense* (6.8%), and *Desmodium canadense* (3.1%).

The NMDS of soil variables showed that soils from plots bordering forest were more similar to one another than were soils from plots bordering agriculture to one another when considering the nine measured soil variables (stress: 0.11) (Table 2.2, Figure 2.4B). PERMANOVA results for soil variables showed land cover edge types were again significantly different ( $F_{1,23} = 8.607$ ,  $P = 0.001$ ), and soil variables were nearly significantly different with distance ( $F_{1,23} = 3.048$ ,  $P = 0.051$ ).



**Figure 2.6 Nonmetric multidimensional scaling (NMDS) of A) plant species composition and B) soil environmental variables, with each point representing a single 1 m × 1 m plot within 24 restored grassland plots, 12 bordering deciduous forest (shown in dark grey) and 12 bordering an agricultural crop field (shown in light grey). Plots were positioned at 10 m increments from boundaries (0-50 m). Ellipses show 95% confidence areas using standard error for forest edge (solid line) and agriculture edge (dashed line).**



### 2.3.4 Field decomposition

Neither freshly senesced litter nor partially decomposed litter showed significant differences in mass loss using RM ANOVA when compared between land cover edge types ( $F_{1,2} = 0.574$ ,  $P = 0.528$ ,  $F_{1,2} = 5.502$ ,  $P = 0.144$ ), respectively, distances ( $F_{5,10} = 2.030$ ,  $P = 0.160$ ,  $F_{5,10} = 1.657$ ,  $P = 0.232$ ), respectively, and testing for an interaction between land cover edge type and distance ( $F_{5,10} = 0.715$ ,  $P = 0.626$ ,  $F_{5,10} = 1.245$ ,  $P = 0.358$ ), respectively. Litterbags composed of freshly senesced litter showed an average percent mass loss of 49.1% while litterbags composed of partially decomposed litter showed an average percent mass loss of 28.3%.

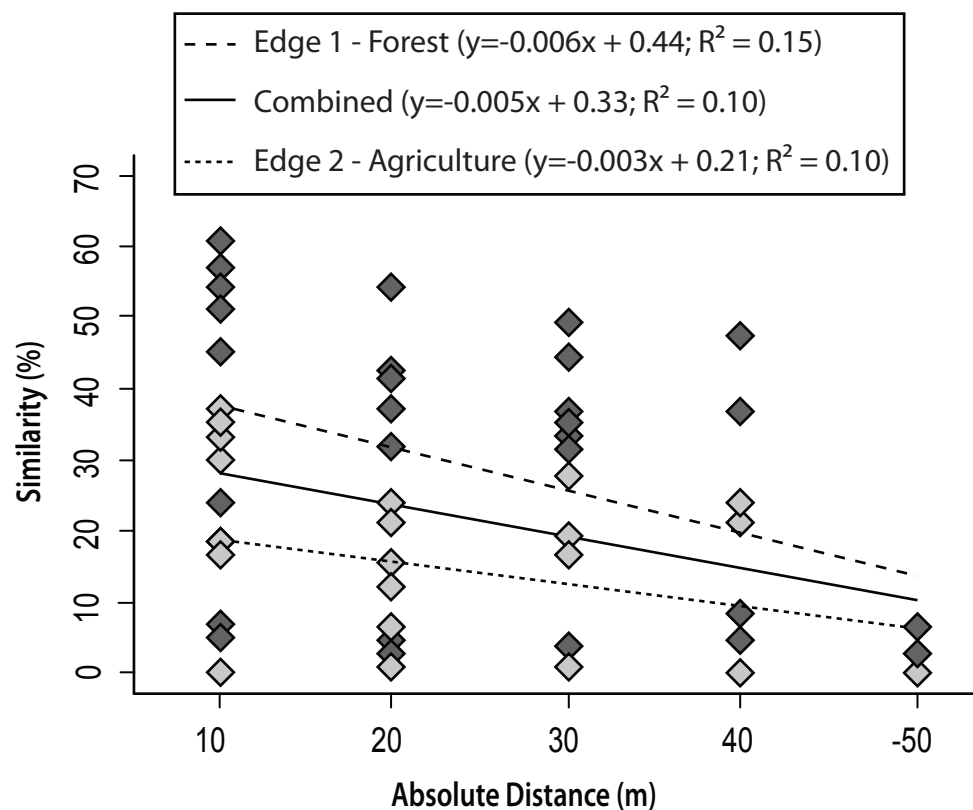
### 2.3.5 Plant, soil, and trait correlations

Results of both Mantel and Partial Mantel tests showed that all pairs of dissimilarity matrices (space, plant composition, environment) were significantly correlated (Table 2.3). Overall, plant composition correlated with absolute distances among plots, the soil environmental variables also correlated with distances, and plant composition correlated with the soil environmental variables, even when controlling for spatial distances. When forest edge and agricultural edge transects were analyzed separately with Mantel tests, forest plant composition significantly correlated with space, and forest environment also significantly, and strongly, correlated with space. However, forest plant composition did not correlate with space while controlling for environment. No significant correlations were found among agriculture datasets, although both Mantel and Partial Mantel tests correlating plant composition and environment were near significant (Table 2.3). Distance decay plots demonstrating the pairwise similarity of plant community composition for the twenty-four DeMaere2 plots, using similarities between pairs of plots within the same transect only (4 transects: 2 forest edge, 2 agriculture edge) (Figure 2.5), demonstrate decreasing plant community similarity with increasing absolute distance between plots.

**Table 2.3 Mantel and partial Mantel test correlations for three matrices including both overall and separate (by land cover edge type): plant community composition, soil environmental variables, and space (Euclidean distance) among 24 sampled plots at the DeMaere2 restored grassland. Twelve plots were located near a deciduous forest and twelve were near an agricultural crop field.**

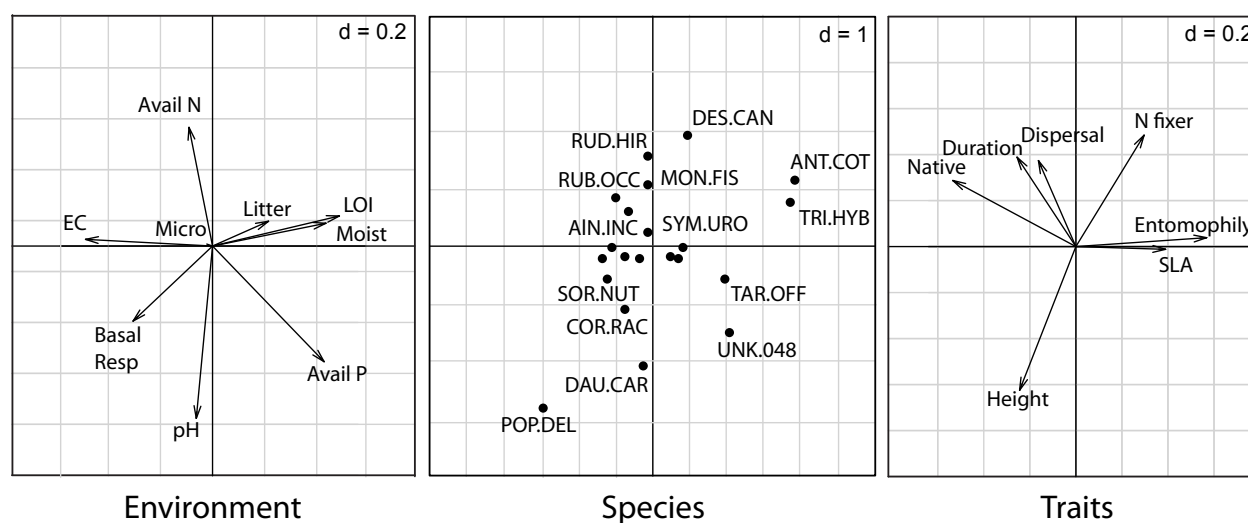
Matrix	Overall		Forest-edge		Agriculture-edge	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>
Plant composition × space	0.456	<b>0.001</b>	0.198	<b>0.048</b>	0.015	0.440
Plant composition × environment	0.334	<b>0.002</b>	0.225	0.126	0.209	0.072
Space × environment	0.347	<b>0.001</b>	0.442	<b>0.002</b>	-0.023	0.498
Plant composition × space <i>(environment)</i>	0.384	<b>0.001</b>	0.113	0.107	0.020	0.405
Plant composition × environment <i>(space)</i>	0.211	<b>0.010</b>	0.156	0.169	0.210	0.067

Data were taken from 24 1 m × 1 m plots in a restored grassland in Norfolk County, Ontario. Partial Mantel tests are shown with the covariate matrix italicized in parentheses.



**Figure 2.7 Distance decay plot showing change in similarity with change in distance between pairs of 1 m × 1 m plots sampled for vascular plant composition (species present and their percent cover) at the DeMaere2 study site in 2015. Points represent pairwise similarity between plots within the same transect only (4 transects: 2 forest edge and 2 agriculture edge). Dark grey symbols represent plot pairs near deciduous forest and light grey symbols represent plot pairs near an agricultural crop field.**

In the final analysis linking plant functional traits to soil environmental variables, the first axis (axis 1) of the RLQ plot explained 76.5% of the total variation while axis 2 explained an additional 12.9% of the total correlated variation among the plant species traits and soil environment variables matrices. However, in this final analysis, plots sampled for vegetation and soil variables were not significantly explained by trait-environmental relationships ( $P = 0.158$ ), and neither were the species sampled significantly explained by their trait-environmental relationships ( $P = 0.249$ ). Together, sampled plots and sampled species were somewhat explained by trait-environmental relationships, however the relationship was not quite significant ( $P = 0.075$ ) (Figure 2.6). Subsequent fourth-corner analysis revealed no positive or negative correlations between plant species traits and soil environmental variables (results not shown).



**Figure 2.8 RLQ biplot showing links between soil environmental conditions and plant species traits given the plant community composition. Results of RLQ analysis plotted to show distribution of variables according to primary and secondary axes for three matrices: A) soil environmental variables, B) plant species, and C) plant species traits. Values for d represent relative scale. Six-letter codes represent the following species: *Alnus incana* (ALN.INC), *Andropogon gerardii* (AND.GER), *Anthemis cotula* (ANT.COT), *Conyza canadensis* (CON.CAN), *Cornus racemosa* (COR.RAC), *Daucus carota* (DAU.CAR), *Desmodium canadense* (DES.CAN), *Monarda fistulosa* (MON.FIS), *Populus deltoides* (POP.DEL), *Rubus occidentalis* (RUB.OCC), *Rudbeckia hirta* (RUD.HIR), *Schizachyrium scoparium* (SCH.SCO), *Solidago canadensis* (SOL.CAN), *Sorghastrum nutans* (SOR.NUT), *Symphyotrichum oolentangiense* (SYM.OOL), *Symphyotrichum urophyllum* (SYM.URO), *Taraxacum officinale* (TAR.OFF), *Trifolium hybridum* (TRI.HYB), Unknown grass 33 (UNK.033), Unknown grass 48 (UNK.048), and Unknown grass 57 (UNK.057).**

## 2.4 Discussion

### 2.4.1 Characterization of the DeMaere2 restored grassland

The DeMaere2 restored grassland in Norfolk County, Ontario has been deemed a ‘success’ since the restoration initiative and seeding of native southern Ontario tallgrass prairie plant species in 2010. Indeed, plant diversity (species richness) is high at this site with a total of 67 species encountered five years post-seeding. Although a large proportion of species seeded was not found in the 2015 survey of the DeMaere2 site, the majority of vegetative cover along the edge that bordered forest was native, seeded species.

### 2.4.2 Role of adjacent land cover in structuring plant community composition

While the high prevalence of seeded, native plant species confers the success of the restoration initiative for the DeMaere2 site, the occurrence of several species suggests that historical land use and the resulting topography is influencing DeMaere2. I found that along the eastern boundary adjacent to forest, plots consisted largely of native, seeded species, and overall I found greater similarity of plot community composition along these transects than for the plots on the sandy slope at the adjacent to the crop field. The plots on this topographical feature contained several exotic and invasive species that may in part be explained by proximity to the adjacent agricultural fields. However, as a sloping, sandy area characterized the plots sampled along this boundary, mechanical seeding was apparently difficult (pers. comm. NCC), and may have contributed to lower seeding success. That said, while overall species richness in both areas was similar, plant community composition differed among the two edges, irrespective of distance from the associated boundary. Both my multivariate analyses of community composition (PCA and NMDS) identified edge type as the primary factor detected structuring plant composition at the DeMaere2 site. Many of the plant species at the edge adjacent to the crop field were broad-leaved weedy forbs, and plant composition at the edge bordering forest was often dominated by graminoids. Defining species near the forest boundary were all species seeded by NCC for

restoration purposes (e.g. *Andropogon gerardii*, *Monarda fistulosa*, *Symphyotrichum oolentangiense*), while more weedy species were common on the slope adjacent to the crop field. For instance, *Anthemis cotula* and *Trifolium hybridum* (common weeds) were only associated with plots adjacent to the crop field; the notorious weedy species *Taraxacum officinale* (common dandelion) was fairly ubiquitous throughout the DeMaere2 site, but more frequent and abundant in plots on the slope near the crop field than plots sampled near the forest. Similarly *Solidago* spp. (goldenrod), which are native species in Southern Ontario and were intentionally seeded at DeMaere2, are considered to be weedy, thriving in fields and hedgerows, among other habitats (Ontario Weeds 2016), and were also found in abundance on the sandy slope. The presence of *Solidago* spp. in these plots contributed highly to the overall dissimilarity of the two edges. Additionally, *Andropogon gerardii*, *Schizachyrium scoparium*, *Monarda fistulosa*, and *Rudbeckia hirta*, found largely or solely in the plots near the forest boundary, explained the high within-edge similarity values there, and were all seeded by NCC.

Much of the dissimilarity between plots near the forest versus on the sandy slope was also driven by the species encountered directly at the edge of the grassland site, which also led to significant distance effects in the multivariate analyses. For instance, along the forest boundary *Cornus racemosa*, unknown grass 48, and *Rubus occidentalis* were found only in 0 m plots. Both 0 m forest edge plots were outliers in the NMDS ordination of community composition, and were composed primarily of typical Carolinian forest species. Similarly, 0 m plots at the agricultural edge were quite dissimilar from other agricultural plots, but the largest outlier in the agricultural community compositional analysis was a particular plot at 10 m that was distinct from all other plots in having two species of *Salix* that were not found in any other plots, likely related to topographic features (i.e. sloping area) at that plot. These direct effects of adjacent land cover types at 0 m corresponds with several environmental factors that were likely also influenced by the adjacent land cover. On the raised berm at the crop field edge (0 m plots), soils sampled were directly at the base of the tree line, had greatest percent cover of vegetation, greater litter inputs, and correspondingly higher organic matter content and basal respiration rates compared to other plots on the same transects, which I attribute to the densely vegetated herbaceous and

shrub layer along this boundary. Plots away from the forest edge generally had greater organic matter and leaf litter than plots alongside the agricultural area, suggesting that proximity to a dense stand of deciduous trees, as expected, would be a distinguishing factor between the land cover types, with the forest producing more leaf litter, which in turn accumulates as soil organic matter. Litter and soil organic matter inputs from the adjacent forest, however, declined around 50 m, as the 50 m plots of both edge types had similar soil environmental characteristics. Deciduous leaf litter inputs in perennial dominated grasslands have been shown to increase the abundance of graminoids over forb species (Letts et al. 2015), which may explain the predominance of graminoids near the forest boundary of DeMaere2.

In several of my *a priori* contrasts, model 3 separating 0 m plots from other distances, was found to be significant, however, whether these plots directly at the edge of the grassland can be considered part of the grassland is debatable. One parameter that was strongly linked to the forest 0 m boundary plots was the community weighted mean of specific leaf area (SLA). High SLA at the forest 0 m plots is attributed to the prevalence of forest understory species found there. Species associated with shady environments (such as forests), and therefore limited in sunlight as a resource, often have a high SLA (Pérez-Harguindeguy et al. 2013). Edge species near the tree line bordering the crop field were not typical understory species. Rather, several agricultural plots near the border had low SLA attributed to the woody perennials that tend to have greater leaf thickness than herbaceous species (Pérez-Harguindeguy et al. 2013).

### 2.4.3 Spatial extent of edge effects

Edge type, the distance from that edge, as well as edge type and distance considered simultaneously, did influence certain vegetative and soil properties within the DeMaere2 study site in 2015. Notably, vascular plant species richness, for vegetation sampled at 10 m incremental distances from the bordering wheat field, followed a similar pattern as was found by Gieselman et al. (2013) in their study of grasslands bordering fruit crops, which was a breakpoint at 30 m. The presence of a ‘breakpoint’ – a notable shift in plant or soil variables at a certain distance from an edge – was tested using two similar *a priori* contrast models (models 1 and 2)



examining the proposed distance of 30 m. This zone of influence up to 30 m has previously been found in other studies. For example, Gieselman et al. (2013) found breakpoints 25–30 m from paved roads and fruit crops, and Taft (2016) saw peak levels of native species diversity and density in remnant prairie 15 m from borders with cropland. Lee and Power (2013) found the penetration distance of soil nutrients and exotic species in grassland to vary somewhat, but effects typically extended up to 20 m from roads. These findings have been attributed to both spatial and environmental factors. Spatial factors include dispersal distance probability functions where dispersal probability decreases after 30 m for many wind-dispersed plants (Vittoz and Engler 2007). Anemochory, or wind-dispersal, is highly correlated with plant height (Thomson et al. 2011), which could also explain why edge effects were often found at greater distances along the forest edge transects than the agricultural edge transects, and overall greater species richness closer to the forest.

Both the plant community composition and the soil environment were spatially structured, suggesting that adjacent land cover also has physical and chemical influence on the DeMaere2 site. Soil environment variables that were strongly influenced by the adjacent forest were soil moisture, leaf litter inputs and available phosphorous. In combination with the Repeated Measures ANOVA, PCA, and NMDS, disentangling the direct effect of adjacent land cover on plant community composition from indirect effects via soil environmental variables was the purpose of the Partial Mantel tests. The Partial Mantel tests suggest that the direct spatial influence was slightly stronger than the indirect influence of a spatially structured environment, as plant composition was more strongly correlated to space while controlling for environment than was plant composition correlated to environment or environment to space. However, the Partial Mantel tests also found significant correlations between plant composition and soil variables, while controlling for space, and when forest edge plots were analyzed alone, both plant composition and environment correlated with space separately, but did not correlate with each other. Meanwhile, there were no significant correlations among plant composition, environment, and space at the crop field boundary. This may be explained by the breakpoint found for forest edge vegetation at 0 m with *a priori* contrasts (essentially forest versus grassland

vegetation), which would correlate space (along transects) and plant composition. Possibly the slightly differing soil variables between the two forest edge transects evident from NMDS would correlate environment (soil) with space (between transects). As for the crop field edge, the near significant breakpoint found at 30 m using *a priori* contrasts (also evident by field observation) explains the near significant correlation between plant composition and soil environment found with Mantel tests, even while controlling for space. These relationships at the crop field boundary were observable even in the field where the soil was noticeably sandier (particularly between 10–30 m), and also populated with different (weedier) species (as compared with plots at 40–50 m).

Several correlations between plant communities and soil environmental conditions have been observed in other grassland studies. Gasch et al. (2013) found that both aboveground and belowground components of grasslands invaded by exotic species differed from those of grasslands vegetated by native species, specifically in having lower species richness and higher soil nitrogen levels. Although not significant, species richness was lower and available soil nitrogen higher in agricultural than forest edge plots at the DeMaere2 site. In a study on the invasive *Bromus inermis*, Vinton and Goergen (2006) similarly found higher nitrogen in invaded soils when compared to soils of native species, which they attributed to faster cycling of nitrogen due to a lower C:N ratio and a higher decomposition rate, giving the exotic species a competitive advantage over native species. In a study of North American shrubland and steppe, nitrogen uptake was found to differ only slightly between native and invasive grasses at low temperatures, but differed greatly at higher temperatures, correlating with leaf N and mass as well as root N and mass (Leffler et al. 2013). The influence of soil nitrogen may likewise influence other soil characteristics such as microbial communities. Birkhofer et al. (2012) found fungal biomass to be positively correlated with soil nitrogen concentration, but found no correlation between abiotic soil properties and yeast or bacterial biomass or diversity.

Plots bordering the two land cover types (forest and crop field) of the DeMaere2 site, in general, differed in their soil environments, but less so than plant community composition. For the NMDS of soil environmental variables, forest edge plots were clustered closely together, but

several crop field edge plots were clustered closely to the forest edge plots. The plots that had high similarity between forest edge and crop field edge transects were mainly distances of 40 and 50 m, again suggesting that there is an edge effect along the crop field boundary, but that this edge dissipates at around 30 m. The similar species richness values between 40 m and 50 m for both forest edge and crop field edge, also suggests there is a general ‘interior’ versus edge habitat, lending support to the presence of a 30 m breakpoint as found by Gieselman et al. (2013). But ultimately it appears that forest and agriculture as adjacent land covers influence vegetation patterns of restoration in DeMaere2 differently, depending on proximity to that land cover.

#### 2.4.4 Trait-based approaches for understanding plant community composition

One goal of this thesis chapter was to apply a trait-based approach to understanding aboveground (plant) and belowground (soil) linkages using plant functional traits. A trait of particular interest was specific leaf area (SLA), which exhibited higher values near the forest border. This observation is consistent as high SLA is often attributed to shaded (forested) environments, and is often observed under or near deciduous canopy. However, in this study, it was *Trifolium hybridum* and *Anthemis cotula* that had higher SLA relative to the other 19 species included in the analysis, which are weedy species not commonly associated with shaded environments. That said, SLA may be highly variable at small spatial scales (Siefert 2011) and can be a highly plastic trait as observed for perennial grass species in response to experimental nitrogen addition (Knops and Reinhart 2000).

Overall, while plant composition was found to correlate with soil environmental variables, my attempt to link these factors using plant functional traits did not reveal any significant relationships. The main method I used was RLQ where I analyzed soil environment – plant trait relationships, using the nine measured soil variables, seven plant traits (two measured and five categorical from trait databases), and 21 species (the top species greater or equal to 0.25 square meters in total percent cover), associated with their location in the DeMaere2 grassland.

Although trait-environment relationships were not found significant with this analysis (RLQ), or by the subsequent Fourth-corner analysis, interesting patterns in trait-environment associations still emerge. For example, the nitrogen-fixing plant *Desmodium canadense* corresponds (positive values for axis 2) with low available nitrogen measured in the soil, where it would have an ecological advantage.

There is some suggestion that RLQ analysis may be more appropriate at larger spatial scales where gradients of environment and plant community compositional turnover are stronger (Oldeland et al. 2012), while others have suggested adding phylogenetic information as a stronger predictor of functional traits (Pavoine et al. 2011). Also using RLQ analysis, Bagaria et al. (2012) did not find plant traits played a major role in species response to environmental variation in Mediterranean grasslands, with no apparent effect of past landscape structure on species frequencies. It is also possible that the soil variables and plant traits selected, both of which were only a subset of possible soil variables and plant traits, were not explanatory due to the traits and variables not having a strong link with one another, or potentially because they explain only part of the full relationship between plants and their belowground environment. The traits selected for the RLQ analysis were chosen according to Laughlin (2014), to incorporate characteristics representing distinct physical plant organs. The selected traits therefore may not fully represent traits related to environment. In addition, intraspecific variation can result in varying responses in one plant trait to the same environmental variable (Kumordzi et al. 2015).

## 2.5 Conclusions

In this study, I saw trends in both plant community composition and soil environmental variables, where an edge was detected at 0 m for both land cover edge types, and a breakpoint is suggested around 30 m from an agriculture border, consistent with the literature. Using a trait-based approach did not increase an understanding of plant community composition as overall my plant functional traits did not significantly correlate with my measured environmental variables. However, the DeMaere2 site studied here is relatively recently seeded as a grassland, therefore

perhaps it is not surprising that plant composition did not naturally associate with environmental gradients. Perhaps in future years, stronger species sorting effects will be detected or become apparent as aboveground-belowground gradients were detected at the site, and there were detectable influences of adjacent land cover.

The findings of this study could have implications for the restoration of grasslands. Delineation of edges could inform the selection of sites for grassland restoration. Area requirements established for habitat restoration, for example, might not in practice be met, if an area seeded is not effectively restored. Edge delineation, in combination with understanding the soil environment at edges, could explain plant composition there (Gieselman et al. 2013), and inform planting patterns, where appropriate vegetation and/or seed could be applied to the grassland interior versus the grassland edges, in order to increase success rates of native species and discourage the establishment of non-native plant species (Funk et al. 2008). Knowledge of plant traits could assist in selecting species for the restoration of grasslands. Determining a breakpoint in vegetation composition, and whether this breakpoint differs between grassland bordering forest versus agriculture is the next step in delineating edges and informing restoration ecology.

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## Chapter 3

### 3 Site-specific differences among restored grasslands prevent generalizations of restoration success

#### 3.1 Introduction

Current and historic intensification of land use for agricultural purposes has led to a global decline in natural ecosystems such as forests and grasslands (Ramankutty and Foley 1999, Ceballos et al. 2010). With this land use change has come the recognition of losses in biodiversity (Sala et al. 2000) and the subsequent consequences for the functioning of ecosystems (Loreau et al. 2001, Hooper et al. 2005). As such, natural habitats have been acknowledged as valuable components of a managed landscape, and the importance of ecologically restoring these habitats has likewise been recognized. Ecological restoration, in particular, is an accepted method to reestablish a previously managed or damaged habitat to its previous historical physical and biological state (Palmer et al. 1997), with the first initiatives often focusing on the reestablishment of plant communities (Lesica and Allendorf 1999). However, restoration ‘success’ requires more than simply planting native plants. Restoration requires an active management to identify and control ongoing causes of ecosystem degradation (Gayton 2001), such as the effects of surrounding non-native vegetation and land use. For instance, Rowe et al. (2013) found the retention of native plant species in remnant grassland patches to be higher when bordered by restored grassland, and vice versa, compared to when these habitats were abutting roads and abandoned lands.

Collectively, physical, chemical, and biological influences of adjacent land cover types or habitats are referred to as edge effects. Edge effects have been examined at natural grassland boundaries with regard to various adjacent land cover types (Gieselman et al. 2013, Lee and Power 2013, Rowe et al. 2013). Within this context, Gieselman et al. (2013) found non-linear

relationships in plant composition and soil variables with distance from adjacent fruit crops and paved roads, with a ‘breakpoint’ at roughly thirty meters from these edges. A breakpoint here can be defined as the point at which, moving away from an edge, rapidly changing vegetative composition transitions to relatively homogeneous composition. Closer to edges, Gieselman et al. (2013) found more introduced plant species and fewer native species. The influence of adjacent land cover types on grassland plant composition has been found to be measurable and significant, especially when considering lands modified by human uses. For example, in a similar study, Lee and Power (2013) found significant direct and indirect impacts of roads adjacent to calcareous grasslands on plant composition of grassland edges, including greater abundances of introduced plant species and less abundant native grassland species at roadsides.

Although previous grassland studies contrasting adjacent land cover types show evidence of edge effects, it is important to consider that each grassland site is unique. Restored grasslands will vary in their planted communities (e.g. seed list), other biotic and abiotic characteristics (e.g. soil environment), as well as in their general environment (e.g. microclimate etc.). Sites will also vary in their previous land use history in ways that can affect planting success. Many of the factors about a site (e.g. soil properties, site history) are often unknown prior to restoration initiatives, because site selection for restoration is often opportunistic. Acquiring a site for restoration will often depend on land availability, and the considerations of the sellers and investors (Gerla et al. 2012). Sites for restoration may also come available through a variety of means (e.g. property sale, donation), and therefore each site presents a unique situation involving a range of variables.

Very little original prairie remains in North America (Rowe et al. 2013). Therefore, many grasslands existing today are restored plant communities that have been seeded with a unique seed mix, and are likely under a specific management regime (e.g. localized spraying, scheduled mowing). Defining restoration ‘success’ is a difficult and subjective task. The mitigation and minimization of edge effects, the establishment of native species, and the re-initiation and maintenance of ecological function appropriate for the site are all aspects of restoration success (Ruiz-Jaen and Aide 2005, Fonseca and Joner 2007). However, establishing an acceptable

breakpoint distance for edge effects may differ with different land cover types, while acknowledging that the native species establishment is subject to differences in seeding mix at the time of restoration will highly influence the outcome of restoration. Therefore, site differences may persist even among restoration projects in close proximity and by the same conservation organization.

To examine edge effects in restored grassland plant composition and diversity bordering forests and agricultural fields, I selected six sites seeded by the Nature Conservancy Canada (NCC) in Norfolk County, southwestern Ontario, Canada. All sites are located on the Norfolk Sand Plain, and all have shared borders with both forest fragments and agricultural crop fields. Despite differing site histories, and unknown environmental variables at each site, the objective was to specifically look for common patterns in plant composition and diversity over changing distances from forest and agricultural edges, as well as the larger scale comparison of vegetation bordering these land covers across the six sites. Specifically, this study tested for breakpoints within the restored grassland plant communities with increasing distance from adjacent land cover borders (edge effects). Vascular plant species richness data, percent cover estimates, and composition using taxonomic identities were collected and analyzed to detect patterns.

The specific hypothesis of a breakpoint in plant composition is based on the observation of edge effects in both plant community and soil variables at a single site (DeMaere2 (HF)) studied extensively in 2015. At this site, a breakpoint was detected at approximately 30 m from an agricultural boundary, while no breakpoint was observed at forest edges (sharp transition at 0 m border). Therefore I hypothesized that there will be a detectable shift in plant community composition (breakpoint) observed at 30 m from an agricultural edge across six restored grassland sites in Norfolk County, despite these sites differing in land use history and seeding mix.

## 3.2 Methods

### 3.2.1 Study area

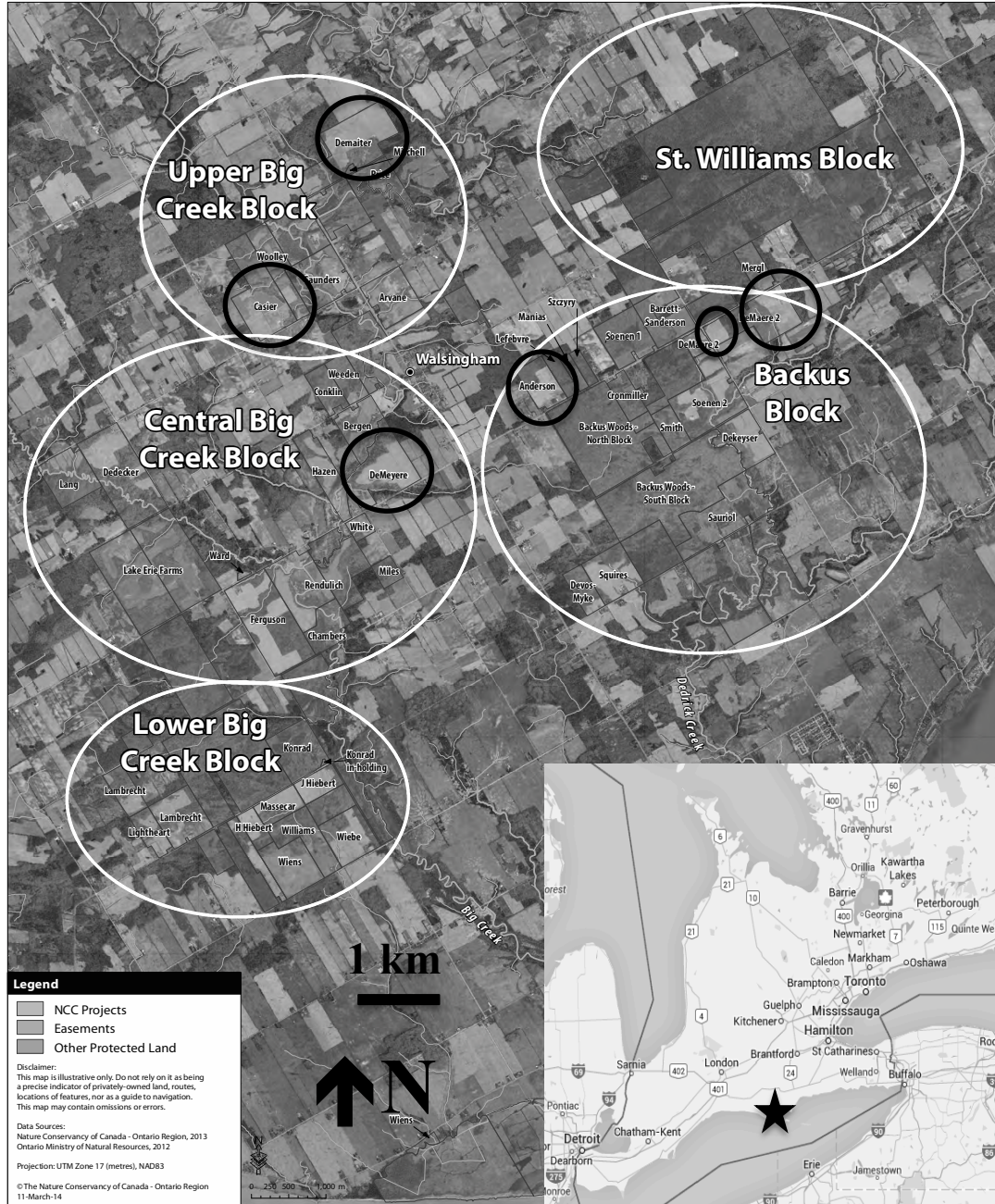
In order to look for patterns in restored grassland plant diversity and composition as influenced by bordering forest and agriculture, six sites were selected from Nature Conservancy Canada's (NCC) Norfolk County region of restored grassland properties, all of which are located within a 150 km<sup>2</sup> area (Figure 3.1). All sites were purchased by NCC for the purpose of vegetative restoration, and seeded between 2010 and 2013 with native southern Ontario graminoids, forbs, shrubs, and trees (Appendix A & C). This region is located on the Norfolk Sand Plain and thus soils are characterized by coarse sand and sandy loam (Chapman and Putnam 1984). Two criteria were used in site selection: 1) at least 0.01 km<sup>2</sup> of continuous grassland, and 2) having shared borders with both forest and agriculture (crop field) along at least 100 m of the grassland perimeter. Within the NCC restoration initiative projects the sites sampled for this study were: Anderson, Casier, DeMaere2 (HF), DeMaere2 (MW), Demaiter, and Demeyere (Figure 3.1). Information on site seeding year, size, geographical coordinates, seeding rates, bordering forest species, forest height, bordering crop, hedgerow species, hedgerow height, and other border features, see Appendix C.

### 3.2.2 Sampling design

Two transects were established at each site: one perpendicular to the bordering forest and one perpendicular to the bordering agricultural field. The orientation of transects was determined by the location of bordering forest and agriculture. Transects were placed at least 50 m from other borders, and originated at a transition in vegetation (crop field edge), the base of the outermost tree or sapling trunk (forest edge), or on the grassland interior side of any road or path that ran closely and parallel to an edge.



## Norfolk Forests and Long Point Wetlands Properties



**Figure 3.1** Map of the Norfolk County, Ontario, Canada region showing the location of Nature Conservancy Canada properties. Designated blocks are indicated by white circles.



**All sites sampled for this study (indicated by black circles) are within NCC project areas. Map provided by and reproduced with permission from NCC. Scale is approximate. The black star on the inset map in lower right shows the location of Norfolk County within southwestern Ontario.**

### 3.2.3 Vegetation sampling

To compare plant diversity and composition among sites, land cover edge types, and distances from adjacent land cover (forest and agriculture), a  $1 \times 1$  m quadrat was positioned at 0 m, 10 m, 20 m, 30 m, 40 m, and 50 m from each boundary, along each transect. Within each plot (72 in total), all vascular plant species were identified and visual cover estimates of each species recorded. As with the 2015 vegetation sampling, overlap was allowed in cover percentages, resulting in some total plot cover estimates greater than 100%. All vegetation sampling in 2016 took place within July 19-27.

### 3.2.4 Statistical analyses

Plant species richness (#spp / m<sup>2</sup>) and total percent cover (%) were analyzed using a repeated measures Analysis of Variance (RM ANOVA) using Statistica 7 software (StatSoft Inc. 2004). Land cover was used as an independent variable, and distances along transects were used as repeating factors to account for spatial non-independence. A principal components analysis (PCA) ordination was performed using all plant composition data for all of the six sites, using the ‘vegan’ and ‘ade4’ packages in R software (Dray and Dufour 2007, R Foundation for Statistical Computing 2013, Oksanen et al. 2015). Case scores for the first two principal components were used in an ANOVA to determine whether land cover edge type or distance from land cover border were drivers of the PCA, factor coordinates (scores) for species were used to further interpret the PCA. A second PCA was performed as described above using a subset of the vegetation community specifically using the most common species (21 species) whose percent cover was greater than 0.5 m<sup>2</sup> in total across all sites.

To further explore patterns in vegetation composition, nonmetric multidimensional scaling (NMDS) was performed using the ‘vegan’ package in R software (R Foundation for Statistical Computing 2013, Oksanen et al. 2015) for plant composition data across the six sites. Two outliers (quadrats) that had dramatically different composition and species unique to only those two plots were removed from the final analysis. The final NMDS was analyzed by

PERMANOVA (permutation ANOVA) to determine whether plots were significantly different among sites, land cover edge types, or distances using the ‘vegan’ package in R (R Foundation for Statistical Computing 2013, Oksanen et al. 2015). Following this, SIMPER analysis of plant species composition (also with outliers removed) was performed to find the average similarity of plots within land cover edge types (forest, agriculture) and the average dissimilarity between land cover edge types. SIMPER also identifies the percent contribution of each species to land cover edge similarity (within land cover edge types) and dissimilarity (between land cover edge types). SIMPER was performed using Primer 5 software (PRIMER-E Ltd. 2001) with a 90% cut-off for species contribution to (dis)similarity.

Finally, to test whether plant species composition at each of the six sites was significantly related to their absolute spatial location, I used Mantel tests in the ‘vegan’ package in R (R Foundation for Statistical Computing 2013, Oksanen et al. 2015). For each site, I ran three Mantel tests. One test used overall site plant composition and spatial data (both transects), and the two other tests used subsets of this data (one using only forest edge data, the other using only agriculture edge data). The Mantel test used a dissimilarity matrix (Bray-Curtis, square root transformed) for plant composition (% cover data for each species observed in a plot at a given site) and a second dissimilarity (distance) matrix (Euclidean, no transformations) based on latitude and longitude GPS data converted to decimal degrees. The Mantel test used 999 permutations to test for a correlation between these two dissimilarity matrices via the Pearson method. To visualize the outcome of the Mantel tests, I plotted percent vegetation similarity (1 – dissimilarity value from the dissimilarity matrices generated for Mantel tests) against absolute distances between sample locations. For these distance decay plots, only similarities between pairs of vegetation plots within the same transect were used (i.e. similarities between forest and agriculture plots were not included and therefore the maximum distance between plot pairs was maintained at fifty meters).

### 3.3 Results

A total of 69 species was recorded from 449 observations across the six sites sampled, of which 54 species were confidently identified to the species level, seven were identified to genus, and eight could only be identified to morphotype. The total numbers of species observed, associated with each edge type within each site, are listed in Table 3.1. Overall, mean species richness per plot was 6.2 species/m<sup>2</sup>, while overall mean percent cover for all plots sampled was 70.8%. Species richness did not differ significantly between land cover edge type ( $F_{1,10} = 0.017$ ,  $P = 0.900$ ), among distances ( $F_{5,50} = 0.736$ ,  $P = 0.600$ ), nor were there interactive effects ( $F_{5,50} = 1.000$ ,  $P = 0.427$ ). Likewise, total percent cover did not differ significantly with land cover edge type ( $F_{1,10} = 2.114$ ,  $P = 0.177$ ), distance ( $F_{5,50} = 0.154$ ,  $P = 0.978$ ), and displayed no interactive effects ( $F_{5,50} = 0.384$ ,  $P = 0.857$ ).

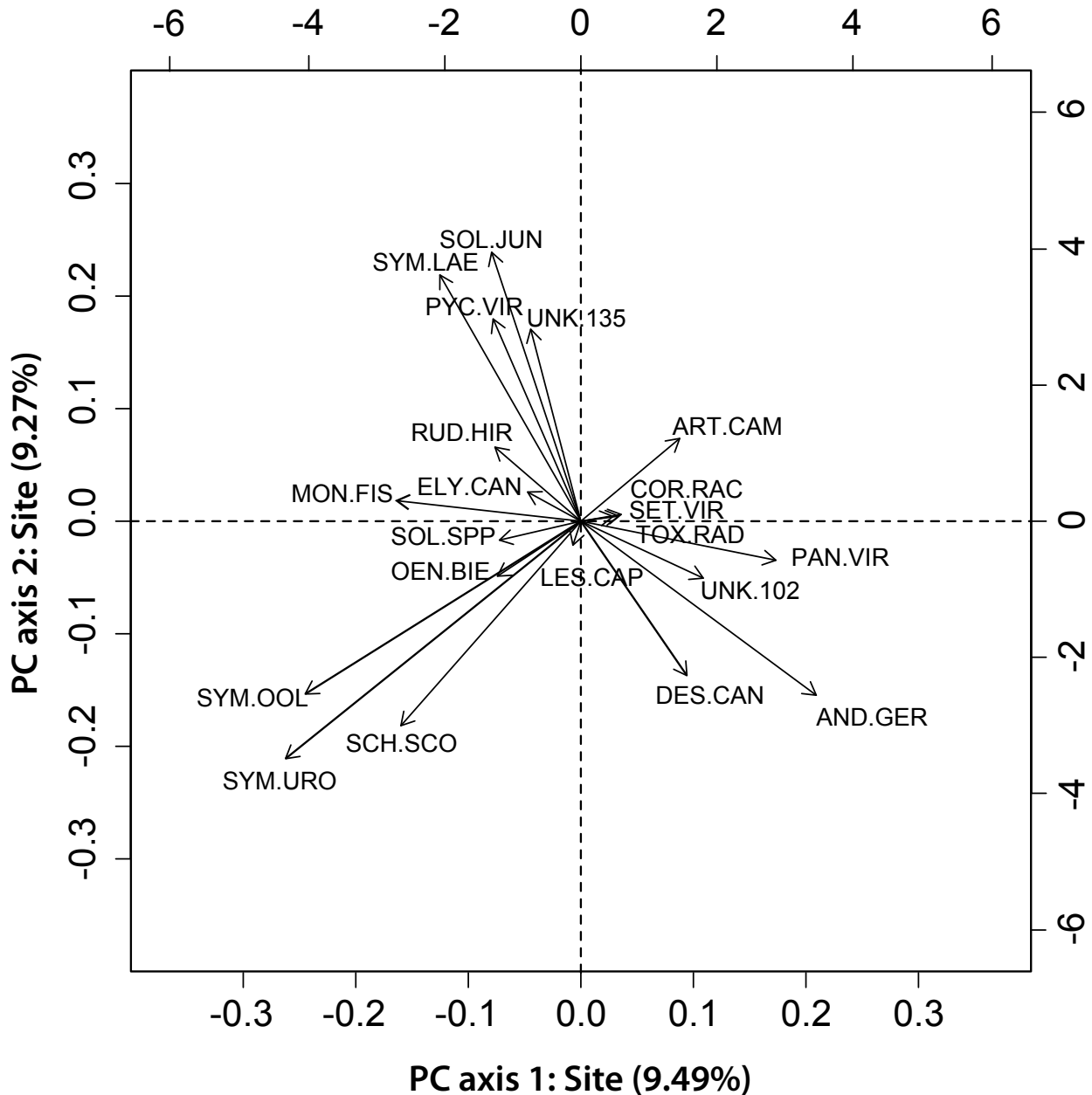
In the PCA ordination of all plant composition data collected in 2016 (Figure not shown), PC axis 1 only explained 2.8% of the variation and was not driven by a known variable, while PC axis 2 only explained an additional 2.0% of the community variation, but was somewhat explained by distance along transects ( $F_{1,71} = 4.027$ ,  $P = 0.049$ ). For this analysis, PC axis 1 was associated with four main species: *Hieracium florentinum* (species score = 0.46), *Prunus serotina* (0.42), *Sporobolus cryptandrus* (0.42), and Unknown forb 133 (0.42) (all positive values). For PC axis 2, positive values were associated with greater distances from edges, and negative values with lesser distances from edges. Negative values of PC axis 2 were associated with six main species: *Elymus trachycaulus* (species score = -0.35), *Poa compressa* (-0.35), *Maianthemum canadense* (-0.35), *Rumex acetosella* (-0.35), *Acer* sp. (-0.35), and *Pinus* sp. (-0.35) (representing distances closer to edges). Positive values of PC axis 2 were not strongly associated with any specific plant species.

**Table 3.1 Observed species richness across six sampled restored grassland sites in Norfolk County, mean ( $\pm$  SE) of species richness and the percent vegetative cover are given for each site observed within 1 m  $\times$  1 m plots along transects away from deciduous forest and agricultural crop field boundaries. HF and MW denote the DeMaere2 home field and Midwest sites, respectively.**

Site	Total species observed	Species richness		Percent cover	
		(#spp/m <sup>2</sup> )		(%/m <sup>2</sup> )	
		(Mean $\pm$ SE)		(Mean $\pm$ SE)	
		Forest	Agriculture	Forest	Agriculture
Anderson	19	4.33 $\pm$ 0.92	3.67 $\pm$ 0.42	66.8 $\pm$ 9.1	76.8 $\pm$ 10.6
Casier	21	5.00 $\pm$ 0.45	8.00 $\pm$ 1.21	67.2 $\pm$ 8.2	82.7 $\pm$ 6.3
HF	38	8.83 $\pm$ 1.40	5.17 $\pm$ 0.87	84.8 $\pm$ 7.2	65.0 $\pm$ 10.8
MW	21	4.17 $\pm$ 0.65	6.00 $\pm$ 0.93	60.7 $\pm$ 9.5	93.0 $\pm$ 17.9
Demaiter	28	8.67 $\pm$ 0.76	7.17 $\pm$ 0.91	51.8 $\pm$ 3.2	65.7 $\pm$ 6.1
Demeyere	27	6.83 $\pm$ 1.01	7.00 $\pm$ 0.86	65.7 $\pm$ 6.4	69.0 $\pm$ 11.2

Sampling was performed at each boundary type within each site and included six 1  $\times$  1 m plots along a 50 m transect placed at 0 m, 10 m, 20 m, 30 m, 40 m, and 50 m from the edge.

The results of the second PCA ordination, including only the most common species ( $n = 21$ ) based on the greatest total percent cover ( $\geq 0.5 \text{ m}^2$ ) across all six sites, show that both axes were explained by site differences (PC axis 1:  $F_{5,66} = 13.45$ ,  $P = <0.001$ , PC axis 2:  $F_{5,66} = 5.054$ ,  $P = <0.001$ ). The first PC axis explained 9.5% variation and the second PC axis explained an additional 9.3% of the total variance in community composition. Positive values for PC axis 1 were driven by *Andropogon gerardii* (species score = 0.36), which was found in greatest abundance at Anderson site, followed by DeMaere2 (MW) and DeMaere2 (HF), and *Panicum virgatum* (0.30) that was found only at DeMaere2 (MW) and Anderson sites. Negative values for PC axis 1 were driven by *Symphyotrichum urophyllum* (-0.45) found in greatest abundance at DeMaere2 (HF), Casier and Demaiter, *Symphyotrichum oolentangiense* (-0.42) found at all sites, but in greatest abundance at DeMaere2 (HF) and Demeyere, and *Mondarda fistulosa* (-0.28) found in greatest abundance at Demaiter and Demeyere. For PC axis 2, positive values were driven by *Solidago juncea* (0.42) found largely at Demeyere and Casier, *Symphyotrichum leave* (0.38) in high abundance at Demeyere and Demaiter sites, and *Pycnanthemum virginianum* (0.31) that was in greatest abundance at Casier and DeMaere2 (MW). Negative values for PC axis 2 were driven by *Symphyotrichum urophyllum* (-0.37) and *Schizocyrium scoparium* (-0.32) with the greatest abundance at DeMaere2 (HF) (Figure 3.2).



**Figure 3.2** PCA biplot of the top 21 species ( $\geq 0.5 \text{ m}^2$  total percent cover) across six restored grassland sites. All sites are within Nature Conservancy Canada project areas in Norfolk County, Ontario, Canada. The analysis used a subset of the 2016 plant compositional data (species vegetative percent cover for  $1 \text{ m} \times 1 \text{ m}$  plots ( $n=72$ )), only including data for the top species (as explained above). Vegetation was sampled at six distances (0 m, 10 m, 20 m,

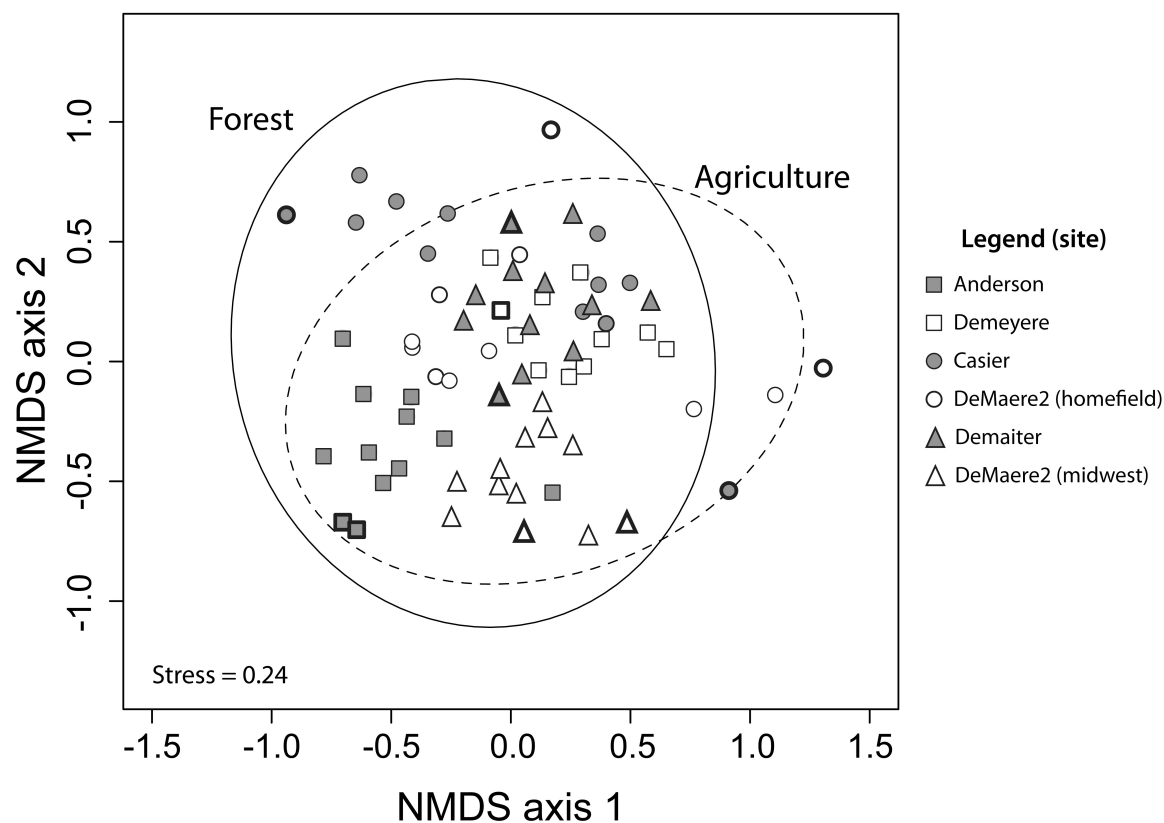
30 m, 40 m, 50 m) from two bordering land cover types (forest, agriculture) within each of the six restored grassland sites. Six-letter codes represent the following species:

*Andropogon gerardii* (AND.GER), *Artemisia campestris* (ART.CAM), *Cornus racemosa* (COR.RAC), *Desmodium canadense* (DES.CAN), *Elymus canadensis* (ELY.CAN), *Lespedeza capitata* (LES.CAP), *Monarda fistulosa* (MON.FIS), *Oenothera biennis* (OEN.BIE), *Panicum virgatum* (PAN.VIR), *Pycnanthemum virginianum* (PYC.VIR), *Rudbeckia hirta* (RUD.HIR), *Schizachyrium scoparium* (SCH.SCO), *Setaria viridis* (SET.VIR), *Solidago juncea* (SOL.JUN), *Solidago* spp. (SOL.SPP), *Symphyotrichum laeve* (SYM.LAE), *Symphyotrichum oolentangiense* (SYM.OOL), *Symphyotrichum urophyllum* (SYM.URO), *Toxicodendron radicans* (TOX.RAD), Unknown grass 102 (UNK.102), and Unknown grass 135 (UNK.135).



For the NMDS, PERMANOVA, and SIMPER analyses, two plot-level outliers were excluded: 0 m plot at the forest edge at the Demeyere site and 10 m plot at the agriculture edge at DeMaere2 (HF) site due to dramatically different vegetation. The 0 m forest edge plot at Demeyere was largely composed of *Setaria viridis*, which was not found in any other plots across the six sites, and thus led to highly significant dissimilarity to any other sampled plot. The 10 m DeMaere2 (HF) plot along the agricultural boundary, as discussed in Chapter 2, was high in *Salix* sp. and occurred on a sandy dune without much other vegetation. Both these plots had a great influence on the first NMDS attempt, which obscured any further patterns — thus the decision was to remove them from this analysis. The NMDS of plant compositional data showed a clear clustering of sites, a slight separation between land cover edge types, and a subtle trend for distance where most 0 m plots were at the perimeter of the cluster (stress = 0.24) (Figure 3.3). Results of the PERMANOVA tests revealed significant differences among sites ( $F_{5,64} = 6.79$ ,  $P = 0.001$ ), but not between land cover edge types ( $F_{1,68} = 1.598$ ,  $P = 0.079$ ) or distances ( $F_{1,68} = 1.726$ ,  $P = 0.066$ ), although both were near significant, and trends for these variables are apparent in the NMDS plot. The SIMPER analysis identified that certain plant species led to generally high similarity within sites, and low similarity among sites (Table 3.2). The average pairwise dissimilarity between site plant composition ranged between 75.1% (Demaiter and Demeyere) and 97.5% (Anderson and Casier), which was on average greater than the dissimilarity between agriculture and forest boundaries (86.0%), but similar to the dissimilarity values among distances. Dissimilarity values among distance categories ranged between 79.4% (most similar: 30 m and 40 m) and 92.0% (least similar: 0 m and 50 m).

Results of Mantel tests correlating plant composition with spatial location at each of the six sites sampled, across all plots and also separated by adjacent land cover type, are shown in Table 3.3. Distance decay plots for individual sites showing significant correlations in Mantel tests (DeMaere2 (all plots) and Casier (all plots and each land cover edge type separately)) are also shown (Figure 3.4). Unlike Mantel tests, the distance decay plots only include pairwise similarities between plots of the same transect, and therefore do not include similarities between plots of different land cover edge types for clarity.



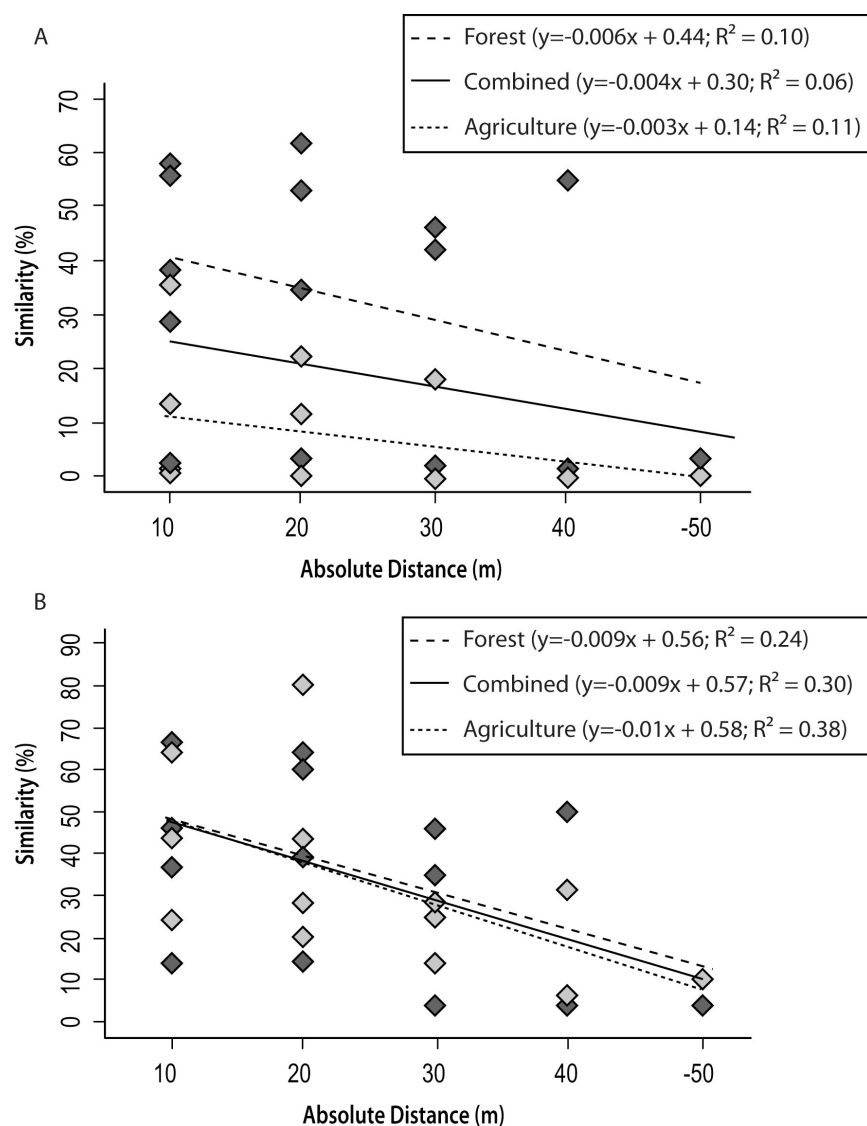
**Figure 3.3 NMDS plot of plant community compositional data from six restored grassland sites within Norfolk County, Ontario, Canada, seeded by Nature Conservancy Canada. Each point represents one  $1 \times 1$  m plot placed at 10 m increments from two land cover edges (forest, agriculture), for a total of 70 plots. Although 72 plots were sampled, two plots were determined outliers and removed from the ordination. Symbols with bold outline represent plots 0 m from an edge. Ellipses show 95% confidence areas using standard error for deciduous forest (solid line) and agriculture crop fields (dashed line).**

**Table 3.2 Results of SIMPER analysis on the similarity of plant community composition within six restored grassland sites sampled from Norfolk County at the 1 × 1 m plot scale. Based on 12 plots per site, with 2 outliers removed (0 m plot at Demeyere and 10 m plot at DeMaere2 (HF)).**

Site	Within site similarity	Top two species drivers of within site similarity (% contribution)
Anderson	43.9%	<i>Andropogon gerardii</i> (89.1%) <i>Desmodium canadense</i> (4.7%)
Casier	20.0%	<i>Oenothera biennis</i> (49.8%) <i>Solidago canadensis</i> (22.6%)
DeMaere2 (HF)	18.7%	<i>Schizachyrium scoparium</i> (41.0%) <i>Andropogon gerardii</i> (25.4%)
DeMaere2 (MW)	38.6%	<i>Artemisia campestris</i> (72.3%) <i>Andropogon gerardii</i> (13.0%)
Demaiter	30.4%	<i>Symphyotrichum laeve</i> (46.8%) <i>Rudbeckia hirta</i> (19.8%)
Demeyere	25.6%	<i>Symphyotrichum laeve</i> (56.6%) <i>Artemisia campestris</i> (13.4%)

**Table 3.3 Mantel test results showing relationships between plant composition (based on percent cover of species within 1 m x 1 m sampling plots) and absolute distances between vegetation sampling plots for six restored grassland sites. Results are shown for all plots at a site as well as edge type individually (deciduous forest or agricultural crop field). All sites are located in Norfolk County, Ontario, Canada and were seeded, and are managed by, Nature Conservancy Canada.**

Site		r	P
Anderson	All plots	-0.085	0.741
	Forest	0.347	0.132
	Agriculture	0.024	0.487
Casier	All plots	0.705	<b>0.001</b>
	Forest	0.429	<b>0.035</b>
	Agriculture	0.571	<b>0.042</b>
DeMaere2 (HF)	All plots	0.232	<b>0.039</b>
	Forest	0.236	0.269
	Agriculture	0.353	0.115
DeMaere2 (MW)	All plots	0.035	0.308
	Forest	0.210	0.225
	Agriculture	0.008	0.486
Demaiter	All plots	0.109	0.158
	Forest	0.344	0.144
	Agriculture	0.336	0.114
Demeyere	All plots	0.151	0.281
	Forest	0.017	0.369
	Agriculture	0.262	0.233



**Figure 3.4** Distance decay plots showing change in similarity with increasing distance between pairs of  $1\text{ m} \times 1\text{ m}$  plots sampled in 2016 for vascular plant composition (species present and percent cover). Shown are sites with significant correlations between plant composition and absolute distance as found with Mantel tests. Plots show only pairwise similarities within the same transect, while Mantel tests used similarities between all plots at a site. Shown are data for sites a) DeMaere2 (HF) and b) Casier. Dark grey symbols represent plot pairs near deciduous forest and light grey symbols represent plot pairs near agriculture crop fields.

### 3.4 Discussion

Native grassland ecosystems have declined worldwide (Samson and Knopf 1994), largely due to land conversion to agriculture, impacting both plant and animal biodiversity (Ceballos et al. 2010). As such, efforts have been made to acquire managed land parcels and restore them to their native state through seeding and sometimes management programs such as burning or mowing (Gerla et al. 2012, Plenzler and Michaels 2015). However, restoration initiatives of native grasslands can be influenced by adjacent land cover (forests, agricultural lands), limitations of seeding mix availability, and site specific differences including land use history. Therefore, the ‘success’ of a restored grassland is difficult to assess and measure, and can be highly subjective (Ruiz-Jaen and Aide 2005). Measures of diversity (richness, abundance), plant community structure (composition), and aspects of ecosystem function (e.g. nutrient cycling) across multiple sites are the suggested methods of assessment (Ruiz-Jaen and Aide 2005). In this study, I focused on the influence of adjacent land cover on plant species richness, abundance (percent cover), and community composition across six restored grasslands in southern Ontario, where native tallgrass habitat is less than 3% of the original range (Bakowsky and Riley 1994) and exists mostly as small remnants (< 2 ha) (Shorthouse 2010).

Adjacent land cover can influence plant composition of restored grassland areas through direct biotic effects (non-seeded plants invading a restored site) or indirectly through physical (e.g. shading), chemical (e.g. nutrient) or management (e.g. pesticide use) factors, with these influences being most pronounced where grasslands and adjacent lands come in contact (edges). In my study, while land cover edge type and distance from adjacent edges were not statistically significant drivers of plant species richness, abundance (percent cover) or composition, several trends were observed that suggest adjacent land cover of forest and agriculture are potential factors influencing grassland plant composition. However, because the results did not show strong differences between land cover edge types, or among distances from edges, this suggests that within-site effects may be a stronger driver of plant composition in restored grasslands.

### 3.4.1 The influence of forests and crop fields on adjacent restored grassland plant communities

Unchanging plant species richness and vegetation cover moving away from adjacent forest or agriculture edges to grassland interiors may indicate no breakpoint in vegetation, at least for these variables. However, this result may not be surprising, because richness and percent cover do not fully take into account the composition of the grassland plant community. When considering plant species identity and their relative abundance (percent cover) together I found compositional change occurring from land cover edge to grassland interior at specific study sites. These results are similar to Gieselman et al. (2013) and Dutoit et al. (2007) who also did not find significant differences in plant species richness or total percent cover when comparing edge vegetation bordering crop fields with non-edge control sites, despite strong patterns in compositional change. This spatial pattern of decreasing similarity between increasingly distant plots away from an alternative land cover edge (distance decay) without a change in species richness, could be due to replacement of native species with exotic species at edges, thus maintaining richness, but with changing species composition (Gieselman et al. 2013). This proposed mechanism is supported by several other studies of grassland edge vegetation that have also found greater proportions of exotic species at edges versus grassland interiors (Tyser and Worley 1992, Cilliers et al. 2008, Taft 2016). So, perhaps it is when composition changes faster than overall distance changes (i.e. a steep slope in distance decay plots), that an edge effect is occurring, and a breakpoint in the rate of change could indicate the end of edge vegetation and beginning of core vegetation.

Composition of plant communities differed greatly among sites (see section 3.4.2), which likely overshadowed the detection of study-wide edge effects. Sites tended to have one or two species that explained the majority of their similarity within, and these explanatory species differed among sites, explaining site dissimilarity. Overall composition across the six sites was also largely explained by these same site-defining species, with composition of course characterized mostly by species found in abundance at multiple sites versus at a single site. Across all sites,

plots bordering forest versus agricultural borders showed only slight differentiation, and the DeMaere2 (HF) and Casier sites mainly drove these differences. When sites were analyzed separately, these two sites in particular demonstrated patterns indicative of edge effects. For instance, the overall significant correlation between composition and space observed at DeMaere2 (HF) was likely due to the high contrast between forest edge and agriculture edge transects, which demonstrated larger differences in vegetation. For both forest and agricultural boundaries at DeMaere2 (HF), the observed distance decay (decrease in composition similarity between plots of increasing distance) (Nekola and White 1999) was largely explained by a distinct vegetation community at the immediate edge (0 m). At several sites, I found plant species immediately adjacent to the forest edge (0 m) that were native to the forest but not seeded grassland species (e.g. *Maianthemum canadense*, *Toxicodendron radicans*, *Vitis aestivalis*, *Betula* sp., *Pinus* sp. and *Acer* sp.), while the remainder of plots adjacent to the forest boundary was largely composed of seeded grassland species. This was observed at DeMaere2 (HF), as well as Casier, Demaiter, and Demeyere. Along the agriculture boundaries, I only found a strong pattern of distance decay at DeMaere2 (HF), where the sampling transect originated at the base of a tree line with a dense shrubby and grassy cover (0 m), separated from the interior of the grassland by a sandy slope with willow trees (10 m) and mostly weedy species (20 m), before transitioning to seeded species (30-50 m) where composition most closely represented the grassland's interior. Overall plant composition at Casier site also demonstrated a fairly strong correlation of plant composition and distance between sample locations. This trend was observed across the entire site as well as within both forest edge and agriculture edge transects separately, indicating an edge effect at both land cover types.

While plant composition decreased in similarity with distance between sample locations at most sites, this was not the case for the DeMaere2 (MW) and Anderson sites, where the vegetation compositional similarity was consistently 30% and 50% similar, respectively, along the lengths of each transect sampled. Overall similarity within these sites was explained by the high abundance and frequency of a single species at each site — Wormwood (*Artemisia campestris*) at DeMaere2 (MW) and Big Bluestem (*Andropogon gerardii*) at Anderson. The Anderson,



DeMaere2 (MW), and Demaiter sites had higher similarity of 0 m plots to more interior plots, possibly due to samples being collected approximately 5 m from the true forest edge due to small dirt roads running parallel to their forest boundaries. DeMaere2 (HF), Casier, and Demeyere sites had high dissimilarity at 0 m plots, which was noticeably distinct in visual observation with the occurrence of native forest species at forest edges and non-native species at crop field edges. However, overall, the 0 m plots sampled at boundaries of both forest and agricultural edges were not only unique to their adjacent land cover type, but also unique to their respective site.

### 3.4.2 Site differences within a spatially and temporally fragmented landscape

While all of the six restored grassland sites chosen for this study were within ten kilometers of each other and shared many physical habitat characteristics (e.g. soil type), clear differences emerged in plant composition among the six sites. It is important to recognize that each of the sites was seeded with a distinct assortment of species. Seed mix composition for these sites varied in species proportions, and certain species were only sometimes present in, or completely absent from, a mix. The dissimilarity of plant composition among sites could have resulted from the physical distance between them as expected from Tobler's First Law of Geography (also called spatial autocorrelation), which, when applied to ecology, predicts decreasing similarity in species composition between locations that are increasingly farther apart in physical space (Tobler 1970, Nekola and White 1999). Consistent with this, I observed that as my sampling scale became finer (from the site level (1-10 km apart), to the land cover level — between forest and agricultural boundaries within the sites (50-500 m apart), to between plots within transects (10-50 m apart)), similarity in vascular plant composition became greater.

Patterns of decreasing similarity with distance can also arise from changes in the physical environment or through spatially-mediated biotic processes such as dispersal. While I did not sample the physical environment for these six sites, I would not anticipate that differences in soil or climate, primary factors structuring plant communities, would differ significantly given the relative closeness of sites. However, at the same time, the proximity of sites did not lead to high

similarity in vascular plant composition that would arise from high dispersal among these sites. While the fragmented nature of the southern Ontario landscape may have slowed dispersal among sites (Nekola and White 1999), dispersal among sites does not seem to be influencing these plant communities, with the exception of several weedy species (not seeded, and both native and introduced), occurring in greatest abundance 0 to 20 m from both forest and agriculture boundary types. Rather, differences in seed lists and in seed banks between sites probably had a much greater impact on the observed species post-restoration, and led to sites that differed in composition.

Differential proportions of seed planted (within and among species) across sites likely affected the plant composition observed in the present study. The intention behind seeding for restoration is to give desirable species a competitive advantage over non-seeded species, which may be present in the seed bank, but undesirable (Bakker and Berendse 1999). The seed mixes for each of the six sites included in this study were all prepared by NCC and contained a high similarity of species for this reason, with seed used at different sites often from the same or similar origin (NCC pers. comm.). However, each site-specific seed list was unique to a degree in terms of species quantities as well as what species were actually included. Each site had noticeable differences upon visual assessment, with most sites having a single or few distinguishing (seeded) species (pers. obs.). However, plant species that were both seeded and actually observed at the study sites in 2016 were generally low, and represented between 15.6% and 20.8% of their respective seed mixes, across sites. There were greater proportions of plant species observed but not seeded (40-50%) based on richness values, but overall most sites contained about 50% observed species as seeded species, which is the typical similarity (50-60%) between restored grassland's plant composition and its associated seed bank (Bakker and Berendse 1999) (Appendix A).

That said, as the objective of this study was not to census the plant communities, rather examine edge vegetation associated with adjacent land cover across six restored grassland sites, an accurate assessment of seeding success was not possible, and it is probable that many species actually present at these sites (seeded or otherwise) were not captured in this study. Although

NCC shared seed weights by species along with their seeding lists, I attempted no further investigation as to the relationship between seeding rates and observed composition, because the relationship between mass of seed planted and actual plant output can vary greatly among species (Guo 2011), and seed amounts were selected by NCC based on knowledge of seeding rates, prior to seeding.

### 3.4.3 Similarities and differences in land-use histories at restored grassland sites

Historical activities in formerly agricultural, fragmented landscapes can strongly influence current vegetation patterns (Lunt and Spooner 2005). All sites used in this study had a history of agricultural use, although the crops used and the time between final crop harvest and restoration seeding differed slightly (NCC pers. comm.). All sites were recently planted with soybeans (for weed control) prior to restoration, except Casier, which was formerly cultivated for asparagus. Formerly agricultural soils can have higher pH, nitrogen, and phosphorus levels than land never farmed, and likely have a higher exotic to native seed ratio (Neill et al. 2015, Schelfout et al. 2015). In addition, management practices associated with certain cropping systems can have long-term impacts (Blair et al. 2006).

Plant compositional and percent seeded species is also expected to change over time since the initial restoration initiative of a grassland (Munson and Lauenroth 2012). For instance, Waldén and Lindborg (2016) found that differing lengths of time between abandonment and restoration disparately affected species compositional change in semi-natural restored grasslands over an eleven-year period. Restored calcareous grasslands in Britain reached composition similar to their natural prototype after two seasons, regardless of three experimental seeding rates (Stevenson et al. 1995). Yet, Redhead et al. (2014) found the return from abandoned and unrestored calcareous grassland to plant composition resembling ancient calcareous grassland to take over a century. While the time since seeding differed among sites in the present study, all were seeded recently and within a few years of one another (2010-2013), and I found no

observable trends among total species richness, percent seeded and observed or percent not seeded and observed with time since restoration (Appendices A and C).

### 3.5 Conclusion

Although species dissimilarity among sites makes the task of finding repeatable patterns in vegetation at grassland edges difficult, dissimilarity within plant communities was shown to increase with space in six restored grassland sites. In future research, comparisons of “core” grassland vegetation in changing space with “edge” vegetation (as captured here) could show different rates (and consistencies) of species turnover that may allow a stronger elucidation of edge effects. I would predict that there would be a steadier, but lower rate of turnover within a grassland interior, versus a rapid change and a breakpoint in composition that would define an edge. Perhaps the reality of unique site characteristics (past, present, and future) will often obscure the outcomes of grassland vegetative restoration, and the associated edge effects. The goal of this study was to find a measurable, possibly predictable, distance at which agriculture and/or forest influence restored grassland vegetation at edges. In restoration, there is a need to identify edge versus core habitat, the latter of which is considered a measure for restoration and conservation of habitat for a variety of species (Gieselman et al. 2013). However, my study suggests that while the impact and degree of edge effects can be informative, aspects such as absolute edge width may need to be handled case-by-case, because each restoration project may exhibit unique characteristics and challenges.

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## Chapter 4

### 4 General Discussion

Tallgrass prairie habitat once covered a significant portion of southern Ontario. Currently, however, it is estimated that less than three percent of the original extent remains, mainly due to land-use of agriculture and urbanization. Land-use changes are the leading cause of biodiversity losses (Sala et al. 2000), and in southern Ontario there are several conservation and restoration initiatives to reverse this loss of prairie and restore biodiversity. Restoration initiatives may identify longer-term forest restoration through vegetative succession as end-goals, while others identify the maintenance of tallgrass prairie or oak savannah (J. Crosthwaite, NCC, pers. comm., 2015). Regardless of the goal, however, because these areas are often small, fragmented patches on the landscape, influences of adjacent land cover may affect restoration success.

In my thesis, I specifically examined restored grassland patches that were bordered by both forest habitat and agricultural areas in Norfolk County, in southern Ontario, Canada. Areas studied were restored and managed by Nature Conservancy Canada between 2010 and 2013. My research included two linked field studies: 1) an in-depth examination of plants, plant traits, and soil characteristics at a single grassland site (DeMaere2 HF), and 2) an examination of plant community composition among six restored grassland sites bordering agricultural fields and Carolinian forest patches. In my first study (Chapter 2), I found evidence of edge type influencing grassland plant composition and soil variables at DeMaere2 (HF) with this zone of influence being two-tiered — direct influences at 0 m, and approximately 30 m away from the bordering land cover type. Patterns in vegetation were linked to several soil variables, rather than being dictated by plant species traits. Using this information, my aim was then to investigate whether these patterns were also detectable at other sites in Chapter 3. However, even though the six selected restored sites were seeded and managed by Nature Conservancy Canada, overall plant composition differed greatly among sites, and I found no detectable, general influence of neighboring forest or agriculture on grassland vegetation composition.

Thus, I concluded that generalizations of restoration success may be difficult to ascertain due to site-specific differences. In the following sections I discuss why the DeMaere2 (HF) may be unique in displaying edge effects, and why differences among sites may supersede detectable differences in plant community composition within sites. I end this chapter with a discussion of future work, caveats and limitations of my research, and general conclusions that can be made.

#### 4.1 A unique case of edge effects: DeMaere2 Home field

Edge effects on grassland habitats from adjacent land cover have been observed in the form of both distinct plant composition (e.g. native versus exotic species) and soil variables (e.g. pH) in numerous previous studies (Bogaert et al. 2001, Gieselman et al. 2013, Lee and Power 2013, Rowe et al. 2013, Taft 2016, Winsa et al. 2015). The distance at which edge effects extend into grassland areas from adjacent land cover types can greatly affect the area of land effectively restored or conserved (Gieselman et al. 2013); thus, many studies, including my own research, have sought to delineate interior or 'core' versus edge habitat by finding a breakpoint in compositional changes reflecting edge effects and homogeneous composition of a grassland interior (Gieselman et al. 2013, Lee and Power 2013, Taft 2016). However, results from the literature and from my study indicate that an absolute distance from a grassland border where the 'edge' stops and true grassland begins may be too simplistic. Instead, there may be multiple factors influencing edge effects, and these factors can be unique to a particular grassland patch, including its history, management, restoration and/or conservation success, and the general nature of the grassland's environment.

A good example of unique site attributes is the DeMaere2 Home field (HF) site that I studied intensively in 2015 (Chapter 2), and again as part of a larger study in 2016 (Chapter 3). Among the six sites examined in 2016, DeMaere2 (HF) was the only site to exhibit edge effect patterns relating to adjacent land cover types, which was apparent in both 2015 and 2016 sampling years. Despite sampling half as many plots in the 2016 for DeMaere2 (HF), I found highly similar results, in particular that plant composition correlated with space using Mantel tests, mainly driven by differences between the forest and agricultural borders in general. These results

suggest that sampling intensity for plant composition along a single transect was sufficient for detecting edge effects. However, my results also suggest that edge effects are largely dependent on site characteristics, as DeMaere2 (HF) was the only site where edge effects were observed. Site characteristics may include differences in soil type, physical landforms, time and successional changes in plant composition, or possibly the success of the restoration initiative itself.

DeMaere2 (HF) was unique in having distinct topological features that differed between forest and agricultural borders. DeMaere2 (HF) has a distinct sandy berm and ‘hollow’ area along the agricultural border where 0 m plots were along a berm of planted hedge-row trees, and 10 m into the grassland along this edge was a sandy hollow filled with willow trees species, and not much other vegetation leading to low values of vegetation percent cover, SLA leaf trait measures, soil moisture, and plant litter inputs. With respect to community composition, these plots were often outliers in the multivariate ordinations in both sampling years. Distinct plant composition at the DeMaere2 (HF) site is at least partially due to the peculiarity of this physical feature, reflected both in measured soil variables of this sandy soil and also explained by low establishment owing to seeding difficulty. That said the forest boundary also displayed indications of edge effects that were likely ‘true’ edge effects related to forest plant species encroachment, shading, and litter inputs.

Several studies of grassland edges found invasion by weedy species at borders with agriculture as well as with roads (Gieselman et al. 2013, Lee and Power 2013, Taft 2016). I found higher cover of exotics at the agriculture border at DeMaere2 (HF), where in general plant composition differed from other sites. The higher cover of exotics at the DeMaere2 (HF) agricultural border could be due to poor establishment of native seeded species, owing in part to planting difficulty in this sandy unstable soil up to 30 m from the edge, and potentially also due to the higher available nitrogen found there, because high nitrogen has been found to correlate with more invasive species (Gieselman et al. 2013, Lee and Power 2013). Although only weakly, nitrogen-fixing species found at this boundary associated with lower available nitrogen in the soil at the DeMaere2 (HF) site in 2015. Other plant-soil relationships were evident at DeMaere2 (HF),

where a 30 m breakpoint in soil variables was reflected in lower plant species richness near the agricultural border. Although low species richness before the breakpoint might be explained by the bareness of the sand dune there, vegetative percent cover was also low near the forest boundary, yet richness there remained high.

Although exotics (non-native plant species) were present at all sites, their abundance was generally low in terms of percent cover, and were mostly found at 0 m, and only for some sites. Important to note is that NCC uses targeted herbicide control of exotics at these sites, which can occur at edges. Overall, I could not conclude that non-native species invasion was a significant issue for the areas studied in this thesis, and in fact, DeMaere2 (HF) is lauded as a restoration success (pers. comm. NCC) due to its high seeding success and low non-native composition. All sites considered in this study were relatively the same age (seeded between 2010 and 2013), and this young age may explain the overall the lack of exotics. Restored grasslands will change with time, and largely due to local propagule sources (Auestad et al. 2015), and often exotics are found at edges in remnant grassland patches where enough time has passed for invasion. It may be that edge effects were detected at DeMaere2 (HF) only because there is low non-native composition within this grassland, and therefore new, exotic species were detectable along edges.

In general, within-site similarity versus between-site dissimilarity generally overshadowed edge effects in my study, although even when observed separately, individual restored grassland sites did not display detectable edge effects with the exception of DeMaere2 (HF). Each site had one or two representative species explaining within-site similarity, and these species differed among sites. Vegetative restoration (in terms of species richness and species seeded) was generally successful at edges, in that seeded, native species were consistently in relatively high abundance, with the exception of the agricultural border at DeMaere2 (HF) as described above. The only other site displaying significant spatial trends in plant community composition was Casier, where significant correlations between plant composition and space were evident in the turnover of species away from edges; this change occurring closer to forest than agriculture boundaries.

## 4.2 Restoration success at grassland edges

Restoration success is subjective despite attempts at a definition (Ruiz-Jaen and Aide 2005). That aside, the goal of this study was not to measure restoration success. I did, however, aim to find breakpoints in plant composition and characterize grassland borders in terms of edge versus core vegetation, and in doing so I consider here the native/non-native and seeded/not seeded abundance of plant species to 50 m from edges. I largely found seeded, native plant communities, save for at 0 m from the edge at many sites and at the DeMaere2 (HF) agricultural border in 2015, where the abundance of seeded native, not seeded native, and exotic species were in roughly equal abundance. For the remainder of sites, native seeded composition predominated, regardless of bordering land cover. Across all sites, although only roughly fifty percent of species observed in the 2016 study were seeded species, the actual percent cover of seeded, native species was quite high, suggesting low invasion of non-native species (Appendix A). This could be because vegetative restoration of the sites sampled in this study in the Norfolk County Sand Plain is still recent (within the last seven years), and it is possible that non-native species could potentially spread over time and invade more deeply into restored grassland patches (Auestad et al. 2015, Conradi and Kollmann 2016).

When considering restoration initiatives, it is important to consider appropriate minimum habitat size with respect to edge effects, because edges reduce the 'effective' size of a habitat (Laurence et al. 2007). Environment Canada (2013) guidelines suggest a minimum grassland patch size is 50 ha, with at least one 100 ha patch within an existing or potential grassland landscape, however these guidelines are based on preserving bird species richness, and are not specific for plant restoration initiatives. All of the six sites sampled in this study were under 50 ha, ranging from only 7 to 45 ha. Edges tend to become a larger component of the grassland as patch size gets smaller because the perimeter : area (or edge : interior) increases in a non-linear manner with decreasing patch size. While many similar previous studies in remnant prairie have found breakpoints roughly between 15 to 30 m from examined edges (Gieselman et al. 2013, Lee and Power 2013, Taft 2016), perhaps edge effects are less of an issue in restored grassland versus remnant grassland due to homogenized seeding and invasive plant management.

### 4.3 Forest versus agriculture as land cover in southern Ontario

Mixed habitats of forest and agriculture in the landscape of southern Ontario demonstrate the historical influences on restored grasslands. Affecting overall restoration success at these sites is their recent history as agricultural fields prior to restoration, yet it is important to recognize when considering the maintenance of these sites as grassland that the natural progression is succession to forest habitat. High grassland species richness has been associated with low soil nutrients (Janssens et al. 1998), thus there may be lasting effects of agriculture including higher nutrients. It could be that agricultural activity, such as soil disturbance and land reconstruction, could possibly play as large, or an even larger role, than the factors hypothesized here. The time for a restored grassland to resemble remnant grassland can range from a few years to nearly a century (Stevenson et al. 1995, Redhead et al. 2014). At the same time, in this particular restoration setting, several of the restored grasslands are hoped to undergo succession to forest stands, connecting existing forest fragments and creating a more robust forest network. Although saplings of woody species were planted at these sites, the early stages of encroachment of woody shrubs and trees from neighboring forest patches may have been observed in the form of small seedlings and saplings, and could continue in the years to come. Thus time between agricultural abandonment, restoration, and time since restoration can all affect composition (Waldén and Lindborg 2016).

### 4.4 Future studies and caveats of the current research

My in-depth study of DeMaere2 (HF) (Chapter 2) revealed several interesting patterns linking aboveground plant communities with belowground (soil) properties, while I did not measure soil properties at the other five sites included in Chapter 3 of this study. Differences in soil properties at forest versus agricultural borders could have been an additional source of variability leading to high between-site dissimilarity in Chapter 3. Although not fully understood, plant-soil feedbacks can either encourage or discourage succession, such as the differential influences of mycorrhizal fungi dependent on plant species, for example (Dickie et al. 2002, Nara and Hogetsu

2004, Nara 2006, Teste and Simard 2008). However, considering microbial relationships with aboveground vegetation, Li et al. (2015) found beta diversity linkages between late successional vegetation and soil microbes, but not between those of early successional temperate forests. Therefore a young, highly diverse grassland, especially because it has been seeded, may not show aboveground-belowground biodiversity relationships. Planting initially leaves less room for natural sorting, although sorting will occur with time (Conradi and Kollmann 2016). A greater understanding of the temporal scale over which aboveground-belowground relationships develop would be helpful in a grassland restoration context.

Future studies could also sample more intensively, perhaps continuously, immediately at edges (e.g. in my study, closer investigation between 0 m and 10 m), which may reveal some borders as narrow transition zones owing to physical disturbance, for example. My research highlighted often distinct composition of most 0 m plots when placed directly at the edge, however, the origin of my transects were often in direct contact with dirt roads, against tree trunks (forest), or at an agricultural field edge. Thus it is not surprising that plant composition was most dissimilar to grassland interior plots at 0 m. Although analyzing these 0 m plots was informative, for consistency in future studies I would establish the delineation of the actual grassland through a zone of planting, and sample more intensively in the first 10 m to determine a standard distance for more spatially consistent comparisons among sites.

Finally, in Chapter 2, I used a trait-based approach to try to understand patterns in plant communities along grassland edges, however this approach was not overly informative. Using more, or more specific plant trait variables may have revealed tighter plant trait-soil environment relationships using RLQ. However, acquiring plant trait data either from direct measurements or through data mining from databases was exceedingly time consuming. That said, the plant functional trait specific leaf area (SLA) was informative in identifying shade-loving and/or forest species at the 0 m forest boundary of DeMaere2 (HF) as clearly part of the adjacent forest communities. Further possibilities for future research include the use of distance decay specifically on plant functional traits such as SLA.

## 4.5 General conclusions

Distinguishing between site specific effects (e.g. unique physical site features, species planted) and potential temporal effects will be important in future analyses of edge effects among restored grassland sites. However, the ratio of edge versus core habitat may need to be determined on a case by case basis (i.e. site by site), as an arbitrary edge distance is likely not universal. Edge effect research in grassland should therefore be site specific and goal oriented, whether surveys preclude restoration plantings to identify potential edge effects in the planning phases or to assess vegetative establishment at edges. After all, restoration success can only be evaluated with goals in place (Waldén and Lindborg 2016). Regardless, the occurrence of non-native vegetation common in grassland edge studies implicates the need for special attention at edges (Gieselman et al. 2013, Lee and Power 2013, Rowe et al. 2013, Taft 2016).

Longer term studies of restored grassland sites that share similar restoration, ecological, and historical contexts, such as the sites studied here, could be highly informative in diagnosis and treatment of edge effects as woody encroachment at forest edges, as well as the growth and establishment of shrubs and trees should change these sites dramatically over time. In time, potential factors such as adjacent forest age and composition, neighboring crop type, and the history of land prior to grassland restoration may show stronger effects than observed here. I would also expect that plant functional traits will correspond more tightly to their environment with increased time since restoration due to increased competitive dynamics among species, dispersal from outside and within sites (as opposed to human seeding), and as species sorting occurs along gradients.

Grassland restoration is a science in progress in a changing environment. Sluis (2002) found species richness in remnant prairies to be always greater than in restored prairies, and suggested that our lack of understanding in how species richness is naturally maintained in ecosystems hinders our ability to re-create plant communities as rich as remnant grassland. Diversity can be increased starting at grassland borders, such as the restoration of grassland habitat adjacent to existing grassland (Rowe et al. 2013). Further understanding with regard to aboveground-



belowground relationships, as well as how plant functional traits determine community structure in a landscape, could bring us closer to understanding the ecological requirements that allow restoration to better emulate natural processes and provide invaluable ecosystem services.

However, understanding the negative impacts on biodiversity require a fuller picture. Although only crop-producing agricultural land adjacent to restored grassland was addressed here, the impact of agriculture on habitat loss is immense, especially when also considering current animal production (Weis 2007). Livestock is the single biggest driver of habitat loss worldwide, and the land used for livestock and feedstock production is growing (Machovina et al. 2015). Land clearing for agricultural uses contributes to climate change through carbon dioxide release (Curtis 2003). Additionally, in an already fragmented landscape, climate change exacerbates conservation challenges, potentially yielding unknown effects in addition to currently insufficient habitat requirements for many species, ongoing species extinctions, and rapid population declines (Donaldson et al. 2017). Restoration goals may then be too short sighted, too small-scale, and too slow-paced if confined within our current climate, within small parcels of land, and only when that land is made available opportunistically.

## 4.6 References

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## Appendices

### Appendix A: Full seeding list and plant species observed for six restored grasslands in Norfolk County, Ontario

Species seeded and observed during sampling of six sites restored and managed by Nature Conservancy Canada in Norfolk County, Ontario, Canada. All sampling took place in 2016. The letter “S” indicates when a species was seeded at a site and “O” indicates when a species was observed during sampling at a site. Hatched areas indicate when species were both seeded and observed at a single site. The full list of species is a combined list of all seeding lists for the sites shown, as well as any additional species identified upon sampling. "HF" and "MW" refer to DeMaere2 Home field and Midwest sites, respectively.

Species	Anderson	Casier	DeMaere2 (HF)	DeMaere2 (MW)	Demaiter	Demeyere
<i>Acer</i> sp.			O			
<i>Alnus incana</i> (L.) Moench			O			
<i>Ambrosia artemisiifolia</i> L.			O		O	O
<i>Andropogon gerardii</i> Vitman	S O		S O	O	O	O
<i>Andropogon virginicus</i> L.			S			
<i>Anemone virginiana</i> L.		S			S	
<i>Antennaria parlinii</i> Fernald		S			S	
<i>Anthemis cotula</i> L.			O			
<i>Aquilegia canadensis</i> L.		S			S	
<i>Arabis glabra</i> (L.) Bernh.		S			S	
<i>Artemisia campestris</i> L.	S	S	S	S O S O S O		
<i>Asclepias incarnata</i> L.			S	S		
<i>Asclepias syriaca</i> L.	S		S			
<i>Asclepias tuberosa</i> L.	S	S	S	S	S	S

<i>Betula</i> sp.										O		
<i>Bromus kalmii</i> A. Gray		[Shaded]						S		[Shaded]		
<i>Ceanothus americanus</i> L.	S	S		S		S		S		[Shaded]		
<i>Carex muehlenbergii</i> Schkuhr ex Willd.				S								
<i>Carex siccata</i> Dewey				S								
<i>Carex vulpinoidea</i> Michx.				S								
<i>Carya cordiformis</i> (Wangenh.) K. Koch	S	S		S		S		S		S		
<i>Carya ovalis</i> (Wangenh.) Sarg.	S	S		S		O		S		S		
<i>Carya ovata</i> (Mill.) K. Koch	S	S		S		S		S		S		
<i>Conyza canadensis</i> (L.) Cronquist		O		O		O				O		O
<i>Cornus amomum</i> Mill.				S				S		S		
<i>Cornus florida</i> L.	S	S						S		S		
<i>Cornus racemosa</i> Lam.	S	S		[Shaded]				S		S		
<i>Cornus stolonifera</i> Michx.				S				S		S		
<i>Corylus americana</i> Walter	S	S		S		S		S		S		
<i>Crataegus pruinosa</i> (Wendl. f.) K. Koch				S		S		S		S		
<i>Crataegus</i> sp.				S				S				
<i>Cyperus lupulinus</i> (Spreng.) Marcks						S						
<i>Daucus carota</i> L.								O		O		
<i>Desmodium canadense</i> (L.) DC.	[Shaded]											
<i>Desmodium paniculatum</i> (L.) DC.	S	S		S		S		S		S		
<i>Desmodium rotundifolium</i> DC.				S								
<i>Doellingeria umbellata</i> (Mill.) Nees	S	S		S		S		S		S		
<i>Elymus canadensis</i> L.	[Shaded]			O		O		[Shaded]		O	[Shaded]	
<i>Elymus riparius</i> Wiegand												S
<i>Elymus trachycaulus</i> (Link) Gould ex Shinnars	S	S		[Shaded]				S		S		S
<i>Elymus virginicus</i> L.												S
<i>Equisetum arvense</i> L.								O				
<i>Erigeron annuus</i> (L.) Pers.								O				
<i>Euphorbia corollata</i> L.		S		S				S		S		

<i>Euthamia graminifolia</i> (L.) Nutt.				S	S			
<i>Fragaria vesca</i> L.	S	S			S		S	S
<i>Fragaria virginiana</i> Duchesne	S	S			S		S	
<i>Gnaphalium macounii</i> Greene	S			S				
<i>Gnaphalium obtusifolium</i> L.	S	S		S	S		S	S
<i>Hamamelis virginiana</i> L.	S	S					S	S
<i>Hedyotis longifolia</i> Gaertn.	S	S		S	S		S	S
<i>Helianthemum bicknelli</i> Fernald	S	S		S	S		S	S
<i>Helianthus divaricatus</i> L.	S	S		S			S	S
<i>Helianthus strumosus</i> L.	S	S					S	S
<i>Heliopsis helianthoides</i> (L.) Sweet		S					S	
<i>Hieracium florentinum</i> All.		O						
<i>Juglans nigra</i> L.								S
<i>Juniperus virginiana</i> L.		S					S	S
<i>Lechea intermedia</i> Leggett ex Britton	S	S		S	S		S	S
<i>Lechea villosa</i> Elliott	S	S		S	S		S	S
<i>Lepidium sativum</i> L.		O		O			O	O
<i>Lespedeza capitata</i> Michx.	S	O	S	O	S	O	S	O
<i>Lespedeza hirta</i> (L.) Hornem.	S	S		S	S		S	S
<i>Lespedeza intermedia</i> sensu Clewell, 1966	S	S		S			S	S
<i>Liatris cylindracea</i> Michx.	S	S		S	S		S	S
<i>Lindera benzoin</i> (L.) Blume		S					S	S
<i>Lobelia cardinalis</i> L.		S					S	
<i>Lobelia inflata</i> L.		S		S			S	
<i>Lotus corniculatus</i> L.		O						
<i>Lupinus perennis</i> L.	S	S		S	S		S	S
<i>Maianthemum canadense</i> Desf.					O			
<i>Medicago lupulina</i> L.					O		O	
<i>Monarda fistulosa</i> L.	S	S	O	S	O	S	O	S
<i>Nyssa sylvatica</i> Marshall		S					S	S

<i>Oenothera biennis</i> L.	S	O	S	O	S	S	O	S	O	O
<i>Oxalis dillenii</i> Jacq.				O		O			O	O
<i>Panicum miliaceum</i> L.	S		S		S		S	S		
<i>Panicum virgatum</i> L.	S	O					O		O	
<i>Parthenocissus quinquefolia</i> (L.) Planch.	S						S			
<i>Parthenocissus vitacea</i> (Knerr) Hitchc.	S		S				S	S		S
<i>Penstemon digitalis</i> Nutt. ex Sims	S		S		S		S	S		S
<i>Physalis heterophylla</i> Nees			S					S		
<i>Physalis virginiana</i> Mill.			S					S		S
<i>Pinus</i> sp.							O			
<i>Plantago major</i> L.									O	
<i>Poa compressa</i> L.							O			
<i>Populus deltoides</i> W. Bartram ex Marshall									O	
<i>Populus</i> sp.							O			
<i>Prenanthes altissima</i> L.					S					
<i>Prunus americana</i> Marshall			S		S		S	S		S
<i>Prunus pensylvanica</i> L. f.										S
<i>Prunus serotina</i> Ehrh.	S		S		S		S	S		S
<i>Prunus virginiana</i> L.	S		S		S		S	S		S
<i>Pycnanthemum virginianum</i> (L.) T. Dur. & B.D. Jacks. ex B.L. Rob. & Fernald	S	S	O	S	O	S	O	S	O	S
<i>Pyrus coronaria</i> L.		S		S				S		S
<i>Quercus alba</i> L.		S						S		S
<i>Quercus macrocarpa</i> Michx.		S		S				S		S
<i>Quercus prinoides</i> Willd.	S		S			S		S		S
<i>Quercus rubra</i> L.	S		S			S		S		S
<i>Quercus</i> sp.		O		O			O		O	O
<i>Quercus velutina</i> Lam.	S		S			S		S		S
<i>Rhus copallinum</i> L.	S		S		S		S	S		S
<i>Rhus typhina</i> L.	S				S		S			S

<i>Rosa blanda</i> Aiton	S	S	S	S	S	S
<i>Rosa carolina</i> L.	S	S	S	S	S	S
<i>Rubus allegheniensis</i> Porter	S	S		S	S	S
<i>Rubus flagellaris</i> Willd.	S	S		S	S	S
<i>Rubus occidentalis</i> L.		S		S	S	
<i>Rudbeckia hirta</i> L.	S O S O S O			S	S O S O	
<i>Rudbeckia laciniata</i> L.		S			S	
<i>Rumex acetosella</i> L.				O		
<i>Salix</i> sp. 50				O		
<i>Salix</i> sp. 51				O		
<i>Schizachyrium scoparium</i> (Michx.) Nash	S	S O S O S O S O S O				
<i>Scirpus atrovirens</i> Willd.			S			
<i>Scirpus cyperinus</i> (L.) Kunth			S			
<i>Setaria viridis</i> (L.) P. Beauv.						O
<i>Silene antirrhina</i> L.	S	S O	S	S O	S	S
<i>Silene</i> sp.				O		
<i>Sisyrinchium montanum</i> Greene		S	S		S	
<i>Smilax herbacea</i> L.			S			
<i>Solidago canadensis</i> L.		O		O		O
<i>Solidago juncea</i> Aiton	S	S O	S	S	S O S O	
<i>Solidago nemoralis</i> Aiton	S	S	S	S	S	S
<i>Sorghastrum nutans</i> (L.) Nash	S		S			
<i>Specularia perfoliata</i> (L.) A. DC.		S	S	S	S	S
<i>Spiraea alba</i> Du Roi		S	S		S	
<i>Sporobolus cryptandrus</i> (Torr.) A. Gray	S	S O	S	S O	S	S O
<i>Symphotrichum ericoides</i> (L.) G.L. Nesom		S	S O	S	S O	S
<i>Symphotrichum laeve</i> (L.) Á. Löve & D. Löve	S O S O S O S O S O S O					
<i>Symphotrichum lanceolatum</i> (Willd.) G.L. Nesom	S O			S		
<i>Symphotrichum novae-angliae</i> (L.) G.L. Nesom				S		S
<i>Symphotrichum oolentangiense</i> (Riddell) G.L. Nesom	S O S O S O S O S O S O					



<i>Symphyotrichum pilosum</i> (Willd.) G.L. Nesom				O	S		S				S	
<i>Symphyotrichum urophyllum</i> (Lindl.) G.L. Nesom	S		S O S O				S		S O S O			
<i>Taraxacum officinale</i> F.H. Wigg.										O		
<i>Thalictrum revolutum</i> DC.					S							
<i>Toxicodendron radicans</i> (L.) Kuntze				O								
Unknown 129												O
Unknown forb 111		O										
Unknown forb 133				O								
Unknown forb 134				O								
Unknown grass 102		O										
Unknown grass 118								O				
Unknown grass 135				O						O		
Unknown grass 48							O					
Unknown mustard 108		O										
Unknown shrub 132												O
<i>Verbascum thapsus</i> L.								O		O		
<i>Verbena hastata</i> L.					S		S					
<i>Viburnum acerifolium</i> L.							S					
<i>Viburnum lentago</i> L.			S				S		S		S	
<i>Viola fimbriatula</i> Sm.					S						S	
<i>Vitis aestivalis</i> Michx.	S		S O			O	S		S		S O	
<i>Vitis riparia</i> Michx.	S		S				S		S		S	
<b>Total Richness</b>	<b>64</b>	<b>19</b>	<b>84</b>	<b>26</b>	<b>75</b>	<b>38</b>	<b>69</b>	<b>21</b>	<b>84</b>	<b>28</b>	<b>77</b>	<b>27</b>
Seeded and observed	9		15		14		12		14		16	
Observed but not seeded	10		11		24		9		14		11	
% seeded and observed	14.1		17.9		18.7		17.4		16.7		20.8	
% observed but not seeded	52.6		42.3		63.2		42.9		50.0		40.7	
% seeded and observed/% observed but not seeded	26.8		42.2		29.6		40.6		33.3		51.0	

## Appendix B: Dominant plant species and their functional traits from DeMaere2 Home field.

Total percent cover (across 24 plots) as well as average trait values of the top 21 species (as determined by  $\geq 0.25$  m<sup>2</sup> total percent cover across all plots), determined by the greatest percent cover. Specific leaf area (SLA) and Maximum height were measured traits based on leaf and height measurements taken at the DeMaere2 (HF) restored grassland site in Norfolk County, Ontario, Canada in 2015. Other plant trait values are from online sources (see text for full list of references).

Species	Total Cover (m <sup>2</sup> )	SLA (cm <sup>2</sup> /g)	Max. Height (cm)	Duration	Nitrogen-fixer	Dispersal	Entomophily	Native
<i>Alnus incana</i>	0.38	139.71	72.7	perennial	yes	wind	no	yes
<i>Andropogon gerardii</i>	2.35	180.56	53.7	perennial	no	animal	no	yes
<i>Anthemis cotula</i>	0.50	239.79	10.8	annual	yes	proliferous	yes	no
<i>Coryza canadensis</i>	0.33	168.01	5.0	annual	no	wind	yes	yes
<i>Cornus racemosa</i>	0.35	176.06	105.0	perennial	no	animal	yes	yes
<i>Daucus carota</i>	0.30	117.22	132.5	biennial	no	wind	yes	no
<i>Desmodium canadense</i>	0.28	245.67	38.6	perennial	yes	animal	yes	yes
<i>Monarda fistulosa</i>	1.44	203.42	51.0	perennial	no	rhizomes	yes	yes
<i>Populus deltoides</i>	0.50	115.35	155.0	perennial	no	wind	no	yes
<i>Rubus occidentalis</i>	0.40	260.83	51.7	biennial	no	animal	no	yes
<i>Rudbeckia hirta</i>	1.12	224.20	29.4	biennial	no	proliferous	yes	yes

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<i>Schizachyrium scoparium</i>	1.80	205.62	42.7	perennial	no	wind	no	yes
<i>Solidago canadensis</i>	2.90	222.63	46.9	perennial	no	wind	yes	yes
<i>Sorghastrum nutans</i>	0.26	168.04	73.5	perennial	no	animal	no	yes
<i>Symphotrichum oolentangiense</i>	0.47	198.80	49.4	perennial	no	wind	yes	yes
<i>Symphyotrichum urophyllum</i>	0.30	147.00	27.9	perennial	no	wind	yes	yes
<i>Taraxacum officinale</i>	0.34	201.16	15.6	perennial	no	wind	yes	no
<i>Trifolium hybridum</i>	0.34	275.51	10.0	annual	yes	animal	yes	no
Unknown grass 33	0.38	169.90	42.3	perennial	no	wind	no	?
Unknown grass 48	0.45	270.14	22.0	annual	no	wind	no	?
Unknown grass 57	0.25	139.87	49.0	perennial	no	wind	no	?

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## Appendix C: Study site information for six restored grasslands in Norfolk County, Ontario.

Geographical location, size, seeding year, and boundary characteristics of the six sites sampled in both 2015 and 2016 studies. Number of species seeded includes all graminoids, forbs, shrubs, trees, and cover species. All sites are owned, managed, and were restored by Nature Conservancy Canada.

Site Name (Lat/Long) Year seeded	Site size (km <sup>2</sup> )	Number species seeded	Forest height (m)	Primary forest tree species	Bordering crop (2015)	Hedgerow species	Hedgerow height (m)
<b>DeMaere2 Home field (HF)</b> 42°41'07.8"N 80°28'12.1"W 2010	0.21	75	20	<i>Quercus velutina</i> <i>Acer</i> sp. <i>Quercus rubra</i> <i>Fagus grandiflora</i> <i>Alnus incana</i> <i>Morus</i> sp. <i>Carya glabra</i> <i>Sassafras albidum</i>	wheat/corn	<i>Quercus rubra</i> <i>Rhus typhina</i> <i>Populus deltoides</i> <i>Prunus serotina</i> <i>Acer</i> sp.	20
<b>DeMaere2 Midwest (MW)</b> 42°40'48.1"N 80°28'33.5"W 2010	0.07	69	13	<i>Rhus typhina</i> <i>Populus grandidentata</i> <i>Pinus</i> sp. <i>Abies balsamea</i> <i>Acer</i> sp. <i>Quercus rubra</i> <i>Tilia americana</i>	corn	<i>Abies balsamea</i> <i>Thuja occidentalis</i> <i>Rhus typhina</i>	6
<b>Anderson</b>	0.13	64	13	<i>Quercus albus</i>	soybeans	N/A	N/A

42°40'20.4"N 80°30'09.5"W 2010				<i>Alnus incana</i> <i>Quercus rubra</i> <i>Acer sp.</i> <i>Populus tremuloides</i> <i>Quercus velutina</i> <i>Crataegus sp.</i> <i>Rhus typhina</i> <i>Populus grandidentata</i>			
<b>Demeyere</b> 42°39'44.3"N 80°31'38.1"W 2011	0.23	55	16	<i>Rhus typhina</i> <i>Acer sp.</i> <i>Juglans nigra</i> <i>Prunus sp.</i> <i>Quercus velutina</i> <i>Vitis aestivalis</i> <i>Rubus occidentalis</i>	corn	<i>Thuja occidentalis</i> <i>Rhus typhina</i>	6
<b>Casier</b> 42°40'56.0"N 80°32'50.9"W 2013	0.34	84	15	<i>Rhus typhina</i> <i>Quercus velutina</i> <i>Acer sp.</i> <i>Juglans nigra</i> <i>Vitis aestivalis</i>	fallow (wheat)	N/A	N/A
<b>Demaiter</b> 42°42'05.0"N 80°32'12.2"W 2013	0.45	84	18	<i>Rhus typhina</i> <i>Acer sp.</i> <i>Quercus velutina</i> <i>Vitis aestivalis</i>	soybeans	<i>Thuja occidentalis</i> <i>Acer sp.</i>	8

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## Curriculum Vitae

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