Rethinking restoration ecology of tallgrass prairie: considering belowground components of tallgrass restoration in southern Ontario

by

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Author's Declaration

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

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Statement of Contributions

This thesis contains five chapters that are collaborative efforts of multiple researchers that will be submitted into peer-reviewed journals. Heather Cray is first author on all contributing papers and therefore was responsible for the development, data collection, data analysis and preparation of each of the manuscripts found in this dissertation. The written portions of all manuscripts, including figures and tables, were completed in their entirety by Heather Cray and edited for content and composition by thesis supervisor Dr. Stephen Murphy and committee members Dr. Andrew Trant, Dr. Greg Thorn, Dr. Rebecca Rooney, and Dr. Andrew MacDougall. Design, development, data collection, vegetation and soil analysis, and writing, including figures and tables, of chapter 4 was done by Heather Cray. The microbial data processing for chapter 4 was done by Dr. Josh Neufeld's lab at cost through a contract and he assisted with the microbial analysis methods section for chapter 4; Dr. Neufeld will be an author on the journal manuscript resulting from that study.

Abstract

Agriculture, urban development, and woody encroachment have reduced the North American tallgrass prairie ecosystem to less than 1% of its historical extent. The remnants of this now rare habitat are currently challenged not only by ongoing human disturbance but by the anticipated ecological regime shifts from anthropogenic climate change. In response, active restoration of tallgrass prairie is ongoing, aiming to re-establish native vegetation communities, often on former croplands. The success of tallgrass prairie restoration has been mixed and many knowledge gaps exist, especially pertaining to soil biota. With the goal of addressing key knowledge gaps identified by restoration practitioners, this thesis investigates the invasive earthworm populations of restored and remnant tallgrass prairie sites across southern Ontario, establishes the dietary preferences of the largest and most widespread invasive earthworm *Lumbricus terrestris* with respect to seeds commonly used in tallgrass prairie restoration, and examines below-ground (soil bacterial community) as well as traditional above-ground (vegetation community) measures of restoration success for different methods of tallgrass prairie restoration. The core significant original contributions of this dissertation are 1) invasive earthworms are present and abundant in all remnant and restored tallgrass prairies in southern Ontario; 2) the largest and most widespread invasive earthworm, *Lumbricus terrestris*, can play an important role in seed granivory in tallgrass prairie habitats, and these effects are uneven across the target and weed species investigated; and 3) above- and below-ground measures of restoration success can tell different stories, and conventional restoration methods do not maintain microbial communities similar to high quality remnant prairie in the short term, whereas sod mat transplants do. Recommendations for practice include considering interactions with invasive earthworms in restoration and ecosystem management plans, considering alternative measures and methods of tallgrass prairie restoration, and broadening the definition of restoration success to encompass the retention and restoration of below-ground ecosystem components. By deliberately engaging the end-users of this research in question development and producing and communicating context-specific results and recommendations that can guide future management decisions, this dissertation is in line with the core tenants of translational ecology, which is suggested as a way forward for the discipline of restoration ecology.

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Chapter 1

Introduction

The overarching goal of this dissertation is to address key knowledge gaps identified by restoration practitioners pertaining to the restoration of the southern Ontario tallgrass prairie ecosystem. Accordingly, the research questions addressed in each manuscript (chapters 2 through 4) were created with input from local land managers and restoration practitioners in addition to a detailed assessment of the available literature. Each manuscript also includes a summary of key findings and specific recommendations, which are summarized in the final chapter (chapter 5). This dissertation is designed to be in line with the core tenants of a translational ecology framework: end-user involvement and producing actionable science to address complex environmental problems. By deliberately engaging the end-users of this research in question development and producing and communicating context-specific results and recommendations that can guide future management decisions, my research achieves both academic and practitioner goals: it advances our scientific understanding of belowground elements of tallgrass prairie restoration (exotic earthworms and soil microbial communities), contributes to the larger narrative of how we can measure and achieve restoration success in tallgrass prairie, and provides actionable, locally relevant results to my collaborators. With the goal of addressing key knowledge gaps identified by restoration practitioners, this thesis investigates the invasive earthworm populations of restored and remnant tallgrass prairie sites across southern Ontario, establishes the dietary preferences of the largest and most widespread invasive earthworm *Lumbricus terrestris* with respect to seeds commonly used in tallgrass prairie restoration, and examines below-ground (soil bacterial community) as well as traditional above-ground

(vegetation community) measures of restoration success for different methods of tallgrass prairie restoration.

Background knowledge related to knowledge gaps in restoration ecology as well as the tallgrass prairie restoration context is provided (chapter 1) in support of the proceeding manuscripts examining the invasive earthworm communities in restored and remnant tallgrass prairies of southern Ontario (chapter 2), granivory preferences of the largest and most common invasive earthworm with respect to tallgrass prairie seed mix (target) and weedy or invasive (non-target) species (chapter 3), and restoration success based on above-ground (vegetation) and below-ground (soil microbial community) metrics using different restoration methods (chapter 4). This is followed by a discussion of translational ecology as a way forward for the field of restoration ecology, concluding with a brief discussion of the thesis and opportunities for future work (chapter 5).

1.1 Knowledge gaps: soil biota

The uncertainties and complexities surrounding below-ground interactions have important implications for the future of restoration ecology, as inadequate understanding of the ecology of the ecosystem can lead to restoration failures (Harris et al. 1996, House and Bever 2018, Hawkins and Crawford 2018). Soils are some of the most diverse and complex habitat on this planet (Kubicek and Druzhinina 2007), and it is only within the last few decades that we have begun to recognize the critical role they play in mediating ecosystem responses to anthropogenic disturbances (Gadd 1993, Staddon et al. 2002, Rillig 2004, Six et al. 2006). Soil ecosystem components also provide valuable ecosystem services such as reduction of surface-water runoff, soil stabilization, erosion control

through mycelial and plant root networks, increased water infiltration, nutrient cycling, and carbon storage (Liski et al. 2003, Zhang et al. 2005, van der Heijden et al. 2008).

Though the importance and complexity of below-ground interactions are just beginning to be understood, we do know that the presence of soil biota, as well as their functional and taxonomic diversity, has a direct bearing on the success of restoration and reclamation projects (Harris et al. 1996, Bever et al. 2001). The roles of below-ground biota in driving ecosystem dynamics are as numerous as they are cryptic. On a broad scale, plant-microbe symbioses have a significant impact on ecosystem function. This is not surprising given that the soil microbiome has been shown to alter plant community structure, productivity (Grime et al. 1987, Klironomos et al. 2000), and the course of succession (Medve 1984, Gange et al. 1990, Deyn et al. 2003, Carbajo et al. 2011). We also know that in some cases, the restored microbial community remains distinct for decades, and varies with soil type even given equivalent historical conditions and the same management and restoration practices (McKinley 2001, Jangid et al. 2010, 2011).

Failure to take the soil microbial community into account can also be a limiting factor in restoring native plant diversity and composition as microbial population dynamics can play a major role in plant species co-existence (Bever et al. 2010). Where the re-establishment of native plant species is a project goal, inoculating an area with native soil microbes has been shown to increase the rate of establishment of native plants (Thrall et al. 2005, Kardol et al. 2007). The degree of redundancy in soil microbial communities is currently unknown, and so net losses in diversity pose problems for future adaptation and resiliency (Peay et al. 2008, Talbot et al. 2014). Unfortunately, faced with the challenge of managing changing ecosystems, it is not currently possible to research the life cycle needs and dynamic function of each individual species of soil microbe, though functional

assessments and comparisons can be made through new eDNA, metagenomics, and barcoding techniques (Cabin 2011).

This has led to a call to create and preserve as much and as varied habitat as possible in a "Noah's Ark" or "save all the pieces" approach (Rosenzweig 2003). While desirable species may be adapted to conditions that we can never fully recreate, the more habitat created on different soils in different microclimates which support different species assemblages, the better the odds that it will fit the unknown habitat preferences of target species. This is particularly important in light of increasing land use and climate change that drive more rapid shifts in ecosystem dynamics and habitat suitability envelopes. Unfortunately, while the techniques to assess below-ground microbial communities continue to improve and becomes less costly (Allen et al. 2003, Arnold et al. 2007, Peay et al. 2008), below-ground interactions and feedbacks and the importance and functional role of elements of the soil microbiome remain a prominent gap in our knowledge base and constrain our ability to conserve or restore "whole ecosystems" that include all associated taxa (Fahselt 2007).

Scaling up, the role of soil macro fauna (earthworms, small vertebrates, arthropods, molluscs, nematodes, etc.) in ecosystem restoration also remains relatively unknown. Earthworms merit special attention here, as invasive species across multiple continents; the effects of earthworm invasion are just beginning to be elucidated but include changes to soil structure, nutrient cycling, water infiltration, rates of decomposition, and seedbank conditions (Brown 1995, Edwards and Bohlen 1996, Forey et al. 2011). Earthworms have direct effects on the composition and function of plant communities, but these vary by ecosystem, and species-specific interactions are common (Shumway and Koide 1994, Eisenhauer et al. 2009c, Clause et al. 2016b). The consequences of earthworm invasion are therefore variable depending on the particular species and ecosystem involved, and as with other invasive species, these pressures are synergistic with other challenges facing ecosystem

conservation and restoration. With respect to earthworms in particular, a major implication of this uncertainty is the impact of exotic earthworms on seed survival, especially where seeding is the main method for ecological conservation and restoration. Clearly, a more complete understanding of below-ground processes would inform how restoration ecology addresses global ecosystem change. A greater understanding of soil ecosystem dynamics could also help ecologists understand why successful restoration methods applied to one situation may have very different results in another (Jangid et al. 2010, Webb et al. 2014).

1.2 The tallgrass prairie context

During 8000-4000 BCE, the eastern third of the North American Great Plains was comprised of an extensive (over 950,000 km^2) and mainly contiguous tallgrass prairie ecosystem where groups of First Nations farmed, hunted, and set management fires (Transeau 1935, Stuckley 1981, Anderson 2006, Umbanhowar et al. 2006). There is considerable uncertainty regarding the exact period and extent of historic tallgrass prairie; pollen evidence suggests that many prairie species were present in the prairie-forest border of North America since the end of the Pleistocene (Benninghoff 1964), but did not form a distinct community until the Xerothermic roughly 8,000 years ago (King 1981). Identifying the drivers of historic tallgrass prairie establishment has been challenged by inaccurate radiocarbon chronologies, difficulty interpreting charcoal records, and the diversity of ecological and climatic events which occurred within this time period (Gill et al. 2012, Leys et al. 2015). Recent research has used paleorecords (e.g. early and middle Holocene sediments from lakes, pollen, charcoal influx) to assess long-term climatic, vegetational, and fire variability to evaluate hypotheses about the prairie's environmental history (Nelson et al. 2006, Umbanhowar et al. 2006). This evidence suggests that spatial heterogeneity is a characteristic feature of the historical tallgrass prairie

landscape, particularly at the prairie-forest ecotone of the northern United States and southern Canada (Danz et al. 2011), and that increasing aridity and fire influenced the prairie-forest transition between 10000 years and 6200 years before present, favoring the establishment and expansion of the tallgrass prairie ecosystem (Nelson et al. 2006, Umbanhowar et al. 2006, Moos and Cumming 2011). Inconsistent evidence based on pollen, spores, charcoal and macrofossils from pond sediments in the unglaciated southeastern United States supports a more directly climatic-driven hypothesis, suggesting that increasing temperatures and carbon dioxide levels, not fire, were primarily responsible for the ecological shift (Jones et al. 2017). Although charcoal variables from paleorecords can be difficult to interpret, fire history reconstructions can provide indicators of local burn areas (Leys et al. 2015). Determining the origin of fire (spontaneous versus First Nations management), however, remains challenging, and there is currently little evidence from the paleofire record to support the Early Anthropocene Hypothesis of human modification (Marlon et al. 2013). Further research investigating the relationships between the quantity of charcoal with fire intensity, vegetation cover, and climatic parameters using the most recent technologies may shed more light on the precise timing and extent of tallgrass prairie in the pre-European landscape of North America (Blois et al. 2011).

The presence of tallgrass prairie communities in southern Ontario in more recent history (prior to European settlement) has been supported by settler survey records and oral histories of First Nations communities (Rodgers and Anderson 1979, Bakowsky and Riley 1994, Faber-Langendoen and Maycock 1994), although these are by no means comprehensive. Determining the extent of tallgrass prairie and the degree to which it was actively managed by First Nations peoples is further complicated by the scarcity of documented oral histories. First Nations peoples including the Mississaugas of Alderville First Nation, the Anishinabe, and the Ojibwe, Potawatomi, and Odawa

peoples of Walpole Island First Nation set management fires to craft the landscape and expand prairie for Bison habitat; First Nations also used tallgrass prairie as a source of medicinally, historically and culturally important plant species (Irving 1956, Russell 1983, Higgins 1986). However, the consequences of European colonization (including widespread disease, death, and disenfranchisement) have resulted in a dearth of land management and traditional knowledge from First Nations oral histories, which is a permanent loss. Neither can the historic extent of tallgrass prairie be inferred from the location of contemporary sites; current tallgrass prairie remnants are often located in areas not representative of the former prairie – instead, they have persisted in those areas that were not appropriate for farming (Allison 2002).

There is also a great deal of uncertainty regarding the current state of tallgrass prairie remnants. Although charity and conservation organizations such as The Nature Conservancy of Canada, Ontario Parks, Halton Conservation, and Tallgrass Ontario are engaged in conservation and restoration activities, these records are not publicly available and many sites exist in small, isolated parcels on private lands which are either not catalogued or intentionally obscured. The last comprehensive attempt to survey tallgrass prairie sites in southern Ontario was in 1994 by Wasyl Bakowsky, working with Gore and Storrie Limited Environmental Planning Services Division, and John Riley, a Ministry of Natural Resources employee. This report categorized prairie and savannah remnants into three classes: extensive intact remnants, large remnants (1-2 ha), and small remnants (> 1 ha). Based on conversations with employees of London and Thames Valley consulting firms, several small remnant tallgrass prairies, particularly those along railway lines, were not included in this report. From my own experience sampling two of the sites listed as large remnants in this 1994 report (the Holland Landing Prairie ANSI and Brachton Prairie Remnant), it is clear that there has been considerable change (in these cases, reduction) in the size and floristic quality of southern

Ontario's tallgrass prairie remnants in the over twenty years since its publication. Compiling an up-to-date, comprehensive survey of restored and remnant tallgrass prairie sites in southern Ontario, including their extent, characteristics, and protection and management status would be a worthwhile endeavor which could inform future planning and management decision making at provincial and local scales. Updating this records would require revisiting each of the 1994 documented sites for reassessment and documenting previously unlisted sites; this would involve collaborating with First Nations communities, local conservation authorities, Tallgrass Ontario, individual landowners, the Ministry of Natural Resources and Forestry, Ontario Parks, and a diverse set of consulting firms to identify, access, and survey each site. Although this mass survey and assessment project is outside of the scope of this dissertation, it presents a valuable opportunity for future work.

In the context of restoration, evidence for factors controlling successful tallgrass prairie establishment are mixed. Some studies suggest that management (composition, diversity, and density of seed mix) and history (site age) are the primary drivers behind creating target prairie whereas site (soil conditions) and landscape factors (connectivity) are rarely important (Grman et al. 2013). Management by controlled burning is generally considered an important factors in tallgrass prairie maintenance, although mowing and grazing regimes and combined approaches with the primary goals of increasing light availability and controlling undesired plant species have also met with success (Collins 2000, MacDougall and Turkington 2007). There is also research to support a more stochastic model, where long-term community assembly is influenced by climatic variations, seed availability, species identity, and disturbance during the initial stages of community establishment (MacDougall et al. 2008).

Prairie ecosystems do appear to be able to resist eutrophication, despite receiving increased atmospheric nitrogen deposition from anthropogenic sources; this is particularly true when sites are

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managed through burning and grazing regimes (Borer et al. 2014, McLauchlan et al. 2014). However, the long-term stability of tallgrass prairie in the context of nitrogen fertilization is uncertain, as fertilization has been shown to weaken the positive effect of species diversity on stability due to an increase in the temporal variation of productivity (Hautier et al. 2014). Because nitrogen is a limiting nutrient in prairie (Seastedt et al. 1991) and the absence of large ungulate grazing combined with frequent burning promotes nitrogen limitation (Anderson et al. 2006), there is some concern that as microbial demand for nitrogen increases under elevated carbon dioxide conditions, nitrogen limitation for plant growth may increase (Rice et al. 1994, Williams et al. 2001); the extent to which increases in atmospheric nitrogen deposition may offset these pressures is currently unknown. It has also been suggested that when assessing and predicting the consequences of anthropogenic nutrient enrichment in grassland systems, we should consider multiple-nutrient constraints which include less studied nutrients such as K and micronutrients (Fay et al. 2015).

In the last 250 years, steel plows, crops, cattle, fertilizers, pesticides, exotic plant introductions, fire suppression, tile drainage, and urbanization have reduced tallgrass prairies to a fragmented series of habitat parcels that represent less than 1% of the original area occupied (Szeicz and MacDonald 1991, Bakowsky and Riley 1994, Packard and Ross 1997). That so little remnant prairie exists can make it difficult to assign suitable reference states for restoration, and creating new habitat that captures the diversity of remaining high quality remnant prairie is an important priority for restoration. The remnants of this now rare habitat are currently challenged not only by ongoing human disturbance but by the anticipated ecological regime shifts resulting from anthropogenic climate change (Morgan et al. 1995, Hobbs et al. 2009).

Remaining tallgrass prairie fragments provide habitat and overwintering sites for many rare and endangered species (Morgan et al. 1995) including twenty-three plant species associated with

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tallgrass vegetation communities that are regulated as Endangered, Threatened, or Special Concern within schedules of the Canadian Species at Risk Act and Ontario Endangered Species Act (Government of Canada 2002, Government of Ontario 2007). Tallgrass prairie also provides important habitat for declining pollinators (McLachlan and Knispel 2005, Whiles and Charlton 2006, Hopwood 2008), and a variety of ecosystem services including erosion control, runoff and rainfall infiltration, below-ground carbon sequestration, and nutrient cycling (Samson and Knopf 1994). For these reasons and more, since the first formal restoration project in 1934 there have been various public, private, and industrial agencies active in tallgrass prairie restoration (Anderson 2006).

Two of the main direct economic values associated with tallgrass prairie habitat are carbon sequestration credits and biofuels. Switchgrass (*Panicum virgatum* L.), a warm-season grass native to the North American tallgrass prairie, has been identified as the most promising source of biomass for cellulosic ethanol and has the potential to become as a major biofuel crop (Bies 2006, Fletcher et al. 2011). Potential ecological benefits of converting marginal agricultural lands to switchgrass production include protection from soil erosion, carbon sequestration, creation of wildlife habitat, and increases in landscape biological diversity (McLaughlin and Walsh 1998, McLaughlin et al. 2002). However, the consequences to biodiversity and ecosystem function are still largely unknown and will likely depend on how the grassland is managed (Bies 2006). Research has suggested that soil microbial activity of switchgrass monocrops is lower than monocrops of other perennial grasses (Haney et al. 2010), and clipping for biofuel harvest may result in significant soil erosion and accompanying losses of soil carbon and nitrogen, which may be further aggravated by climate warming (Xue et al. 2011). There is also concern that summer harvests of switchgrass could result in the destruction and abandonment of nests by birds, analogous to tilling effects in corn fields (Best 1986, Fike et al. 2006, Fletcher et al. 2011). As the creation of nesting habitat for grassland bird

species such as Bobolink (*Dolichonyx oryzivorus* L.) and Eastern Meadowlark (*Sturnella magna* L.) is an explicit goal of some tallgrass prairie restoration projects (Fletcher and Koford 2002, McCracken et al. 2018), this represents a potential conflict. Based on the current unknowns surrounding switchgrass biofuel production, further assessments would be required to determine the net ecosystem consequences of switchgrass biofuel production.

Resources for tallgrass restoration include several manuals about the techniques of tallgrass prairie restoration (Ahrenhoerster and Wilson 1981, Schramm 1992, Morgan et al. 1995, Packard and Mutel 1997, Environment Canada 2002, Quinlan 2005), each of which offers different approaches, best practices, and recommendations. Those who undertake restoration projects are themselves varied and include non-profit organizations, university researchers, private companies, government agencies, and private landowners (Rowe 2010). Perhaps owing to this variety, restoration methods and reported successes are highly variable (Morgan et al. 1995, Packard and Mutel 1997, Rodger 1998, Wilson and Hartnett 1998, Rowe 2010), and documentation is often located within the grey literature or entirely absent. Based on a review of the literature, conversations with practitioners, and recommendations offered by restoration manuals, restoration of tallgrass prairie has typically focused on seeding native plant species into retired agricultural fields (Kindscher and Tieszen 1998, Rowe 2010), and measures of restoration success have traditionally focused on plant community metrics including coefficients of conservatism (a measure of the degree to which a species exhibits faithfulness to remnant natural plant communities) and the floristic quality index (FQI, a measure of habitat quality based on the number and value of desired plant species present) (Swink and Wilhelm 1994, Freyman et al. 2015, Murphy 2018). If assessed by floristic quality, the outcomes of tallgrass prairie restoration have been disappointing (Kindscher and Tieszen 1998, Martin et al. 2005, Rowe 2010), and many knowledge gaps exist, especially pertaining to soil biota. There are also substantial communication gaps, where

the activities of practitioners are seldom informed by the results of restoration research, and practitioners rarely document their actions or collate their data in forms accessible to researchers (Anonymous 2007).

The question of what constitutes "success" in ecological restoration is the subject on ongoing discussion in the literature (Ruiz-Jaen and Aide 2018, Wortley et al. 2018, Higgs et al. 2018). Some have suggested that the purpose of restoration is to return an ecosystem from a disturbed or totally altered condition to a previously existing "natural" one (Jordan et al. 1988, Berger 1990, Hamilton 1990, Lewis 1990, Palmer et al. 2006). More recently, there has been a shift in the conceptualization of restoration success to recognize that global, irreversible changes have occurred and that restoring to historic conditions is not realistic (e.g. Hobbs et al. 2013, Mascaro et al. 2013). This has led to a more functional approach to restoration, emphasizing restoration of key ecosystem functions, taxa, or general species communities (Wyant et al. 1995, Miller and Hobbs 2007, Comin 2010, Cardinale et al. 2012). As the exact conditions that characterized pre-colonial tallgrass prairie in southern Ontario are unknown, the definition of restoration success used in this thesis reflects a functional approach. Using existing reference sites which are within the same locality and considered to typify the ecosystem functions and species communities of the southern Ontario tallgrass prairie ecosystem, restoration success in this thesis is measured as the similarity of restored sites to these reference sites, including presence and abundance of endangered species, species community, and soil characteristics.

With the goals of bridging the research-practice divide and addressing these knowledge gaps in mind, thesis chapters 2 through 4 each address a key question or questions pertaining to soil biota that were identified as research priorities by land and project managers engaged in tallgrass prairie restoration in southern Ontario (Halton Region Conservation, Ontario Parks, the Ministry of Natural Resources and Forestry). While chapter 2 shows for the first time that exotic earthworm invasion is

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widespread in remnant and restored tallgrass prairie ecosystems across southern Ontario, it leaves

open the question of what effects these organisms have on the restoration of tallgrass ecosystems.

This is particularly important to tallgrass prairie restorations as most are initiated by seeding, and

earthworm effects in tallgrass restoration had been previously unqualified. Specifically, chapter 2

addresses the research questions: what portion of the diverse restored and remnant tallgrass prairie

sites sampled hosted earthworms?; in what densities are earthworms present at these sites, and how

does this compare to highly invaded forest systems in northeastern Canada and the United States?;

what is the age (juvenile/adult) and size class structure of the earthworms present at each site?; how

many and what size of earthworm middens were present at each site?; and, which earthworm species

were confirmed present at each site? I address the ecological effects of exotic earthworms in my

subsequent chapter (chapter 3) which examines exotic earthworm granivory by the most widespread

and largest invasive earthworm species in southern Ontario tallgrass prairie, *Lumbricus terrestris*, on

species of a typical tallgrass prairie seed mix used by practitioners as well as on problem weed seeds.

This chapters answers the questions: do *L. terrestris* prefer seeds of native species used in ecological

restoration or seeds from exotic and 'weedy' species usually found in the seedbank of former

cropland?; what is the relationship between seed morphology and ingestion of seeds of different

species?; how does earthworm weight affect seed ingestion rates?; is there a protective effect afforded

by seed trichomes?; and, what are the implications of selective ingestion of seeds for ecological

restoration planning and management? Chapter 4 uses an agency-led (Ministry of Transportation and

Ministry of Natural Resources and Forestry) restoration based around an infrastructure project to

assess the success of different methods of tallgrass prairie restoration based on above-ground

vegetation and soil microbial communities. The specific research questions addressed in this chapter

are: do different methods of tallgrass prairie restoration show differential outcomes and 'success' with

respect to soil bacterial communities?; is the sod mat transplant method an effective technique for tallgrass prairie restoration?; and, do aboveground (vegetation) and below-ground (soil bacteria) metrics of tallgrass prairie indicate consistent interpretations of community restoration success? This research represents the leading edge of a still-few research studies linking above-ground vegetation communities to soil microbial community metrics of restoration success in tallgrass prairie, and one of only a handful of documented examples of large sod mat transplants being used for grassland restoration, a technique with the potential to preserve the seed bank, the soil microbiome, and other edaphic characteristics of remnant prairie sites. Recommendations for practice and future research are embedded in their respective chapters and expanded upon in the final chapter.

Chapter 2

Assessing invasive earthworm populations in restored and remnant tallgrass prairies of southern Ontario

2.1 Summary

In eastern North America, exotic earthworms have become ecologically dominant organisms that can alter plant community trajectory and composition. This will affect management efforts to conserve and restore these ecosystems. The North American tallgrass prairie ecosystem represents one such situation as it is an ecosystem of high conservation and restoration priority (less than 1% of peak area remains). Despite research showing that invasive earthworms are spreading across North America, their presence, populations, and community structure have remained largely undocumented across a diversity of environments. Upon testing a range of the sites available in southern Ontario (17 restored and 5 remnant prairies), I found widespread earthworm invasion. Invasive earthworms were recorded at all sites, despite the diversity of prairie size, age, soil texture, soil pH, adjacent land use, and management history represented. The average earthworm density was 79.2 m-2; comparable to highly invaded forest systems in the northeastern United States and southern Canada. I found a high proportion (average of 94%) of juvenile earthworms and a diversity of species, with the ecologically influential *Lumbricus terrestris* species present at all sites; this suggests stable, resilient populations that may challenge conservation and restoration efforts. New conservation and restoration strategies are needed to explicitly address the effects of invasive earthworm species.

2.2 Introduction

2.2.1 Ecosystem context: tallgrass prairie

Agriculture, urban development, and woody encroachment have reduced the tallgrass prairie ecosystem to less than 1% of its historical extent by area (Bakowsky and Riley 1994, Samson and Knopf 1994). An ecosystem unique to eastern North America, tallgrass prairie developed as a distinct, extensive assemblage during approximately 8000-5000 BCE following the Wisconsinan glaciation (Anderson 2006). In southern Ontario, it is estimated that tallgrass prairie covered at minimum 800 km^2, and possibly greater than 2,000 km^2 of the landscape (Rodger 1998). Excluding three large remnants (Walpole Island, Ojibway Prairie Complex, and the southern edge of Lake Huron), remaining tallgrass prairie exists as small, isolated parcels of less than 2 ha (Bakowsky and Riley 1994, Rodger 1998). Active restoration of tallgrass prairie is ongoing, aiming to re-establish native vegetation communities through seeding, often on former croplands (Kindscher and Tieszen 1998). In Ontario, these restoration sites vary in size and inter-site connectivity, but most are isolated and under 3 ha in size. The success of tallgrass prairie restoration efforts has been mixed, and the restoration of the original highly diverse vegetation community has proved challenging (Kindscher and Tieszen 1998). The native plant species richness of tallgrass prairie that has been restored is usually lower than remnants and often declines over time, whereas exotic plant species richness is higher and increases with time (Leach and Givnish 1996, Sluis 2002, Camill et al. 2004, Martin et al. 2005, McLachlan and Knispel 2005).

Historically, tallgrass prairie communities existed on a variety of soils including clay and clay-loam soils, but since these soil types were targeted for agricultural development, most remaining prairie remnants in southern Ontario are found on the less desirable sandy soils (Bakowsky and Riley

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1994, Faber-Langendoen and Maycock 1994). Soil fertility in this ecosystem is typically low, and reduction of soil fertility is often an explicit goal of tallgrass prairie restoration (Kindscher and Tieszen 1998, McLachlan and Knispel 2005). Compared with tallgrass communities in the mid-western USA, Ontario's communities often have a high forb: grass ratio and several key grass species form the basis of the plant community (Rodger 1998).

2.2.2 Earthworms in southern Ontario and the tallgrass prairie ecosystem

In Canada and the northern United States, native earthworms (Oligochaeta: Lumbricidae) did not survive the Wisconsinan glaciation that receded approximately 11,000 years ago (Gates 1982, Reynolds 1994, Edwards and Bohlen 1996). Of the 21 species recorded in Ontario today, 19 are introductions from Europe and Asia, and the two native species are provincially rare; *Bismastos parvus* Eisen 1874 is known exclusively from arboreta and *Sparganophilus esieni* Smith 1895 from aquatic or semi-aquatic mud (Reynolds 2014).

As Ontario's exotic earthworm species naturally expand their range by only 5–10 m per year (Addison 2009), their distribution into new areas is mainly mediated by anthropogenic soil transfer or bait dumping (Callaham et al. 2006, Hale 2007). In addition to the history of dispersal by humans (Edwards and Bohlen 1996), the spatial distribution of earthworms in soil is affected by soil pH, texture, moisture, and availability of food (including leaf litter, vegetation, and consolidated organic matter) (Guild 1952, Murchie 1958).

As a result of their high consumption rates, burrowing activity, and large body size, earthworms are influential soil macro organisms that alter fundamental ecosystem processes of soil structure, nutrient cycling, water infiltration, rates of decomposition, and seedbank conditions and drive the resource availability for other soil biota (Brown 1995, Edwards and Bohlen 1996, Forey et

al. 2011). As such, earthworm presence and distribution is an important consideration for ecosystem management. In the context of prairie restoration in particular, the impact of exotic earthworms on seed dispersal and consumption may be exacerbated as tallgrass prairie restoration is typically initiated by a single seeding event. Earthworms are increasingly recognized as important and under-studied post-dispersal seed predators (Eisenhauer et al. 2010, Forey et al. 2011, Drouin et al. 2014) with selection pressures affecting the dispersal, survival, and establishment of seed (Forey et al. 2011, Clause et al. 2016b). These pressures include behaviour- driven choices of selective ingestion as well as uneven digestion (Shumway and Koide 1994, Eisenhauer et al. 2009c, Clause et al. 2016b), accelerated or inhibited germination (Decaëns et al. 2003, Clause et al. 2011a), and seed transport (Mcrill and Sagar 1973, Thompson et al. 1994) of various seed species. Earthworms have direct effects on the composition and function of plant communities, but these vary by ecosystem, and species-specific interactions are common (Shumway and Koide 1994, Eisenhauer et al. 2009c, Craven et al. 2016, Clause et al. 2016b). As these pressures are additive to the other post-dispersal challenges to seed establishment (e.g. granivory by birds, rodents and insects, competition with ruderal weeds, water availability) (Moles and Westoby 2006, Eisenhauer and Scheu 2008, Forey et al. 2011) it is critical to understand the distribution and density of earthworms in order to effectively manage and restore invaded ecosystems.

Previous work in Ontario has focused on adding individual observation records to create a province-level distribution map of earthworm species (Reynolds 1977, 2011b, 2011a, Reynolds and Reynolds 1992), or earthworm-driven change in forest ecosystems (Jennings and Watmough 2016, Cassin and Kotanen 2016, Craven et al. 2016, Choi et al. 2017). While the negative effect of industrial tillage practices on earthworm populations in agricultural fields are well established (Jill Clapperton et al. 1997, VandenBygaart et al. 1999, Simonsen et al. 2010, Briones and Schmidt 2017),

there is currently no estimate available of the average biomass of earthworms in Ontario soils and no comprehensive survey of earthworm species, densities, or biomass has been completed. Earthworm population structure and density outside of a few study forests are largely unknown, and their presence within the tallgrass prairie ecosystem in Ontario has not been previously investigated. New research using nested PCR (polymerase chain reaction) to improve detection of earthworm eDNA in Canada is promising, but has yet to be widely implemented (Jackson et al. 2017a); if viable, this method would likely be the most effective in generating a comprehensive survey of earthworm distribution in the future. Currently, although the establishment and spread of non-native earthworm species in North America has been ongoing for centuries, the distribution of individual earthworm species remains patchy (Reynolds 2014). I do recognize that research conducted in the Midwestern US (e.g. Callaham et al. 2001, 2003, Loss et al. 2017) is relevant to the Ontario situation as that area and parts of southern Ontario and Manitoba form the current northern range limit of tallgrass prairie. However, the Ontario context is distinct because Canada had no widespread native earthworm communities following glaciation (Reynolds 2014), the northern tallgrass prairie plant community of Ontario forms a distinct subtype (Rodger 1998), and Ontario tallgrass prairie conservation remnants and restorations occur on a much smaller scale (most under 1 hectare) (Bakowsky and Riley 1994) and within a different land-use context as compared to the Midwestern US.

This research investigates the earthworm population of restored and remnant tallgrass prairie sites in southern Ontario. This is the first analysis of earthworms in Ontario tallgrass prairie of which I am aware.

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2.3 Methods

2.3.1 Site information

Twenty-two tallgrass prairie sites were selected for sampling, including five remnant, two restored-remnants, and 15 restored sites (Figure 2.1; Table 2.1). Restored-remnants describe prairie that re-established unexpectedly from the seedbank following accidental fire or large-scale brush cutting. In an attempt to represent the diversity of tallgrass prairie sites across southern Ontario, study sites were selected which varied in geographic range, management history, restoration age, adjacent land use, parcel size, and soil characteristics. Prairies sampled for this research include privately owned lands, areas within the public parks system, municipally owned lands, and properties managed by conservation authorities and non-profit organizations. Study site vegetation communities included ruderal weeds, invasive plant species, and expected southern Ontario tallgrass prairie plants including both grasses (*Andropogon gerardi* Vitman; Sorghastrum nutans (L.) Nash; *Panicum virgatum* L.; *Schizachyrium scoparium* (Michx.) Nash; *Elymus canadensis* L.) and forbs (*Monarda fistulosa* L.; *Pycnanthemum virginianum* (L.) B.L. Rob. & Fernald; *Rudbeckia hirta* L.; *Ratibida pinnata* (Vent.) Barnhart; *Asclepias* spp.; *Penstemon* spp.; *Lespedeza capitata* Michx.; *Liatris spicata* (L.) Willd.; *Symphyotrichum* spp.; *Solidago* spp.; *Desmodium* spp.).
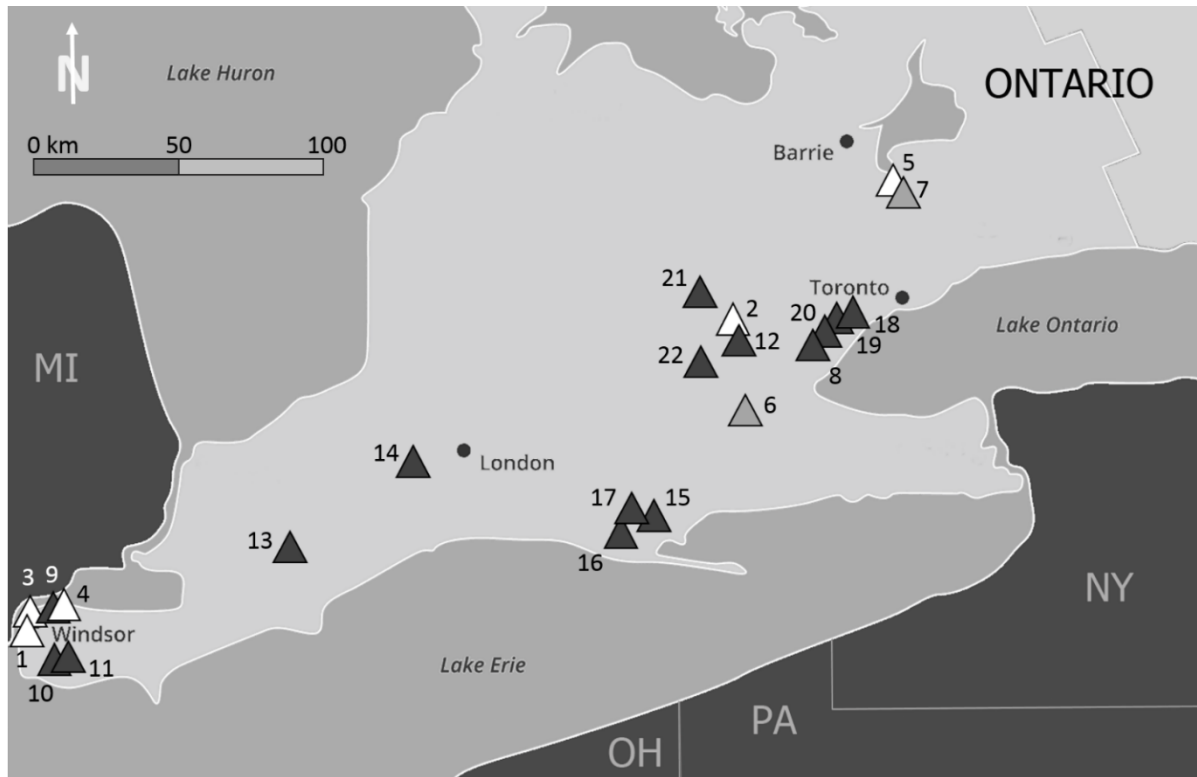
Figure 2.1 Location of study sites. Restored sites (dark triangles), remnant sites (white triangles), and restored-remnant sites (grey triangles). United States in dark grey, Canada in light grey, and waterbodies in medium grey. Adapted from Google Maps.

Table 2.1 Site characteristics and management history of restored and remnant tallgrass prairie sites sampled including the approximate site area, adjacent land use, year that restoration was initiated, method of restoration, year of most recent prescribed burn, and site management through herbicide, the removal of woody plants, or cattle grazing.

Site No.	Area (ha)	Status	Adjacent land use	Year begun	Method	Most recent burn	Herbicide?	Removal of woody plants?	Grazed?
1	17.5	Remnant	P, H			2010			
2	1.2	Remnant	P, H, A			2010		Y	
3	1.3	Remnant	I, P, H			2012	Y	Y	
4	1.9	Remnant	I, P, H			2014	Y	Y	
5	3.5	Remnant	P, H					Y	
6	3.3	Restored-Remnant	P, H	2006	Seeded	2015		Y	
7	0.6	Restored-Remnant	P, H	2015			Y		
8	3.3	Restored	P, A, H	2015	Seeded		Y	Y	
9	1.6	Restored	I, H, A, R	2013	Seeded				
10	0.3	Restored	I, H	2013	Planted		Y		
11	2.1	Restored	I, H	2013	Seeded + Planted		Y		
12	1.2	Restored	P, R, H, A	2006	Seeded + Planted	2010	Y	Y	
13	21.5	Restored	A, I	2010	Seeded		Y	Y	
14	2	Restored	A, P	2011	Seeded		Y	Y	Y
15	36	Restored	P, A	2013	Seeded		Y	Y	
16	14.5	Restored	P, A	2012	Seeded		Y	Y	
17	14	Restored	P, A	2011	Seeded		Y	Y	
18	6.1	Restored	P, I, H	2012	Seeded		Y		
19	6	Restored	P, I, H	2013	Seeded		Y		
20	6.3	Restored	P, I, H	2014	Seeded		Y		
21	16	Restored	P, H	2010	Seeded	2015			
22	23.5	Restored	P, A	2011	Seeded	2015		Y	
Code: Suburban housing (H), Protected Area (P), Resource Extraction (E), Agriculture (A), Major infrastructure (I)									

2.3.2 Research methods

Fieldwork was conducted in October 2015 and 2016. At each study site, five (2015 data) or ten (2016 data) plots were pre-assigned using satellite imagery so as to be evenly distributed across the entire prairie area and not within 10 metres of any edge. Due to a severe flooding event that led to standing water on the sampling area of sites 1, 3, 4, 9, 10, and 11 for several weeks preceding the 2016 sampling period, 2015 data is presented for sites 1, 3, 4, 9, 10, and 11; these sites are therefore represented by five sampling plots instead of ten for sites sampled in 2016. Field sampling was conducted during the day when soil temperatures were above 10˚C and no rain had fallen in the previous 24 hours. Earthworms were collected using mustard liquid extraction (Lawrence and Bowers 2002) from sample plots measuring 20 cm x 20 cm. At sites with litter cover, the surface litter was first removed and searched for earthworms, then 2 L of mustard solution (10 g/L of Weston Inc. Bulk Barn® hot mustard powder) was applied to the plot over a period of 10 minutes, and any emerging earthworms were collected for 15 minutes following application. Due to the inability of juvenile (sub-adult) earthworms to be reliably identified to genus or species level based on physical (as opposed to genomic) traits, earthworm body size and number were used to characterize the earthworm populations. Comparisons of these data are therefore presented in place of traditional community analyses (e.g. comparisons of species diversity) which would require species identifications. Earthworms were measured after becoming active in a collection container to obtain a length estimate (average of stretched and rest, ignoring the much shorter defensive/inactive length). Adult earthworms were identified by the presence of the clitellum. Because Enchytraeidae (microdriles: Oligochaeta, Annelida) strongly resemble young earthworms (megadriles) and grow to between 10-20 mm (Coleman and Wall 2015), any sampled annelids which were unpigmented and <2 cm were not counted in the data. At each site, a voucher specimen of any adult earthworm that could not be identified in the field was collected and immediately placed in a 75% isopropyl alcohol solution to obtain minimum species counts (i.e. the number of identifiable species) for each site. After being identified using physical attributes (Hale 2007), these adult specimens (n = 29) were donated to The Barcode of Life

project at the University of Guelph and are curated at that institution. The adult earthworms identified (representing a fraction of the total earthworms recorded) were used to create a minimum species list which represents the lowest number of species which have been verified to occur at the study site.

The largest earthworm species in Canada (Reynolds 1977), *Lumbricus terrestris* L., has a large impact on invaded systems both as a granivore and ecosystem engineer (Shumway and Koide 1994, Zaller and Saxler 2007, Clause et al. 2016b). An anecic earthworm, *L. terrestris* create a permanent or semi-permanent vertical burrow system which may extend several meters into the soil profile, and is thus likely to be under-sampled using typical extraction methods appropriate for other species – including the mustard extraction method used in this research (Hamilton and Sillman 1989, Edwards and Bohlen 1996). To reconcile this bias, I used midden counts as an additional metric to achieve representative sampling. Middens are the distinctive piles of cast, organic, and inorganic materials that an individual *L. terrestris* creates around the opening to its vertical burrow. They are surface structures unique to this species in southern Ontario and readily recognizable (Butt and Grigoropoulou 2010, Stroud et al. 2016). The diameter of each midden which was contained wholly or in part within the 20 x 20 cm sample plot was recorded in this study.

To quantify the soil characteristics at each site, soil samples were collected at each plot using a 3 cm diameter soil corer to a depth of 20 cm. Each sample was stored in a Whirl-Pak® sample bag and frozen until processing. Soil cores from each plot were homogenized and subsampled for analysis of pH, organic matter content, and texture following McKeague (1978).

I used the non-parametric Spearman's Rank Correlation to test for associations between earthworm density and soil properties (pH, texture, organic matter content) and earthworm midden measurements (area, density). To account for potential overestimation related to sub-surface lateral movement of mustard solution, I calculated the number of earthworms per square meter using an assumed plot area of 25 cm x 25 cm rather than the actual plot area (20 cm x 20 cm). All means are presented with standard deviations (SD).

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2.4 Results

The Anderson-Darling Normality Test showed that all variables were non-normal. Soil properties varied widely across the study sites (Figure 2.2) and no statistically significant associations were found between the abundance or density of earthworms or middens and soil characteristics. Soil organic matter content ranged from 1.65% to 4.3% ($\bar{x}$= 3% +/- 0.980). Soil pH values were between 5.27 to 7.67 with a mean value of 6.27 +/- 0.677. Soil textures encompassed a wide range, from sand to silty clay (Figure 2.2).
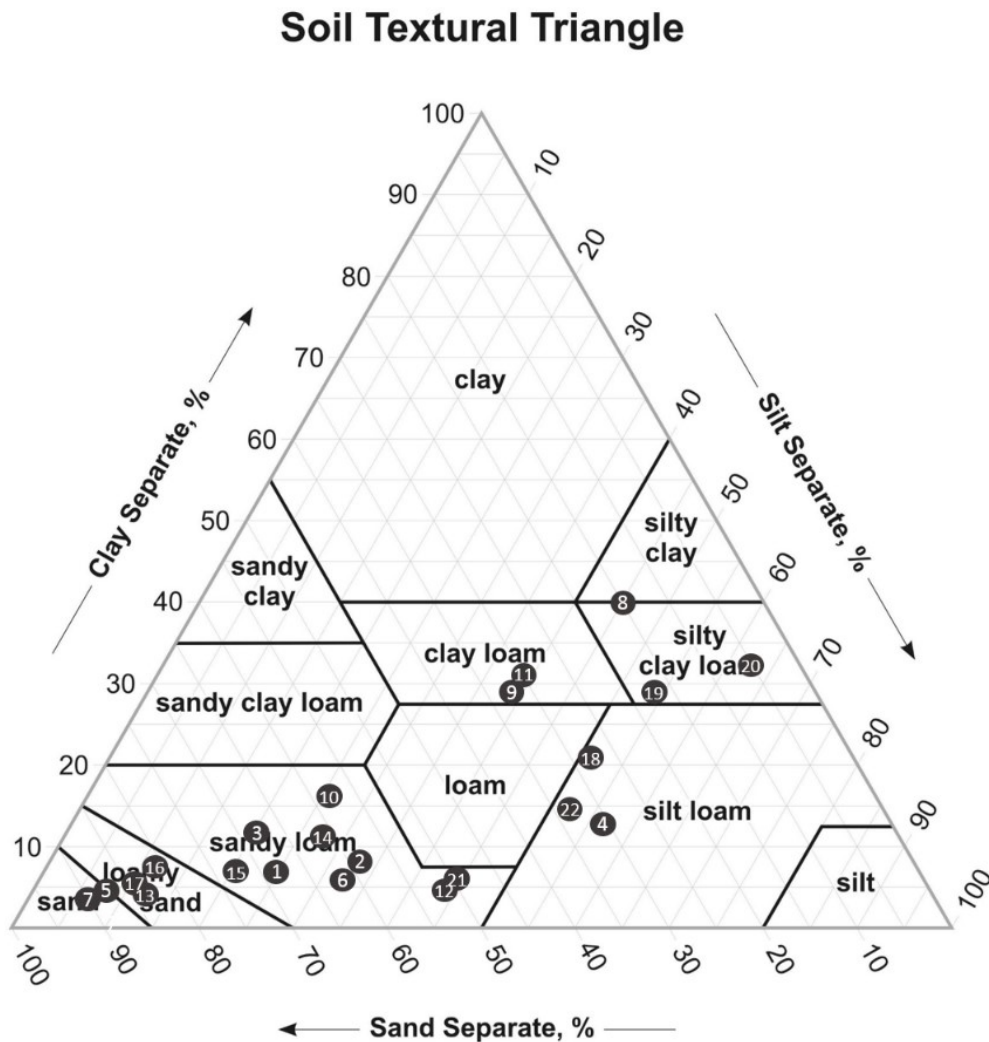
Figure 2.2 Soil texture triangle illustrating the soil texture of prairie sites sampled (sites 1-22 as labelled). Soil texture triangle adapted from USDA (2017).

Although the uneven sample sizes in this study limit the strength of the result interpretation and hamper precise comparisons, earthworms were found at every tallgrass prairie site in this study. The total number of earthworms per site varied substantially between 5 and 108, with a mean earthworm count of 36.6 +/- 29.3 (Table 2.2). No significant associations were found between the number or density of earthworms or middens and site condition (remnant, restored-remnant, restored). The mean earthworm density ranged between 8 and 345.6 earthworms m^{-2} ($\bar{x}$ for all sites = 79.2 +/- 81.2). The majority of earthworms recorded were juveniles ($\bar{x}$= 94%, +/- 6.5). The highest percentage of adult earthworm recorded was 17%; no adult earthworms were collected at 8 sites (Table 2.2).

Table 2.2 Total number of earthworms (EW) per site, mean number of earthworms m^{-2} +/- standard deviation (SD), and percent of adult earthworms.

Site No.	Total EW #	Mean EW m^{-2}	SD	% adult EW
1	35	56	54.0	2
2	28	44.8	44.5	5
3	31	99.2	30.8	15
4	35	112	75.9	4
5	9	14.4	15.9	0
6	5	8	17.3	0
7	18	28.8	14.7	4
8	9	14.4	21.9	0
9	37	118.4	95.1	16
10	70	224	125.5	0
11	108	345.6	285.2	17
12	44	70.4	43.5	11
13	5	8	11.3	0
14	7	11.2	16.9	0
15	79	126.4	82.8	13
16	49	78.4	58.2	3
17	7	11.2	26.2	0
18	45	72	42.8	9
19	68	108.8	69.0	16
20	81	129.6	52.0	15
21	32	51.2	23.6	8
22	6	9.6	17.2	0

The distribution of the total earthworms recorded by size class varied considerably between sites (Figure 2.3). Earthworms between 5 and 10 cm were the most abundant overall ($\bar{x}$= 39.3% +/- 15.7, absent from 4 sites), followed by 1-5 cm ($\bar{x}$= 32.5% +/- 25, absent from 1 site), 10-15 cm ($\bar{x}$= 23.4%, +/- 24.6, absent from 3 sites), and those greater than 15 cm ($\bar{x}$= 4.9% +/- 6.3, absent from 10 sites).
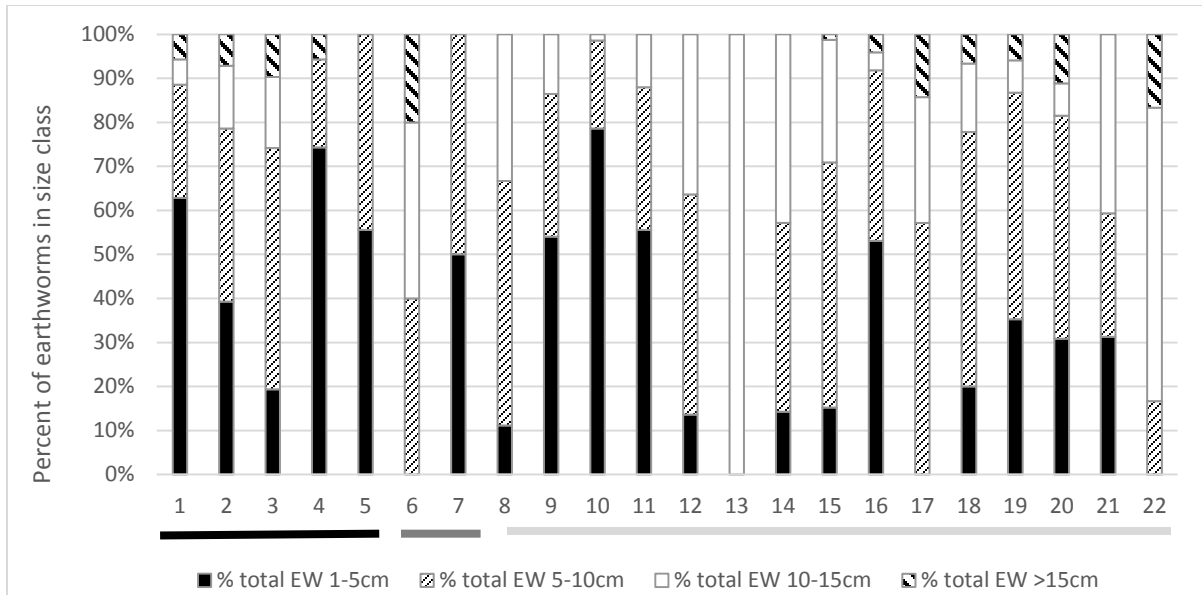
Figure 2.3 Distribution of earthworms across size classes per site. Percent of total earthworms found in each site between 1-5 cm (black), 5- 10cm (thin dash), 10- 15cm (white), and over 15 cm in length (thick dash). Bars below the x-axis indicate the status of the site: black for remnants, dark grey for remnant-restored, and light grey for restored tallgrass prairie.

The number, size, and mean area of middens varied by site (Figures 2.4, 2.5). On average, larger mean midden areas and numbers were recorded in restored tallgrass prairie compared to remnant prairie sites. There is a positive correlation between the number of earthworms in each plot and the area ($r = 0.257$, $p = 0.0003$, $DF = 193$) and number ($r = 0.264$ $p = 0.0002$, $DF = 193$) of middens partially or wholly contained in each plot.
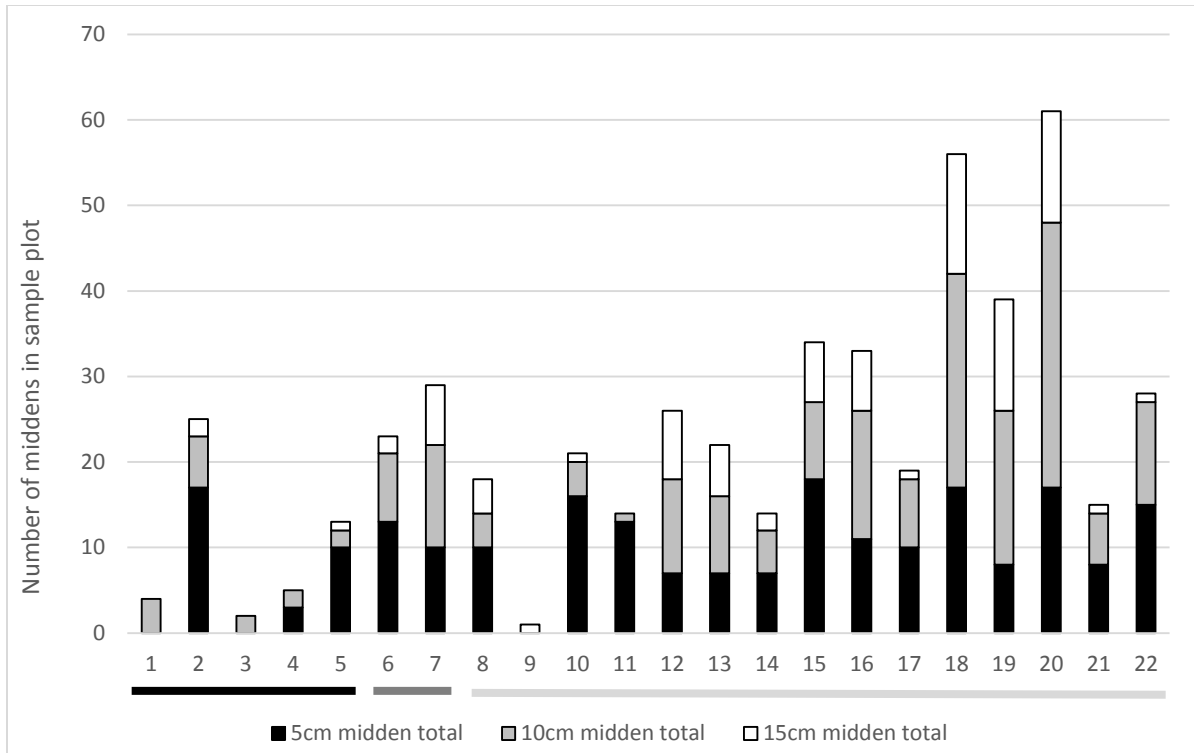
Figure 2.4 Number, by mean area category, of earthworm middens partially or wholly contained in sample plots, per site. 0-5cm mean total area (black), 5-10cm mean total area (grey), and 10-15+cm mean total area (white). Bars below the x-axis indicate the status of the site: black for remnants, dark grey for remnant-restored, and light grey for restored tallgrass prairie.
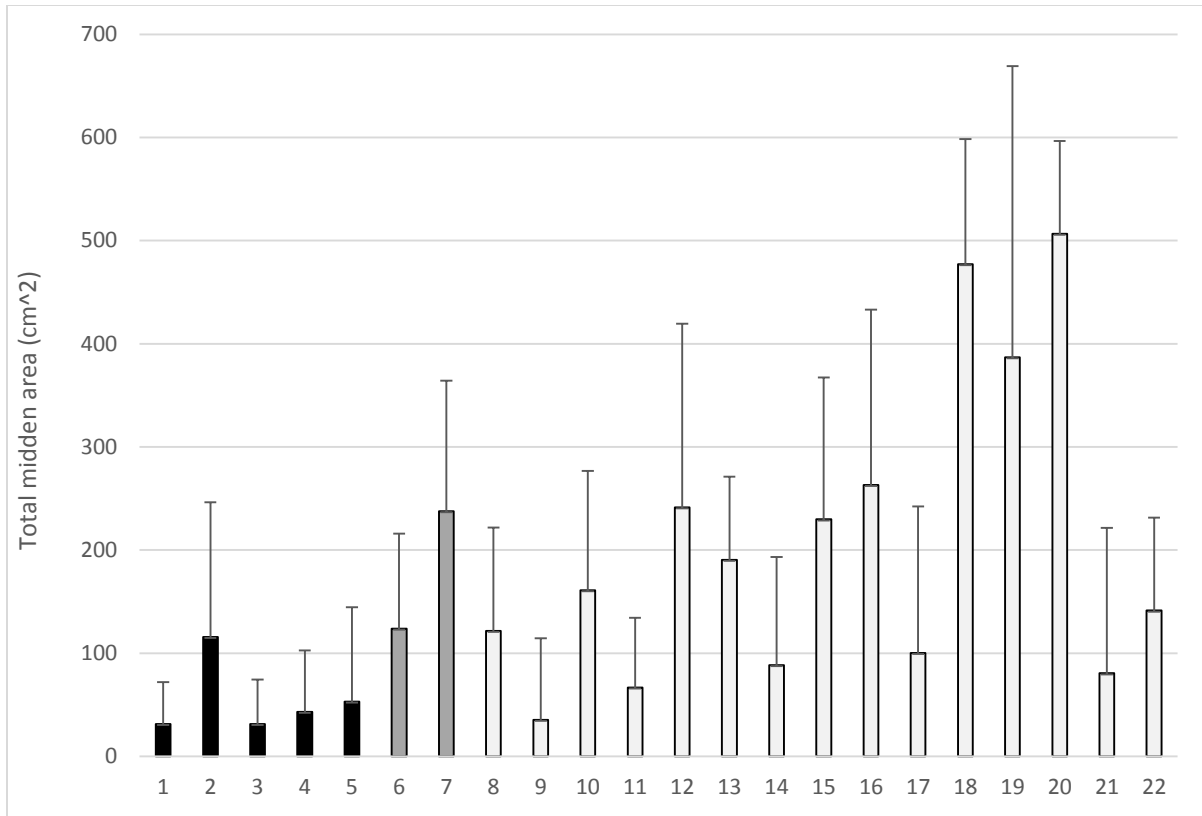
Figure 2.5 Mean total midden area (square centimeters) and standard deviation for each site. Bars fill shading indicates the status of the site: black for remnants, dark grey for remnant-restored, and light grey for restored tallgrass prairie.

The minimum number of earthworm species present (based on the adult specimens only, those that were identifiable to the species level) at each site varied between 1 and 5 ($\bar{x}$= 2.5, +/-1.3), and *L. terrestris* was the only species observed at every site (Table 2.3). Compared with previous earthworm surveys (Reynolds 2014), this study is the first record of *Dendrobaena octaedra* Savigny and *Lumbricus rubellus* Hoffmeister in Waterloo and Halton counties, respectively, and contributes the second or third record in nine other instances (Table 2.4). No native earthworms were identified in this study.

Table 2.3 Identities of confirmed earthworms and minimum number of species of earthworm at each site. Minimum number of species based on taxonomic verification of adult individuals and is less than total earthworms recorded per site. A. = Allolobophora; Ap. = Aporrectodea; D. = Dendrobaena, L. = Lumbricus; O. = Octolasion

Site No.	Min. no. of species	A. chlorotica	A. sp.	Ap. longa	Ap. rosea	Ap. tuberculata	D. octaedra	L. rubellus	L. terrestris	O. tyrtaeum
1	2							Y	Y	
2	4					Y	Y		Y	
3	1								Y	
4	1								Y	
5	1								Y	
6	3		Y						Y	
7	1								Y	
8	2								Y	
9	2			Y					Y	
10	1								Y	
11	2		Y						Y	
12	5				Y	Y		Y	Y	Y
13	3					Y			Y	
14	1								Y	
15	3		Y					Y	Y	
16	2					Y			Y	
17	1								Y	
18	4	Y				Y		Y	Y	
19	4	Y				Y		Y	Y	
20	4	Y				Y		Y	Y	
21	4	Y						Y	Y	Y
22	3		Y					Y	Y	

Table 2.4 Number of previously published species observations by County and earthworm species. A. = Allolobophora; Ap. = Aporrectodea; D. = Dendrobaena, L. = Lumbricus; O. = Octolasion

County	A. chlorotica	A. longa	A. rosea	A. tuberculata	D. octaedra	L. rubellus	L. terrestris	O. tyrtaeum
Brant						2*	4*	
Chatham-Kent				2*			3*	
Essex		2*				19*	18*	
Halton	1*			6*		0	2*	
Middlesex							2*	
Norfolk				5*		1*	2*	
Waterloo	1*		5*	8*	0	3*	6*	4*
York							6*	
* Reynolds 2014								

2.5 Discussion

Despite a wide range of site soil texture, soil pH, soil organic matter content, management history, adjacent land use, and age, invasive earthworms were found in all tallgrass prairie sites studied. Due to the vertical stratification of earthworm communities, the complexity in life cycle phenology of each species of earthworm, and the selected sampling method, the numbers presented here are likely underestimates in general and likely underrepresent the number of endogeic and anecic species in particular due to their burrowing habit (Edwards and Bohlen 1996). With a mean of 79.2 earthworms m^{-2}, we can expect that in comparison to pre-invasion conditions, earthworms now make up a substantial proportion of animal macrofauna in tallgrass prairie soils (Forey et al. 2011).

Comparisons between studies of earthworm populations are complicated by variations in timing, method of collection, the uneven distribution of earthworm populations, and the fact that studies may report the densities of only certain study species. With respect to earthworm sampling

methodologies, hand sorting is usually considered a superior method for quantifying earthworms present at a site; in comparison, the mustard extraction method will tend to underestimate earthworm numbers (Pelosi et al. 2009). The main argument against the hand extraction method is that it necessitates digging up, breaking apart, and sieving an entire column of soil for each sampling plot, each measuring 25 cm x 25 cm x 50-75 cm (Nordström and Rundgren 1972). This manual processing has consequences for the sampling plot, including homogenization of the soil profile and disturbance to root networks, fungal hyphae, and soil dwelling organisms. Once infilled, the hand sorting method also often leaves behind a patch of bare ground, which some land managers worry will provide a colonization opportunity for weedy or invasive species (Ontario Parks employee, pers. comm., 2015). As the majority of the sites used in this research were protected areas, the hand sorting method was rejected on the basis that it would disturb the soil and habitat in ways that not be acceptable to research partners. The mustard extraction method employed in this research is a low-disturbance method particularly suitable for use in sensitive, conservation-focused habitats; the consequence of this decision is that the earthworm quantities reported likely represent underestimates of the true population size. When coarsely compared to other ecosystem types, mean earthworm densities I recorded in tallgrass prairie appear to be within the range documented in other ecosystems (Table 2.6).

Table 2.6 Comparison of mean earthworm densities (earthworms m-2) of different ecosystem types.

System	Location	Mean EW m-2	Source
Maple-dominated second-growth forest without history of agricultural activity	New York State	38-46.7	Bohlen et al. 2004
Maple-dominated second-growth forest with history of agricultural activity	New York State	57.6-111.3	Bohlen et al. 2004
Tallgrass prairie	southern Ontario	79.2	This study
Agroforestry	southern Ontario	95-182	Price and Gordon 1998
Hardwood forest	Central New York State	128	Shakir and Dindal 1997

The scope and detail of the analyses presented in this paper is constrained by the inability to identify juvenile (sub-adult) earthworms to the genus or species level. Being that the majority (~94%) of earthworms recorded in this study were juveniles and no adult earthworms were recorded at eight study sites, the number of adult earthworms (those specimens that were identified to the species-level) was small and so patterns of earthworm community variables (e.g. related to soil types, site type) could not be established.

The positive correlation between the number and area or middens in a plot and the number of earthworms recorded has two likely causes. First, some of the anecic, midden-forming *Lumbricus terrestris* were successfully collected by the mustard extraction method; a positive correlation between midden density and the midden-forming *L. terrestris* was therefore expected. Second, middens themselves may be centers of activity for other earthworm species, meaning that the invasion of *L. terrestris* may facilitate the invasion and proliferation of other earthworm species on a micro-scale (Butt and Lowe 2007), and plots with higher numbers and/or area of middens could be expected to contain higher numbers of earthworms.

While the maximum lifespan of earthworms in the field is unknown, it has been hypothesized to be two years or less (Edwards and Bohlen 1996). Given this, the high percentage of juvenile earthworms of varying size recorded in this study indicates that these populations are reproducing successfully and likely represent a persistent population. Whereas some species can only breed sexually (most species in the genera *Aporrectodea, Allolobophora, Lumbricus*), others can reproduce parthenogenetically (*Octolasion, Aporrectodea rosea, Dendrobaena*), and all genera in Ontario are hermaphrodites (Edwards and Bohlen 1996). With this reproductive flexibility in mind, it is likely that even in the event of a periodic disturbance such as flooding or fire, the earthworm populations recorded in this study are resilient.

Some land managers in southern Ontario had surmised that soil texture (sand or silty clay) would act as a barrier to the spread of invasive earthworms into threatened grassland ecosystems (C. Brdar, pers. comm., 2017). I found this was not true. Exotic earthworms in southern Ontario do not appear to be limited by differing soil textures in their dispersal into tallgrass prairie conservation remnants and restored sites. The ecologically important *L. terrestris* were found to have invaded all soil texture types represented. This included even sand and heavily compacted silty clay. Plant community trajectory and composition is affected by earthworm species-specific interactions with seeds, including ingestion and digestion, accelerated or inhibited germination, and seed transport through the soil profile. I suggest that land managers in systems previously considered immune to earthworm invasion effects should be monitoring for earthworms and begin mitigation planning to inform their future landscape management plans.

For successful ecosystem management in the context of earthworm invasion, we require details first about the earthworm population in a specific ecosystem, and second, knowledge of the ecosystem-specific earthworm interactions. This research fulfils the first priority by documenting the

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invasive earthworm populations within tallgrass prairie, an ecosystem that has dwindled to 1% of its original range (Bakowsky and Riley 1994). Future investigation of prairie-specific earthworm interactions, particularly earthworm-seed interactions, will improve our capacity to manage and restore tallgrass prairie into the future. Although the data presented herein is ecosystem-specific, these conservation and management challenges are echoed for the ongoing, widespread earthworm invasion occurring at a global scale.

2.6 Conclusions

Non-native earthworm invasion is a major driver of ecological change that can alter plant community trajectory and composition, and one whose effects are often ecosystem-specific. Currently, both the documentation of earthworm population characteristics and our understanding of earthworm-plant feedbacks in specific ecosystems is limited. While species distribution maps based on preserved specimens (Reynolds 2014) and research in specific forested areas are informative, earthworm densities and population characteristics outside of a few forest sites were previously unknown, limiting our ability to promote effective long-term management of these invaded systems. I found populations of invasive earthworms in all tallgrass prairie sites studied, including remnant and restored prairie across a range of soil texture, pH, age, size, adjacent land use, and management history. Overall earthworm densities were comparable to other invaded ecosystems in the region, and population density varied between sites. Given the rarity and high conservation value of the tallgrass prairie ecosystem coupled with the lack of knowledge regarding prairie-earthworm interactions, the high densities of invasive earthworms recorded in this study support Forey et al.'s (2011) call for more investigation of above- and belowground multitrophic interactions. I suggest that future research focus on quantifying the ecosystem-specific interactions between invasive earthworms and invaded

ecosystems, with a goal of facilitating the development of effective conservation strategies in the context of earthworm invasion.

Chapter 3

Weed versus tallgrass prairie: Seed preferences of *Lumbricus terrestris* L

3.1 Summary

Depending on seed and earthworm species identities, earthworms can be important seed predators which consume or bury large amounts of surface-applied seeds. As seeding is the most common practice for initiating tallgrass prairie restoration, earthworm-seed interactions likely contribute to restoration outcomes. This chapter represents the first feeding experiment focusing on tallgrass prairie restoration seed mixes. Nine native tallgrass prairie species frequently used in tallgrass prairie restoration and four common weed species were selected for this experiment. The results demonstrate that the widespread invasive *Lumbricus terrestris* earthworm has strong and consistent preferences for invasive plant, undesirable weed, and target restoration seed species. In terms of seed destruction, *L. terrestris* likely digested over 50% of the target tallgrass prairie seeds ingested in this study. As earthworm invasion of tallgrass prairie habitats is uneven but probably accelerating, understanding the effects of exotic earthworm granivory can contribute to more effective restoration.

3.2 Introduction

Seed predation during the post-dispersal period can act as an ecological filter, influencing the future species composition of an ecosystem (Chambers and MacMahon 1994, Fenner and Thompson 2005, Larios et al. 2017). This is particularly true in the context of ecosystem restoration, where the initial seeding activities often serve as the sole influx of target seeds until the system is capable of self-seeding. Selective seed predation may shift an ecosystem away from restoration targets; this could be exacerbated if the seed predator is an exotic species. While small mammals, birds, and insects are

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perhaps better studied (Hulme 1998), earthworms are important post-dispersal seed predators (Eisenhauer et al. 2010, Forey et al. 2011, Drouin et al. 2014).

Earthworm-mediated selection pressures vary in intensity and include selective ingestion and digestion (Shumway and Koide 1994, Eisenhauer et al. 2009b, Clause et al. 2016b), accelerated or inhibited germination (Decaëns et al. 2003, Clause et al. 2011b), and seed transport, including burial (Mcrill and Sagar 1973, Thompson et al. 1994). Interactions are known to be seed- and earthworm-specific (Eisenhauer et al. 2009b, Clause et al. 2011b), and are likely driven by seed chemical and morphological properties (Clause et al. 2011b, 2016b). In general, earthworms seem to prefer small seeds (Shumway and Koide 1994, Eisenhauer et al. 2009b, Clause et al. 2011b), although some large-seeded plant species are favoured (Regnier et al. 2008). The cumulative effects of morphological seed traits such as shape, mass, and seed coat characteristics (e.g. presence of trichomes) on generalized earthworm palatability are not well understood (Grant 1983, Regnier et al. 2008, Clause et al. 2011b, 2016b).

The net effects of earthworms on seed survival remain uncertain for both ingested and buried seeds (Milcu et al. 2006, Eisenhauer et al. 2009a, Dávalos et al. 2014, Nuzzo et al. 2015, Clause et al. 2016a). Variation in the impact on seed survival may relate to species-specific interactions between individual earthworm species and plant seeds (Eisenhauer et al. 2009b), whereby specific earthworm species display strong preferences for certain seed species which are distinct from the preferences of other earthworm species. Post-dispersal seed selection pressures in an ecosystem likely vary with the composition of both the plant community (the relative numbers and sizes of seeds and the different species present) and the earthworm community (which earthworm species are present and dominant), in addition to edaphic factors. A major implication of this uncertainty is the impact of exotic earthworms on seed survival, especially where seeding rather than transplanting is the main vehicle

for ecological conservation and restoration. The North American tallgrass prairie represents an important case for investigation, as most tallgrass restoration is initiated by seeding.

During approximately 8000-5000 BCE, the eastern third of the North American Great Plains was comprised of an extensive and mainly contiguous tallgrass prairie ecosystem where groups of First Nations farmed, hunted, and managed the landscape with fire (Transeau 1935, Lajeunesse 1960, Wright 1968, King 1981, Stuckley 1981, Bakowsky and Riley 1994, Samson and Knopf 1994, Anderson 2006). In the last 250 years, steel plows, crops, cattle, fertilizers, pesticides, exotic plant introductions, fire suppression, tile drainage, and urbanization have reduced tallgrass prairies to a fragmented series of habitat parcels that represent less than 1% of the original area occupied (Szeicz and MacDonald 1991, Bakowsky and Riley 1994, Samson and Knopf 1994, Packard and Ross 1997, Paiero et al. 2010). As a result, plant and animal species that rely on the habitat have become scarcer, endangered, or extirpated in their historic ranges (COSEWIC 2000, 2010, IUCN 2008). The current range of tallgrass prairies is primarily within a rough triangle bounded by Oklahoma, southern Ontario, and southeastern Manitoba (Paiero et al. 2010). Current tallgrass prairie habitat, comprised of remnant historic and restored areas, is challenged by ongoing human disturbance as well as the ecological regime shifts caused by anthropogenic climate change (McLachlan and Knispel 2005, Whiles and Charlton 2006).

Restoration of tallgrass prairie usually focuses on seeding native plants on ex-arable lands that do not have an established target-species seed bank (Kindscher and Tieszen 1998, Rowe 2010). Generally, the success of these restoration efforts has been equivocal (Kindscher and Tieszen 1998). In re-assessments, the target (native) species richness of restored prairie is usually lower than remnants even decades following restoration, and desired species richness and dominance has been shown to decline over time in both restored and remnant prairies, whereas exotic species richness is

higher and increases with time (Leach and Givnish 1996, Sluis 2002, Camill et al. 2004, Martin et al. 2005, McLachlan and Knispel 2005).

Invasive exotic species of earthworms have been recorded in high densities at both remnant and restored tallgrass prairie habitat in southern Ontario (chapter 2). This is particularly true for the largest earthworm in Ontario by per unit mass, the anecic *Lumbricus terrestris* L. (Oligochaeta: Lumbricidae) (Reynolds 2014). Their role in post-dispersal seed predation in this ecosystem is largely unknown—although it is reasonable to expect that high densities of earthworms will exist in the former croplands used as tallgrass prairie restoration sites, their interactions with restoration seed mixes are unknown. Previous feeding experiments have demonstrated that *L. terrestris* show strong dietary preferences for certain seeds in both laboratory (Mcrill and Sagar 1973, Eisenhauer et al. 2010, Quackenbush et al. 2012), microcosm, and field exclusion experiments (Cassin and Kotanen 2016). If these patterns of seed preferences vary between the components of a target restoration seed mix and the undesirable weedy or invasive plant species at a site, earthworm granivory may be an important contributor to restoration success of failure in earthworm-invaded tallgrass prairie.

I designed this research project to be the first feeding experiment focusing on prairie restoration seed mixes; this adds to the earthworm-seed interaction literature by providing the first investigation of species specific to the tallgrass prairie ecosystem. While I focus on the initial seeding stage of restoration efforts, these earthworm effects may also be important to long-term management of the tallgrass prairie ecosystem through interactions with the autumn seed rain and related seed bank formation in established restorations.

I used laboratory cafeteria experiments to determine if the invasive exotic earthworm *L. terrestris* acts as an important and selective seed predator in tallgrass prairie, particularly during restoration establishment. Although cafeteria feeding experiments do not account for the effects of

caching or earthworm cast chemistry on seed survival, they offer the clearest assessment of ingestion

and egestion rates and allow for multiple seed species (e.g. small seeds, seeds with a low germination

rate) to be recovered with a high degree of accuracy; they are therefore the standard method used to

compare earthworm seed ingestion preferences (Mcrill and Sagar 1973, Hartenstein and Amico 1983,

Eisenhauer 2009b, Eisenhauer et al. 2010, Quackenbush et al. 2012, Clause et al. 2011b). Although

various explanatory factors for differences in seed ingestion and egestion have been suggested (e.g.

Grant 1983, Regnier et al. 2008, Clause et al. 2011b, 2016b), a consensus of generalizable patterns

has not been reached. Based on the contradictory findings of previous research on earthworm

granivory, I included parameters of seed morphology (Shumway and Koide 1994, Clause et al.

2011b, Cassin and Kotanen 2016), earthworm weight (Clause et al. 2011b, 2016b), and seed

trichomes (Regnier et al. 2008, Clause et al. 2011) in this study to allow for comparisons in ingestion

and egestion rates of *L. terrestris*. My specific research questions were:

1. Do *L. terrestris* prefer seeds of native species used in ecological restoration or seeds from
   exotic and 'weedy' species usually found in the seedbank of former cropland?

2. What is the relationship between seed morphology and ingestion of seeds of different
   species?

3. How does earthworm weight affect seed ingestion rates?

4. Is there a protective effect afforded by seed trichomes?

5. What are the implications of selective ingestion of seeds for ecological restoration planning
   and management?

3.3 Methods

3.3.1 Seed selection

Nine native tallgrass prairie species frequently used in tallgrass restoration (Morgan et al. 1995; Diboll 1997; N. Finney, pers. comm., 2016) were selected for this experiment. These include three grasses (*Andropogon gerardi* Vitman; *Panicum virgatum* L.; and *Schizachyrium scoparium* (Michx.) Nash), and six forbs (*Lespedeza capitata* Michx.; *Monarda fistulosa* L.; *Rudbeckia hirta* L.; *Pycnanthemum virginianum* (L.) B.L. Rob. & Fernald; *Liatris spicata* (L.) Willd.; and *Solidago juncea* Ait.). Of these native species, *L. spicata* is listed as a Species at Risk (Threatened) in Ontario (COSEWIC 2010). Four weed species which are common early-establishment competitors (*Taraxacum officinale* F.H. Wigg. [native]; and *Trifolium pratense* L. [introduced]) or problematic invasive species (*Melilotus albus* Medik.; and *Cirsium arvense* (L.) Scop.) in tallgrass prairie remnants and restorations were also included (Gucker 2009; Almquist and Lym 2010; Anderson 2013; N. Finney pers. comm., 2016).

Seeds from nine tallgrass prairie species were purchased from the major provincial supplier of tallgrass prairie seeds used for restoration projects in southern Ontario (St. Williams Nursery). Seeds of the four weed species were collected from a restored tallgrass prairie site in southern Ontario, or purchased from an Ontario commercial seed supplier (*T. pratense*; Ontario Seed Co.). All seeds were stored at 6˚C.

In selecting individual seeds for the granivory trials, a visual inspection was conducted comparing each seed to the bulk average, and unusually large, small, or disfigured seeds were not used. In addition to a visual assessment of each seed, the pinch test was used to verify the presence of an embryo and spot checks to verify the presence of an embryo were conducted by dissecting one in

every ten seeds using a 10x hand lens; all dissected seeds that passed the pinch test had an embryo. As *M. albus* may have 1-3 embryos within its seed coat (Turkington et al. 1978), 400 *M. albus* seeds were dissected for examination. Only one double embryo was found and it noticeably distended the seed coat. I am confident that all seeds used for this research contained a single embryo and so an egested embryo lacking a seed coat represents one ingested seed.

For seeds with a prominent and feathery pappus (*S. juncea*, *L. spicata, T. officinale*), seeds as a bulk group (min. 20,000, stated per guarantee from the supplier) were examined for whether the pappus persisted with agitation; if so, this suggests the pappus would persist through seed sorting, mixing, and application in field conditions. For *T. officinale* and *L. spicata*, the fragile pappus was absent on a majority of the seeds after agitation and so seeds lacking a pappus were used for the granivory trials. For *S. juncea*, the pappus structure was persistent and so seeds with an attached pappus were used in the experiment.

3.3.2 Feeding trials

Lumbricus terrestris individuals were commercially sourced from a local bait shop from refrigerated units kept on a commercial substrate, and no individual was used more than once in the experiment. The species *L. terrestris* were selected for this experiment due to their exceptional traits and widespread distribution. *Lumbricus terrestris* is the largest earthworm species in Canada (Reynolds 1977), is common to all restored and remnant tallgrass prairie sites studied in southern Ontario (chapter 2), and is widely distributed in Ontario (Reynolds 2014). As an anecic earthworm, individuals of *Lumbricus terrestris* create an often permanent vertical burrow system extending up to several meters into the soil profile, and feed on material foraged from the soil surface (Hamilton and Sillman 1989, Edwards and Bohlen 1996). As much of the foraged material is buried, this species

likely has the greatest potential impact on seed survival through direct (ingestion) as well as indirect (burial, deposition at depth after ingestion) factors of all of the invasive earthworm species present in Ontario. Due to its high consumption rate and burrowing habit, *L. terrestris* may have a disproportionate effect on the soil system relative to its abundance (Butt and Nuutinen 2005). In selecting *L. terrestris* for the experiment, active, well-coloured individuals without signs of disease or previous injury (e.g. scarring, unusual dimpling) were chosen, and if a batch of earthworms showed signs of disease (e.g. lethargy, thin, pale, irregularly coloured), no earthworms from the batch were used. Each *L. terrestris* was weighed immediately before being placed in the petri dish containing the seeds (fresh weight); the minimum weight post-fasting for earthworms used in this experiment was set at 3.1 g to ensure that the individuals were comparable to other feeding studies using *L. terrestris* (Clause et al. 2011b).

All stages of the feeding trails used a 15 cm diameter petri dish lined with moistened Whatman Qualitative Filter Paper (Grade 1) trimmed to size and a single *L. terrestris* individual fasted for the previous 24 hours. A 24 hour fasting period was used in the interest of standardizing gut content and hunger levels, as the gut transit time for *L. terrestris* has been estimated at as little as eight hours, and long fasting periods may only increase the chance of re-ingesting material egested during the fast (Hartenstein and Amico 1983). Several small diameter air holes were drilled in each petri dish lid to ensure sufficient oxygen availability. The growth chamber used for this experiment was kept at 18˚C and 70% RH, which was the minimum temperature and maximum humidity that could be maintained for the duration of the experiment. Other studies (Eisenhauer et al. 2009b, Clause et al. 2011b) have used 15˚C for feeding experiments with *L. terrestris*.

Two types of feeding trials were conducted. For the first, the single-species experiments, 30 seeds of one species were placed in each petri dish (n = 20 petri dishes). This design was repeated for

all 13 seed species. To test whether the ingestion of grass seeds was affected by their seed coat (presence of trichomes), *A. gerardi* seeds were tested twice, once with the seed coat intact and again with the seed coat removed (hulled). As some restoration methods (e.g. drill seeding) or seed preparation techniques (e.g. sifting, sorting, hand-broadcast seeding using burlap sacks) may result in the loss of the seed coat, whether the seed coat provides protection from earthworm granivory is directly relevant to ecosystem restoration in earthworm-invaded systems.

For the second type of feeding trial, the choice experiments, 20 seeds each of four different species were placed in each petri dish (n = 40 petri dishes). Three of these trials were conducted; the first using the most palatable native species as indicated by the single-species trials, the second using the four weed species, and the third using the two most preferred weed species as indicated by the previous choice experiments and the top preferred native species plus *L. spicata*. The decision to include *L. spicata* instead of the second-ranked native seed was based on its status as a threatened species with low germination rates and high procurement cost, meaning that it is both a priority conservation species and more difficult to establish at restoration sites given a fixed budget than other species.

After being loaded with the seeds and earthworm, the petri dishes were placed in a growth chamber for 18 hours. The 18 hour ingestion window was selected to give a more conservative ingestion estimate and reduce the chance that egested seeds may be re-ingested during the experiment; this ingestion window has been used in previous research (Mcrill and Sagar 1973, Grant 1983, Willems and Huijsmans 1994, Quackenbush et al. 2012). After this time had elapsed, earthworms were rinsed and transferred to a clean, lined petri dish and returned to the dark growth chamber for 48 hours to egest (Eisenhauer et al. 2009b), and the number of remaining seeds was recorded. After the egestion period had elapsed, the earthworms were rinsed and removed from the

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petri dish, casts were gently broken apart with water *in situ*, and the number of egested seeds was recorded. The difference between the number of ingested seeds and the number of egested seeds was considered the number of digested seeds.

3.3.3 Seed measurements

Length, width, and depth (thickness) measurements (mm; precision of two decimal places) were recorded at the widest point for 30 seeds of each species using digital calipers; calipers were re-calibrated after every five measurements (Table 3.1). For *S. juncea*, a separate pappus length measurement was also recorded, and for *A. gerardi* and *S. scoparium* a separate awn length was recorded. The seed mass of each species was obtained by weighing 100 seeds of each species to 0.001 g accuracy on an analytic balance. Mean seed surface area was calculated in the program ImageJ using a scanned image of the seeds at 24,000 dpi. As this image was binarized for processing, the surface area of *A. gerardi* and *S. scoparium* is a slight underestimate as it does not include many of the seed coat hairs - it does include the awns.

Table 3.1 Mean seed length, width, depth, pappus length, awn length (mm, +/- SD), seed mass (g), and surface area (cm-2) measurements for each of the 14 seed types. Andropogon gerardi with seed coats removed are listed as *A. gerardi. Weed seed species are indicated by (w) and native seed species are indicated by (n).

Seed type	Length	Width	Depth	Pappus length	Awn length	Mass	Surface area
Melilotus albus (w)	3.21 (0.32)	1.86 (0.19)	1.03 (0.13)	-	-	0.2352	0.037
Taraxacum officinale (w)	3.60 (0.31)	0.90 (0.12)	0.50 (0.06)	-	-	0.0474	0.022
Rudbeckia hirta (n)	2.17 (0.18)	0.55 (0.07)	0.47 (0.05)	-	-	0.0203	0.008
Trifolium pratense (w)	1.97 (0.13)	1.42 (0.14)	0.98 (0.08)	-	-	0.1827	0.021
*A. gerardi (n)	4.07 (0.45)	0.85 (0.11)	0.85 (0.11)	-	-	0.1804	0.031
Liatris spicata (n)	5.19 (0.40)	1.42 (0.19)	0.94 (0.10)	-	-	0.2824	0.046
Cirsium arvense (w)	2.96 (0.28)	0.96 (0.17)	0.63 (0.09)	-	-	0.06541	0.025
Solidago juncea (n)	1.32 (0.15)	0.37 (0.07)	0.37 (0.07)	1.73 (0.34)	-	0.011	0.005
Pycnanthemum virginianum (n)	1.15 (0.11)	0.42 (0.07)	0.42 (0.07)	-	-	0.0091	0.0035
Monarda fistulosa (n)	1.67 (0.15)	0.73 (0.08)	0.53 (0.06)	-	-	0.041	0.0083
Panicum virgatum (n)	5.24 (0.39)	1.93 (0.27)	1.09 (0.20)	-	-	0.2223	0.054
Lespedeza capitata (n)	2.76 (0.21)	1.68 (0.12)	1.03 (0.07)	-	-	0.3493	0.036
Schizachyrium scoparium (n)	6.62 (0.54)	4.11 (0.93)	0.72 (0.08)	-	5.90 (1.30)	0.1853	0.12
Andropogon gerardi (n)	7.13 (0.74)	2.87 (0.69)	1.00 (0.12)	-	8.25 (1.79)	0.3152	0.10

3.3.4 Statistical analysis

For all statistical analyses, normality was assessed using the Anderson-Darling Test and equal variances using Levene's Test. All statistical tests were conducted in Minitab® 18.1 at $\alpha = 0.05$, with the exception of the multiple comparisons of relative seed ingestion that used a Bonferroni corrected critical value of $\alpha = 0.008$ to conserve an overall familywise error rate of 0.05.

For the single species feeding trial, mean seed ingestion (% of total seed) was compared across seed type (14 treatment levels) using Welch's Test and the Games-Howell post-hoc test because of violations of the equal variance assumption of a standard One-Way ANOVA. Mean seed egestion (% of ingested seed) was compared across seed type (12 treatment levels, omitting *S. scoparium* and *A. gerardi* completely due to low overall ingestion/egestion and any replicates for other seed types when no ingestion occurred) using a One-Way ANOVA and Tukey's HSD post-hoc test. Two groups of correlations were assessed for both individual seed types and all pooled samples: associations between earthworm fresh weight (g) and seed ingestion (% of total seed) and between earthworm fresh weight and seed egestion (% of ingested seed). I used the Spearman Rank Correlation because of deviations from the bivariate normality assumption of parametric Pearson Product-Moment Correlation.

For the seed morphology, the associations between either seed ingestion or egestion and 10 seed characteristic measurements were assessed using the Spearman Rank Correlation (see above). The 10 seed characteristics included length, extended length (which included the pappus length for *S. juncea* and awn length for *A. gerardi*), Width, Depth, Mass, Surface Area, Eccentricity Index, Eccentricity Index calculated using Extended Length, Flatness Index, and Flatness Index calculated using Extended Length.

I used the Eccentricity Index and Flatness Index from (Cervantes et al. 2016) to quantify various aspects of seed shape. The Eccentricity Index (EI) is a simple and frequently used index defined by the length (L)/width (W) ratio of a seed:

$$EI = \frac{L}{W}$$

The Flatness Index quantifies seed shape based on the three principal axes of length (L), width (W), and depth (D), generating values close to 1 for spherical seeds and values exceeding 2 for spindly seeds:

$$FI = \frac{(L + W)}{2D}$$

For the choice experiments, multiple pairwise-comparisons were made of relative seed ingestion (% of total seed ingested) between each unique pairing of four seed types using Two-Tailed Paired Sign Tests with a Bonferroni Correction for multiple comparisons.

3.4 . Results

Multiple seed coats and half cotyledons were found in the egestion plates, supporting the assumption that seeds were digested and destroyed as opposed to having a longer gut residence time.

3.4.1 Single-species trials

3.4.1.1 Seed ingestion and egestion

Seed ingestion was found to be significantly different between seed types (Welch's Test, $F_{13, 98.5403}$ = 45.23, $p < 0.001$, $R^2 = 65.04$ %). Mean seed ingestion ranged from a maximum of 74.5 ± 27.0 % (*M. albus*) to a minimum of 0.3 ± 1.0 % (*A. gerardi*). Seed ingestion was highest for four species including *M. albus*, *T. officinale* (57.8 ± 21.1 %), *R. hirta* (57.7 ± 25.8 %), and *T. pratense* (56.2 ± 25.7 %). Seed ingestion was relatively low for both *S. scoparium* (0.5 ± 1.6 %) and *A. gerardi* (0.3 ± 1.0 %) (Figure 3.1).

Minimum seed egestion (% of ingested seed) was significantly different between seed types (One-Way ANOVA, $F_{11, 185}$ = 7.52, $p < 0.001$, $R^2 = 30.91$ %). Seeds of *S. scoparium* and *A. gerardi* were excluded from this analysis due to low ingestion rates. Egestion ranged from 84.3 ± 16.8 % (*C. arvense*) to 27.6 ± 26.2 % (*S. juncea*). In general, the distribution of egestion measurements was more consistent across the 14 species than the ingestion rates and there was considerable overlap in the groups assigned through post-hoc testing (Figure 3.2).
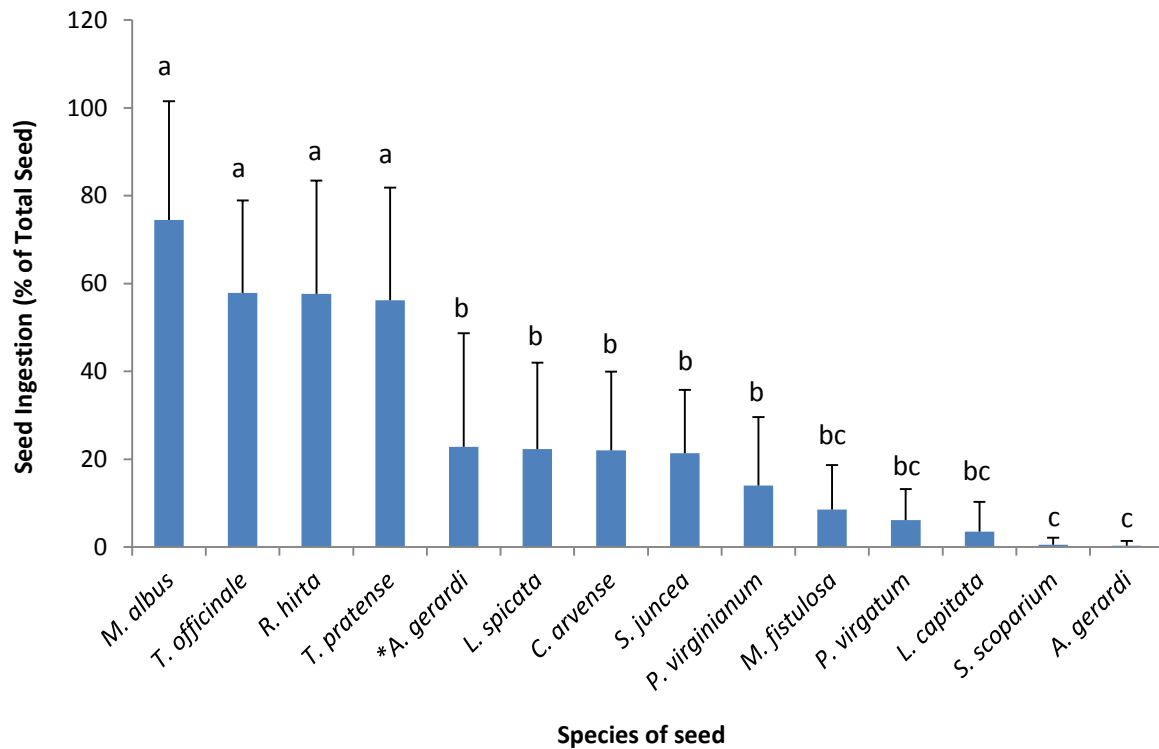
Figure 3.1 Bar chart of mean ingestion (% of initial 30 seeds) by individual *Lumbricus terrestris* of 14 seed types offered in a no-choice, single seed type cafeteria experiment (n = 20 cafeteria trials per seed type). Error bars depict standard deviation (SD). Letters denote groups determined by the Games-Howell post-hoc test. Means that do not share a letter are significantly different. *A. gerardi* with seed coats removed are listed as **A. gerardi*. Seed species: *Melilotus albus, Taraxacum officinale, Rudbeckia hirta, Trifolium pratense, Andropogon gerardi, Liatris spicata, Cirsium arvense, Solidago juncea, Pycnanthemum virginianum, Monarda fistulosa, Panicum virgatum, Lespedeza capitata, Schizachyrium scoparium.*
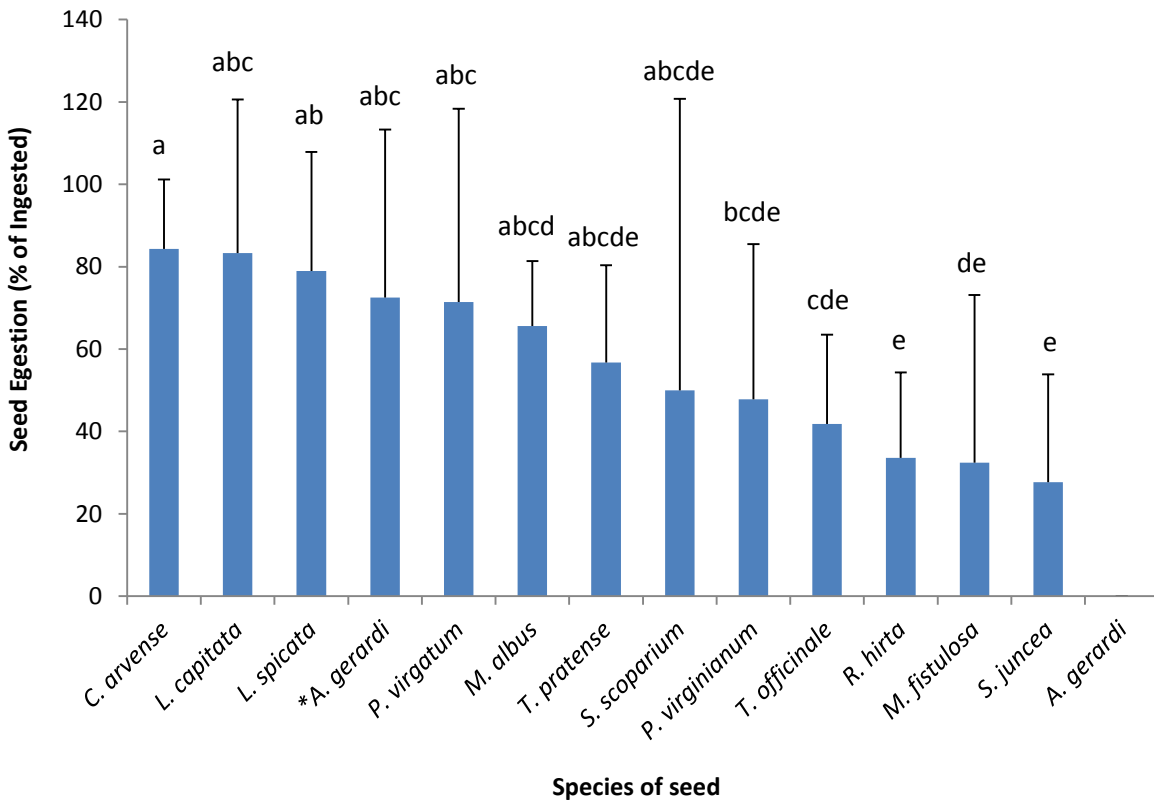
Figure 3.2 Bar chart of mean egestion (% of ingested seeds) by individual *Lumbricus terrestris* of 14 seed types offered in a no-choice, single seed type cafeteria experiment (replicates excluded if no seeds were ingested, from left to right n = 18, 7, 16, 18, 13, 20, 20, 2, 15, 20, 20, 12, 17, 2). Error bars depict standard deviation (SD). Letters denote groups determined by Tukey's HSD post-hoc test. Means that do not share a letter are significantly different. *S. scoparium* and *A. gerardi* are included in this figure but were not included in the statistical comparison of means or post-hoc test due to low sample sizes. *A. gerardi* with seed coats removed are listed as *A. gerardi. For full names of plant seeds, refer to caption to Fig. 3.1.

Mean egestion as a percent of ingestion was 61.5% for weed seeds and 49.5% for target seed species. Egested seeds varied in condition upon egestion; some were noticeably scarified (*R. hirta* and *L.* spicata) whereas others had begun to germinate (*M. albus* and *T. pratense*). Although seed digestion is difficult to quantify with certainty due to varying gut latency time, seed digestion was obvious in some cases (where a partial cotyledon or empty seed coats were egested, e.g. *R. hirta* and *P. virginianum*). Based on these observations, larger seeds as measured by width, depth, mass, and surface area had overall higher intact egestion rates than small seeds, and *L. terrestris* likely digested over 50% of the target tallgrass seeds ingested in this study. Calculated as simple ingestion – egestion = digestion, no plant species was digested above 72.4%, and seed species with the highest digestion rates did not tend to match those with the highest ingestion rates.

3.4.1.2 Earthworm weight

Earthworm weight was not significantly correlated with overall seed ingestion (Spearman Rank Correlation, r_s (278) = 0.458, p = 0.458) (Table 3.2). However, upon testing by individual species, significant (if 'moderate') correlations were found for *R. hirta* (r_s (18) = 0.514, p = 0.021) and *M. fistulosa* (r_s (18)= 0.581, p = 0.007). For these species, larger earthworms tended to ingest greater quantities of seed.

Similarly, earthworm weight was not significantly correlated with overall seed egestion (Spearman Rank Correlation, r_s (199) = -0.129, p = 0.068) (Table 3.2). However, two seed type-specific instances of a significant earthworm weight-seed egestion correlation were found, with one positive association for *R. hirta* (r_s (18) = 0.455, p = 0.044) and one negative association for hulled *A. gerardi* (r_s (16) = -0.506, p = 0.032). For *R. hirta*, larger worms tended to both ingest more seeds and

to egest a greater proportion of these ingested seeds. In contrast, larger earthworms tended to egest fewer seeds of hulled *A. gerardi*.

Table 3.2 Spearman rank correlation outputs for the association between earthworm fresh weight (g) and either seed ingestion (% of total seeds) or seed egestion (% of ingested seeds) in no-choice, single seed type cafeteria experiments for all seed types combined and each of 14 seed types individually. P-values that are significant at α = 0.05 and associated species name and correlation coefficients are in bold print. Andropogon gerardi with seed coats removed are listed as *A. gerardi.

Seed Type	Seed Ingestion (% of Total Seed)			Seed Egestion (% of Ingested Seed)		
	Spearman Rank Correlation Coefficient (r_s)	*p*-value	DF	Spearman Rank Correlation Coefficient (r_s)	*p*-value	DF
All Seed Types	0.044	0.458	278	-0.129	0.068	199
Melilotus albus	0.277	0.237	18	-0.092	0.700	18
Taraxacum officinale	0.287	0.219	18	-0.022	0.926	18
Rudbeckia hirta	**0.514**	**0.021**	18	**0.455**	**0.044**	18
Trifolium pratense	0.032	0.892	18	0.072	0.763	18
*A. gerardi	0.409	0.073	18	**-0.506**	**0.032**	16
Liatris spicata	0.019	0.937	18	0.007	0.980	14
Cirsium arvense	0.021	0.930	18	-0.251	0.315	16
Solidago juncea	0.184	0.438	18	0.035	0.893	15
Pycnanthemum virginianum	0.333	0.151	18	0.246	0.377	13
Monarda fistulosa	**0.581**	**0.007**	18	-0.206	0.520	10
Panicum virgatum	-0.378	0.100	18	0.157	0.592	12
Lespedeza capitata	-0.292	0.211	18	0.490	0.264	5
Schizachyrium scoparium	-0.352	0.128	18	-	-	-
Andropogon gerardi	0.174	0.462	18	-	-	-

3.4.2 Seed morphological traits

No significant correlations were found between seed ingestion and any of the measured seed traits (Table 3.3). Notably, seed length was not significantly correlated with seed ingestion, however it was observed that the two seeds with the lowest ingestion rates (i.e. *S. scoparium*, *A. gerardi*) both had seeds greater than 2 mm in width, while the remaining seed – which also included several species with low ingestion rates but were dominated by seeds with higher ingestion rates – all had seeds less than 2 mm in width (Figure 3.3).

Moderate positive correlations were found between seed egestion and four seed traits related to the overall "size" of the seed: width, depth, mass, and surface area. Seeds that had larger width, depth, mass, and surface area tended to be egested in greater numbers (relative to ingestion).
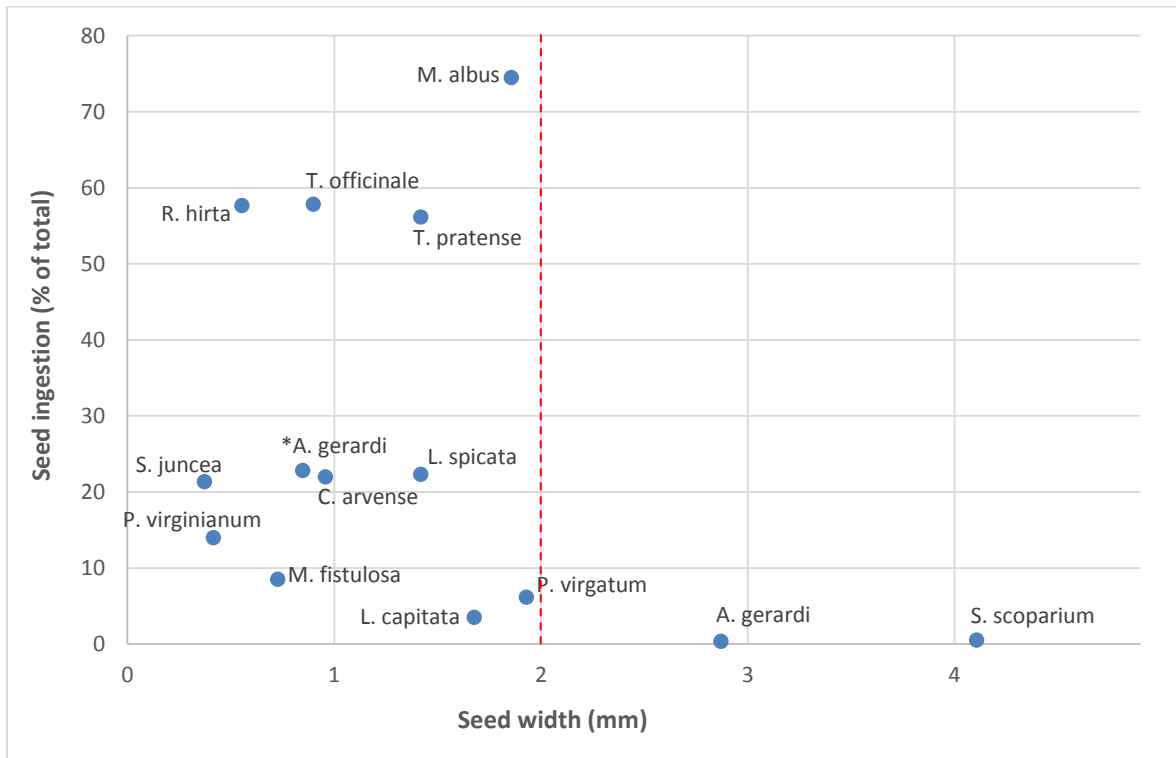
Figure 3.3 Scatterplot of average seed ingestion (% of total seed) from single type cafeteria trials and average seed width (mm, based on average of 32 seeds per seed type) for 14 seed types. The dashed red line denotes the 2 mm seed width that has been postulated as a threshold for earthworm seed ingestion. *Andropogon gerardi* **with seed coats removed are listed as *A. gerardi. For full names of plant seeds, refer to caption to Fig. 3.1.**

Table 3.3 Spearman rank correlation outputs for the association between mean measures of granivory (seed ingestion, seed egestion) and 10 seed morphological traits measured from 14 seed types (n = 14, DF = 12). P-values that are significant at α = 0.05 and associated species name and correlation coefficients are in bold print.

Granivory Measure	Seed Trait	Spearman Rank Correlation Coefficient (r_s)	p-value
Seed Ingestion (% of Total Seed)	Length	-0.257	0.375
	Extended Length	-0.275	0.342
	Width	-0.371	0.191
	Depth	-0.165	0.573
	Mass	-0.288	0.318
	Surface Area	-0.385	0.175
	Eccentricity Index (EI)	0.411	0.144
	Eccentricity Index (EI), Extended Length	0.099	0.737
	Flatness Index (FI)	-0.196	0.503
	Flatness Index (FI), Extended Length	-0.292	0.311
Seed Egestion (% of Ingested Seed)	Length	0.367	0.197
	Extended Length	0.226	0.436
	Width	**0.622**	**0.018**
	Depth	**0.600**	**0.023**
	Mass	**0.692**	**0.006**
	Surface Area	**0.538**	**0.047**
	Eccentricity Index (EI)	-0.380	0.180
	Eccentricity Index (EI), Extended Length	-0.332	0.246
	Flatness Index (FI)	0.187	0.523
	Flatness Index (FI), Extended Length	0.024	0.935

3.4.3 Choice trials

Earthworms ingested unequal quantities of different seed types when offered a choice between four species at a time. Median relative seed ingestion (% of total seed consumed in a given cafeteria experiment) differed significantly between seed type in the weed choice (One-Way Repeated Measures ANOVA, $F_{2.54,99.21} = 14.86$, $p < 0.001$, $\varepsilon_{HF} = 0.85$), native choice (One-Way Repeated Measures ANOVA, $F_{1.67,65.14} = 148.00$, $p < 0.001$, $\varepsilon_{GG} = 0.56$), and mixed choice trials (One-Way Repeated Measures ANOVA, $F_{2.07,80.64} = 32.25$, $p < 0.001$, $\varepsilon_{GG} = 0.69$) (Table 3.4, Figure 3.4).

Table 3.4 Results of multiple pairwise comparisons between median relative seed ingestion (% of total seed ingested, 80 total seeds available per trial, 20 of each of four seed types) of seed types in three four-way seed type choice experiments: weed choice, native choice, and mixed choice (n = 40 per choice experiment). Cells contain p-values generated by 2-tailed Paired Sign Tests between seed types testing (H0: $\eta_A - \eta_B = 0$). p-values that are significant at a Bonferroni corrected α = 0.008 are in bold. Andropogon gerardi with seed coats removed are listed as *A. gerardi.

Weed Choice			
	Melilotus albus	*Taraxacum officinale*	*Trifolium pratense*
Taraxacum officinale	**0.003**	-	-
Trifolium pratense	**< 0.001**	0.073	-
Cirsium arvense	**< 0.001**	**< 0.001**	0.143
Native Choice			
	Rudbeckia hirta	*Solidago juncea*	*Liatris spicata*
Solidago juncea	**< 0.001**	-	-
Liatris spicata	**< 0.001**	**< 0.001**	-
*A. gerardi	**< 0.001**	**< 0.001**	**< 0.001**
Mixed Choice			
	Melilotus albus	*Rudbeckia hirta*	*Taraxacum officinale*
Rudbeckia hirta	0.256	-	-
Taraxacum officinale	0.014	0.418	-
Liatris spicata	**< 0.001**	**< 0.001**	**< 0.001**

In the weed choice experiments, a higher proportion of *M. albus* seed (η = 34.9 %, IQR = 10.0 %) was ingested compared to *T. officinale* (28.0 %, 10.5%), *T. pratense* (21.8 %, 12.4 %), and *C. arvense* (17.0 %, 11.5 %) (Figure 3.4a). All seeds were ingested in some quantity and the median ingestion of the most highly preferred seed (*Melilotus albus*) was only twice as high as the lowest median ingestion (*C. arvense*).

In the native choice experiments, the distribution of seed ingestion across seed types was less even, with a large percentage of the ingestion occurring for *R. hirta* (65.5 %, 26.1 %), a moderate amount for *S. juncea* (25.8 %, 16.0 %), and low amounts for *L. spicata* (6.4 %, 11.9 %) and hulled *A. gerardi* (0.0 %, 4.1 %) (Figure 3.4b).

In the mixed choice experiments, the majority of seed ingestion occurred for three seed types including *M. albus* (34.0 %, 12.0 %), *R. hirta* (29.2 %, 15.0 %), and *T. officinale* (28.6 %, 11.1 %), with lower ingestion for *L. spicata* (7.6 %, 10.2 %) (Figure 3.4c).

Overall, the order of seed ingestion in no-choice, single species trials was consistent with the general order of relative seed ingestion in the three choice experiments (Table 3.5). The seed types with the highest ingestion values in the no-choice trials also had the highest relative ingestion values in the choice experiments (*Melilotis albus*, *T. officinale*, *R. hirta*).

Figure 3.4 Boxplots of relative seed ingestion (% of total seeds consumed) of different seed types by earthworms in choice cafeteria experiments offering 20 seeds each of four different seed types that were a) weed seeds; b) native seeds; or c) a mix of two weed seeds and two native seeds (n = 40 per choice experiment). Letters denote groups determined by multiple 2-Sample Sign Tests between all possible pairs of species within a choice test. Grey boxes represent the interquartile range; bottom and top whiskers extend to the 10th and 90th percentiles, respectively; black dots represent points beyond the 10th and 90th percentiles. Solid horizontal lines are median values. Medians that do not share a letter are significantly different. _A. gerardi_ with seed coats removed are listed as *_A. gerardi_. For full names of plant seeds, refer to caption to Fig. 3.1.
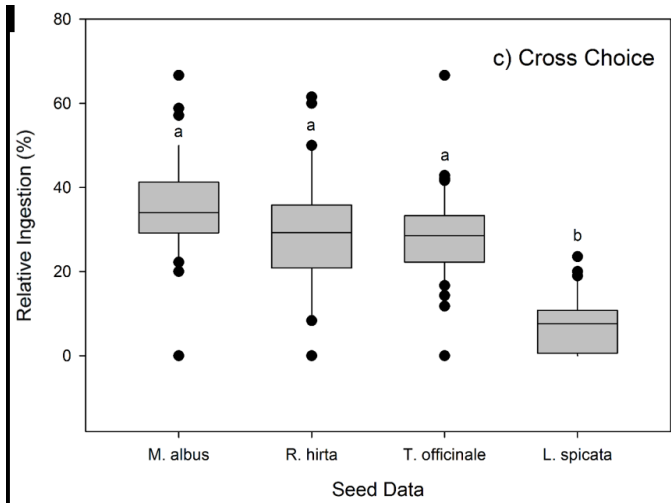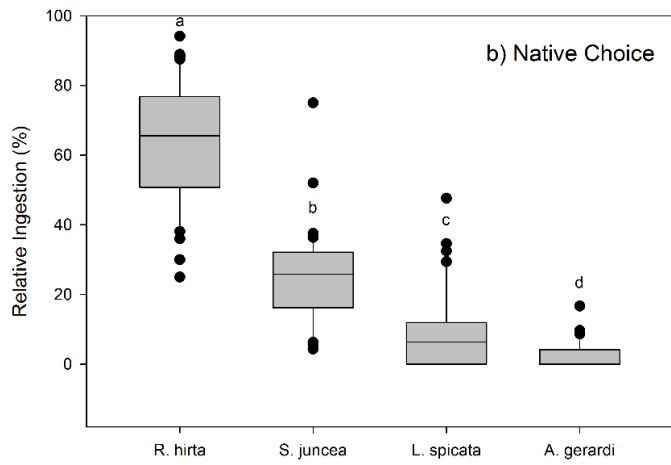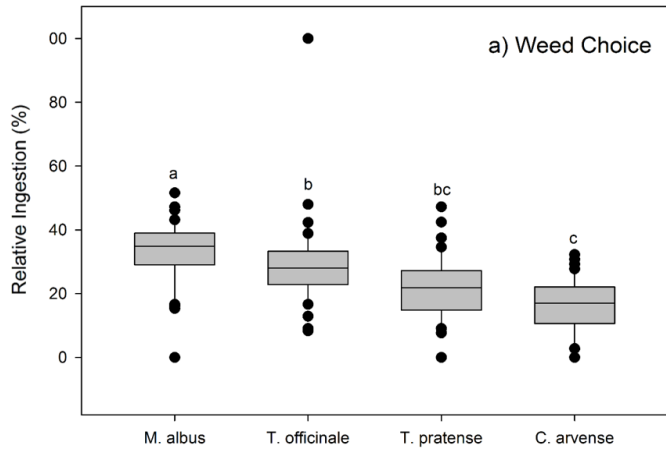
Table 3.5 Summary of groupings assigned by post-hoc tests following comparisons of: a) mean seed ingestion (% of total seed) in single seed type, no-choice trials, groups assigned using Games-Howell post-hoc test; and b) median relative seed ingestion (% of total ingested seed) in four-way choice trials of weed, native, or mixed seed types, using multiple paired Sign Test comparisons with a Bonferroni p-value adjustment. Group letters were assigned alphabetically to groups with decreasing magnitude (i.e. μa > μb > μc …). Andropogon gerardi with seed coats removed are listed as *A. gerardi. Native seed species are indicated by (n) and weed seeds by (w).

Seed Type	Single Seed Type	Weed Choice	Native Choice	Mixed Choice
Melilotus albus (w)	a	a	-	a
Taraxacum officinale (w)	a	b	-	a
Rudbeckia hirta (n)	a	-	a	a
Trifolium pratense (w)	a	bc	-	-
*A. gerardi (n)	b	-	d	-
Liatris spicata (n)	b	-	c	b
Cirsium arvense (w)	b	c	-	-
Solidago juncea (n)	b	-	b	-
Pycnathemum virginianum (n)	b	-	-	-
Monarda fistulosa (n)	bc	-	-	-
Panicum virgatum (n)	bc	-	-	-
Lespedeza capitata (n)	bc	-	-	-
Schizachyrium scoparium (n)	c	-	-	-
Andropogon gerardi (n)	c	-	-	-

3.5 Discussion

As expected, *L. terrestris* in this study demonstrated strong preference for ingesting seeds of certain species over others. These preferences were consistent, following the same general pattern of preference in single, no-choice experiments as when presented with four different seed species in

choice experiments, particularly in the case of the weed seeds tested. Seed egestion, as a percent of seeds ingested by earthworms, was also significantly different between the seed types used in this study, supporting the results of other studies (Mcrill and Sagar 1973, Eisenhauer et al. 2009b, Clause et al. 2016b). Overall ingestion by earthworms was high – in some cases 100% of the seeds presented were ingested within the 18 hour ingestion window. Earthworm activity may thus be an important factor in plant population dynamics, floristic composition, and weed control (see Grant 1983).

3.5.1 Do L. terrestris prefer seeds of native species used in ecological restoration or seeds from exotic and 'weedy' species usually found in the seedbank of former cropland?

Based on the single choice experiments, the highest levels of ingestion recorded in this research were for three of the four weed seeds tested, meaning that *L. terrestris* preferred to ingest problem weeds seeds more than the majority of the native seeds that are included in a standard southern Ontario tallgrass prairie seed mix. Considering the cross-choice results, when given the option of two preferred weed species and two target native species, *L. terrestris* shows a preference for ingesting the two weed species at an equal level to the most preferred target species (*R. hirta*), but significantly lower ingestion of the high conservation value *L. spicata*. Given the low germination rates and high cost of *L. spicata* seeds, its ranking as the lowest preferred seed in the cross-choice experiment is good news for land managers who include this species in their restoration mix.

The preference for invasive or weed seed species has also been demonstrated in previous research with *L. terrestris*; in forest systems, *L. terrestris* have been shown to prefer exotic *Alliaria petiolata* (M. Bieb.) Cavara & Grande to some seeds of some native plant species (Quackenbush et al. 2012), and in cropland systems of the USA, *L. terrestris* has been shown to collect more seeds of the problem weed *Ambrosia trifida* L. than 11 other large-seeded species (Regnier et al. 2008). On the

surface, this may appear to benefit native species and tallgrass restoration efforts. However,

depending on the identity of the seed species and if seeds are egested intact, earthworm ingestion may

provide benefits to the seed including protection from above-ground predators, assistance in seed

bank formation, and nutrient enrichment at the burrow site (Regnier et al. 2008). With respect to

grassland systems, Clause et al. (2015) found that for a weed-invaded California grassland,

earthworm abundance was positively correlated with non-native seedling emergence, and suggested

that there may be a positive feedback between non-native plants and non-native earthworms. In their

study of grasslands in Germany, Eisenhauer et al. (2009a) found that legumes in particular benefited

from earthworm presence (shoot biomass increase), and suggest that earthworms and legumes may

form a loose mutualistic relationship affecting essential ecosystem functions in temperate grasslands,

in particular driving litter decomposition and enhancing nutrient availability. If this mutualism

extends to the tallgrass prairie ecosystem, it would likely favour the non-target/ruderal weedy

legumes *M. albus* and *T. pratense*, with the potential for a positive feedback loop between *L.*

terrestris abundance and the success of *M. albus* and *T. pratense* at restoration sites.

3.5.2 What is the relationships between seed morphology and ingestion of seeds of different

species?

3.5.2.1 Ingestion

I did not find a significant relationship between seed ingestion and seed morphology (Table 3.3). This

contrasts with previous experiments which have shown that *L. terrestris* preferred small, narrow, light

seeds (Shumway and Koide 1994, Clause et al. 2011b, Cassin and Kotanen 2016). However, beyond

an earthworm's inability to ingest seeds larger than their mouth (a threshold usually set at > 2mm for

L. terrestris) (Shumway and Koide 1994), recent work by Clause et al. (2016b) showed that seed size

alone does not explain seed selection by earthworms, and the apparent effect of seed length is explained by high seed oil content and not seed size. My results suggest that the ingestion preferences of *L. terrestris* are not driven by seed size or shape for the plant species tested, hence the strong species preferences that occur may be caused by chemical properties. This hypothesis will have to be tested further, given the work of Clause et al. (2016b). I note that a few seeds with a width greater than 2 mm were consumed and egested by my specimens. However, given that the lowest ingestion rates observed in this study were for the two species above the assumed 2 mm width threshold, my results do support the use of 2 mm width as an approximate threshold of seed ingestion for *L. terrestris*. In the context of restoration, this suggests that larger seeds having a width of > 2 mm may be at lesser risk of ingestion (although not necessarily burial) from invasive earthworms present at restoration sites.

While adult *L. terrestris* were used in this experiment, earthworm feeding behavior can change with age (Curry and Schmidt 2007). This is noteworthy because the majority of earthworms recorded in southern Ontario's tallgrass prairie sites are juveniles (chapter 2) and may have different feeding preferences or maximum seed width thresholds. While this needs to be tested, the high overall earthworm (79.2 m^{-2} +/- 81.2) and *L. terrestris* midden (39.6 m^{-2} +/- 24.7) densities at these sites and the tendency of adult *L. terrestris* to remain under-sampled due to their burrowing habit suggests that the feeding preferences of adult *L. terrestris* have important implications for tallgrass prairie ecological restoration.

3.5.2.2 Egestion

In agreement with previous work (Mcrill and Sagar 1973, Eisenhauer et al. 2009b, Clause et al. 2016b), *L. terrestris* in my experiment egested only 56% of the seed ingested. Once ingested by

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earthworms, seed morphological traits may also factor into the ultimate fate of seeds. For example, small seeds have been shown to be digested or rendered non-viable in greater proportions than large seeds as a result of the physical and chemical damage they sustain during passage through the earthworm gut (Marhan and Scheu 2005, Curry and Schmidt 2007, Aira and Piearce 2009). This has been explained as a function of their gut passage time, where small seeds are likely to take longer to be egested, and to be more physically damaged in the gizzard or digested by enzymatic activity than large seeds (Clause et al. 2016b). My results support the existing body of research showing that larger seeds as measured by width, depth, mass, and surface area had higher intact egestion rates than small seeds (Curry and Schmidt 2007, Aira and Piearce 2009, Clause et al. 2016b).

Although earthworm gut transit time varies (Clause et al. 2016b), the observed egestion of empty or partial seed coats and fragmented cotyledons (e.g. in the case of *R. hirta* and *P. virginianum*) suggests that digestion of the seed material occurred in this experiment. Digestion in laboratory feeding experiments is usually considered the difference between ingestion and egestion (Mcrill and Sagar 1973, Eisenhauer et al. 2009b), and depending on the specific plant species, *L. terrestris* have been shown to digest or destroy up to 100% of ingested seeds (Willems and Huijsmans 1994, Eisenhauer et al. 2009b). In this study, no plant species was digested above 72.4%, and in contrast to previous studies (Mcrill and Sagar 1973, Eisenhauer et al. 2009b, Clause et al. 2016b), seed species with the highest digestion rates did not tend to match those with the highest ingestion rates, and so my results do not support the idea that *L. terrestris* prefers to ingest seeds that it can digest.

3.5.3 How does earthworm weight affect seed ingestion rates?

No significant correlation between earthworm weight and overall seed ingestion or egestion was found, unlike previous work that showed a positive correlation between *L. terrestris* weight and overall seed ingestion and egestion (Clause et al. 2016b), but in agreement with other work showing no relationship between earthworm size (length, width, weight) and seed digestion (Clause et al. 2011b). Considered together, my study and others suggest that egestion is likely a function of the seed species identity more reliably than earthworm size.

As earthworm weight is linked to overall size and thus to mouth size, it has also been suggested that larger and heavier earthworms are likely to have larger mouths and ingest more seeds (Clause et al. 2016b). In this study, I found a positive correlation between ingestion and earthworm weight for seeds of only two native plant species, *Rudbeckia hirta* and *Monarda fistulosa*; for these species, larger earthworms did ingest greater quantities of seed, and for *R. hirta* larger earthworms tended to both ingest more seeds and egest a greater proportion of ingested seeds. As these species are both native, target species in the restoration seed mix, higher ingestion rates with earthworm weight may affect the successful establishment of these species. The ingestion/egestion benefits and risks to seeds are discussed further in section 3.5.5.

3.5.4 Is there a protective effect afforded by seed trichomes?

My results suggest that the trichomes (long or dense spines) present on the seed coat of certain seeds may provide protection from direct earthworm granivory. Intact versus hulled *Andropogon gerardi* was shown to have significantly different ingestion rates, and intact *Schizachyrium scoparium* was also ingested at very low levels, suggesting that the bristled seed coat of certain species of grass seed may offer a measure of protection from earthworm granivory, whether by simply increasing the seed

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width beyond the 2 mm threshold of mouth size, or through physical obstruction. The effect of trichome presence/absence does seem to be a matter of degree, as the much smaller, more subtle bristles present on one end of *Taraxacum officinale* seeds did not protect it from ingestion.

Although Clause et al. (2011) found that the presence of trichomes did not significantly affect the feeding habits of two earthworm species, including *L. terrestris*, they do suggest that trichome presence is likely important, as all highly palatable seeds in their study lacked trichomes. Differences in seed coat texture may also explain the documented preference for forb seeds over grass species (Zaller and Saxler 2007, Eisenhauer et al. 2009b). The presence of trichomes may also have an effect on earthworm collection, where seeds bearing long or dense spines are collected in fewer numbers (Regnier et al. 2008).

To improve the generalizability of these and related findings, it may be useful to establish an index of seed-coat related palatability for earthworm species. Future investigations of chemical seed properties influencing ingestion (oil content, moisture, protein content, etc.), may benefit from testing seed both with and without their seed coat to isolate the effects of chemical versus physical factors related to palatability. Future research opportunities aside, these findings suggest that unless specific seed species require scarification to facilitate germination, practitioners of restoration activities should avoid physical abrasion or removal of the seed coat to maintain this protective effect.

3.5.5 What are the management implications for restoration activities?

Anecic earthworm activity may affect seed dispersal and germination success in five ways; by selective ingestion, egestion, and digestion of seeds (this study); by dispersing seeds vertically in the soil profile (Shumway and Koide 1994, Zaller and Saxler 2007, Regnier et al. 2008), by depositing seeds in their surface casts which form the burrow entrance (Milcu et al. 2006), by transporting seeds

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back up to the surface from the seedbank (Willems and Huijsmans 1994, Drouin et al. 2014), and by inhibiting (Grant 1983) or promoting germination by passage through the earthworm gut (Ayanlaja et al. 2001). With respect to earthworm effects, the ultimate fate of seeds applied during restoration activities is therefore a function several different earthworm-mediated factors (Figure 3.5), of which this research was designed to test selective ingestion and egestion. Both establishment and dispersal filters affect the assembly of restored prairie plant communities, and propagule availability in the initial stages of a restoration is critical to its success (Poulsen et al. 2007, Grman et al. 2015). Combined with interactions among climate, plant species identity, and disturbance during the initial stages of establishment, seed availability can influence community assembly (MacDougall et al. 2008), and so the additional filter of earthworm granivory may have important effects. *L. terrestris* individuals in this study ingested up to 100% (up to 30/30 in single choice, 70/80 in cross choice) of the seed offered within the 18 hour ingestion window. Although the plant-herbivore equilibrium points for tallgrass prairie plant species and exotic earthworms are unknown and earthworms such as *L. terrestris* may consume alternative food sources in field conditions (e.g. detritus), given the high densities of earthworms recorded at restored tallgrass prairie sites (chapter 2), the level and speed of ingestion documented in this study suggests that earthworms likely act as dispersal filters by reducing seed densities at prairie restoration sites. Earthworms should thus be considered alongside other filters such as rodent, insect, and bird herbivory, competition with ruderal weeds, changes in temperature and precipitation, and establishment conditions (e.g. organic matter and sand content of soils, land use history, soil moisture, fire frequency) in tallgrass prairie restorations (Grman and Brudvig 2014, Orrock et al. 2015, Grman et al. 2015, Moles and Westoby 2006, Eisenhauer and Scheu 2008, Forey et al. 2011), and research into the stability and nature of the earthworm-propagule density relationship is warranted.
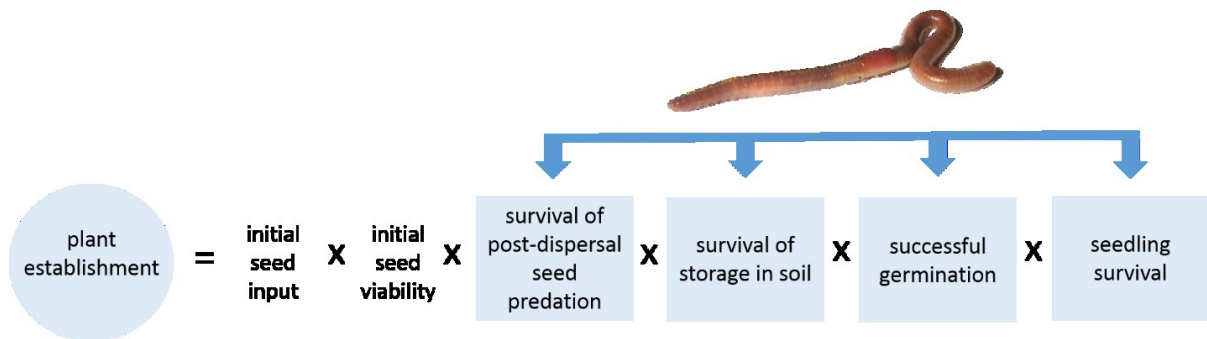
Figure 3.5 Plant establishment following restoration as a function of the challenges to seed survival, with emphasis on factors directly influenced by earthworms (modified after Moles and Westoby 2006; Eisenhauer and Scheu 2008; Forey et al. 2011)

Earthworm seed caching and transport behaviour may vary with seed species (Milcu et al. 2006), hence I may have overestimated the ingestion rates compared to field conditions. While the impacts of earthworm caching were not measured by this study design, this controlled experiment represents many key features of actual field conditions at the time of seeding, i.e., prevalence of bare ground; large, one time deposition of above-ground seeds; little to no surface organic matter or vegetation.

Milcu et al. (2006) found that *L. terrestris* buried or ingested 95% of the seeds applied to the soil surface, regardless of seed size (large vs small) in less than 48h, and Eisenhauer and Scheu (2008) found *that L. terrestris* buried or consumed all seeds irrespective of seed size during the first week. This suggests that burial and direct granivory are both important behaviours to consider in determining the ultimate fate of restoration seed mixes applied to earthworm-invaded habitats. Future

research exploring these preferences in mesocosm and field studies would provide a clearer picture as to the outcome when earthworms have a choice of behaviours.

Ingestion is not always fatal to a seed, and it may even be beneficial to some species by scarifying the seed coat or protecting from aboveground predation and environmental threats (Mcrill and Sagar 1973, Ayanlaja et al. 2001, Traba et al. 2006, Eisenhauer and Scheu 2008, Eisenhauer et al. 2009b). Still, many seeds are killed by ingestion, either by digestion, damage during earthworm gut passage (Eisenhauer et al. 2009b), through burial at depths from which successful germination is impossible (Traba et al. 2004, Forey et al. 2011, Donath and Eckstein 2012, Quackenbush et al. 2012, Drouin et al. 2014), or by ingestions following burial, either post-germination (feeding on germinated seedling) or following partial decomposition of the seed (Lee 1985, Shumway and Koide 1994).

If ingested seeds avoid digestion or destruction and are egested as viable propagules, their ability to establish as seedlings will depend on species-specific traits (persistence, ability to germinate without light, maximum viable germination depth), environmental conditions, burial depth, and possibly burial mode (Burmeier et al. 2010). Seeds of many species that are very small or unable to germinate without light (e.g. *P. virginianum*, *R. hirta, M. fistulosa*), and even seeds of burial-tolerant species are unlikely to have the resource stores necessary to emerge if buried more than a few centimeters (Fenner and Thompson 2005).

For small seeded species, which are more likely to be persistent in the seed bank (Westoby et al. 1992, Thompson et al. 1993, Fenner and Thompson 2005), intact egestion of seeds by earthworms may facilitate the formation of a persistent seed bank (Schmiede et al. 2009, Laossi et al. 2010). In these species, eventual successful germination will depend on later transport in the soil column by *L. terrestris* or physical forces, such as freeze/thaw cycles. Milcu et al. (2006) found that unwanted weed seeds from the soil bank of their mesocosm experiment were transported upwards through the

soil column and to a depth where they were able to successfully germinate, and it has been estimated that in temperate regions the upper 15 cm of soil, containing most seeds of the seed bank, may be turned over completely every 10–20 years by earthworms (Edwards and Bohlen 1996).

This has important implications for restoration practitioners, as the expected versus actual germination and establishment timeframe of the applied seed mix may vary considerably. Delayed establishment of target species through egestion-associated burial or deposition could also facilitate a competitive advantage for non-target invasive or ruderal species. For example, a mesocosm experiment by Eisenhauer and Scheu (2008) showed that more invader plants established in the bare ground treatment than the grass, legume, or mixed community. Laossi et al. (2010) suggest a context-dependent effect of earthworms on seedling emergence, where in a litter-free environment seedling emergence will be lower due to earthworm granivory or transport within the soil profile beyond the level of successful emergence.

As many restoration sites are pre-treated (herbicide, tilling, etc.) former cropland with little litter cover, earthworms may reduce seedling emergence to a greater degree as compared to a naturalized, established system where litter cover may have a protective effect (Laossi et al. 2010). Delayed germination of small-seeded target species could influence the direction of future plant community composition, particularly in the initial years of a restoration project when bare ground is the dominant cover and many 'gaps' are open for establishment. This may favour ruderal and invasive species already present in the seed bank by giving them a chance to self-seed and spread, outcompeting the target plant community.

Importantly, seeds are egested in casts, which may provide favourable environments compared to the surrounding soil environment (Regnier et al. 2008), likely related to the higher soil moisture, organic matter, and nutrient content of cast material (Edwards and Bohlen 1996). Surface

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casts, including those that constitute the midden structure, may also be relatively stable, and may remain at the soil surface for more than one year thereby forming a pool of seeds ready to germinate after disturbances (Decaëns et al. 2003). However, earthworm mucus and the conditions provided by casts are selectively beneficial, so whether the casts inhibit or promote seed success varies with earthworm and plant species (Eisenhauer et al. 2009b).

Seeds in this study varied in condition upon egestion; in some cases many of the egested seed had begun germinating (*M. albus* and *T. pratense*), while in others the seeds were noticeably scarified (*R. hirta* and *L. spicata*). The germination within hours by *M. albus* and *T. pratense* could be either advantageous or disadvantageous to seedling survival. The relatively large seed body resources of these species means that egested seeds which germinate may be able to emerge from depths beyond the capability of smaller seeds or seeds which require light to germinate. Conversely, these seedlings may be predated upon underground by earthworms or other soil biota.

Shumway and Koide (1994) demonstrated subterranean seed germination of *T. pratense* in chambers created by *L. terrestris* in each of four mesocosm replicates at depths of 10-20 cm. Cotelydons had been severed on many of the seedlings, and two individual earthworms were observed feeding on sprouted seedlings during the excavation of the experiment. Although the occurrence of this behaviour *in situ* is not known, this seedling predation behaviour demonstrates that the belowground soil habitat (seedbank) is not a static environment nor necessarily a safe haven for egested seeds. Post egestion, further deterioration of seeds by microbial communities (Aira et al. 2005) might impact seed viability across time. If undesirable species are transported by earthworms back up to the surface (having been protected by burial through underground egestion or gut transit time), or germinate following burial or egestion by earthworms (particularly for *M. albus* and *C. arvense* which are the more persistent, long-term management issue species) in favourable conditions

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(i.e., casts), then the *L. terrestris* preference for these seeds could result in the persistence of these species in the seed bank and complicate control measures.

After ecological restoration, the seed bank of grassland habitats build up slowly, with the target species' seed density in the soil bank declining with soil depth (Schmiede et al. 2009). Even following several years post-restoration, the seed bank of restored grasslands may be dominated by species characteristic of agrestal and ruderal species from the former crop use (Schmiede et al. 2009). This suggests that in addition to the large application event of broadcast seeding target species, earthworm translocation of non-target seeds from the seed bank is likely to play a role in the vegetation composition of the site, which in turn is itself mediated in part by species-specific earthworm-plant seed interactions. The complexities of interactions between target and non-target seed transport from the seedbank to the surface by earthworms and the differential benefit or challenge of casts further illustrates the importance of understanding the role that earthworm communities play in specific ecosystems, particularly in the context of restoration activities.

In light of the effects of earthworm granivory and seed transport, a possible alternative to seeding worthy of future investigation is the planting of greenhouse raised plugs. Although seldom used due to establishment challenges (watering, soil type match, etc.), plugs may be particularly appropriate for establishing high cost or rare tallgrass species whose seeds have high earthworm palatability.

Earthworm invasion of tallgrass prairie habitats is uneven but probably accelerating (chapter 2). In plant communities not adapted to earthworms, earthworm-mediated effects may be stronger (Forey et al. 2011) or affect the restoration and resultant plant community in complex and unexpected ways. As the exploration of interactions between earthworm and plant communities continues to

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develop as a field of ecological research, it is critical that restoration practitioners be included in research activities to facilitate adaptive management of current and future restoration sites.

3.6 Conclusions

In comparison to other secondary seed dispersers, *Lumbricus terrestris* may be among the first animals to encounter seeds in many temperate soils both because of its high populations in invaded systems and its proximity to freshly dispersed seeds. Depending on seed and earthworm species identities, earthworms can be important seed predators which consume or bury large amounts of surface-applied seeds within 48 hours. When seeding is used as the main source of introducing the target plant community, such as for establishing tallgrass prairie restorations, seed mix applications usually represent a one-time, large influx of seed on the surface of ex-arable land. Particularly at restoration sites with a high bare ground cover, earthworm seed predation may represent a significant and previously unquantified and unmitigated challenge to the establishment of the desired plant community.

My experiment demonstrates that the widespread invasive *L. terrestris* earthworm has strong and consistent preferences for invasive plant, undesirable weed, and target restoration seed species. In terms of seed destruction, *L. terrestris* likely digested over 50% of the target tallgrass prairie seeds ingested in this study. Potential protective effects of burial aside, this high level of ingestion and digestion of the seed mix species is cause for concern. While the preference of *L. terrestris* for ingesting seeds of invasive or undesirable forbs may benefit future management of tallgrass prairie restoration sites, disentangling the complex relationships between earthworm burial effects and seedbank formation for this and other species will require further investigation.

As organizations undertaking restoration are typically working with limited budgets and restrictive timelines, studies such as this one can alert restoration practitioners to potential issues with their restoration methods, help explain unanticipated outcomes, and inform future monitoring and planning activities. Knowing that seed predation varies based on the earthworm and plant species in question, future work targeting these relationships in the context of restoration activities will inform effective restoration practices. In tallgrass prairie and other earthworm-invaded systems, we can no longer afford to ignore earthworm-mediated impacts when it comes to ecosystem restoration.

Chapter 4

Aboveground and below: tallgrass prairie restoration using sod mats, seeding, and transplants as assessed by aboveground vegetation and microbial communities

4.1 Summary

Complex communities of plant-associated microbes are an untapped reservoir that can support tallgrass prairie restoration. Restoring beneficial biotic soil conditions remains a challenge in ecosystem restoration, and as species-rich grasslands contain unique microbial communities which drive ecosystem processes, successfully establishing these communities through restoration is a priority. This paper investigates the success of three methods of tallgrass prairie restoration in establishing a vegetation and soil bacterial community that converges with target remnant prairie sites five years post-restoration: sod mat transplants, seeding, and seeding plus individual plant transplants. The results show that sod mat transplants, while not clearly advantageous when assessed by traditional above-ground vegetation measures, are a promising method of creating a bacterial community similar to target high quality remnants. This experiment reinforces the idea that aboveground vegetation assessment alone can be misleading, and that a more accurate picture of restoration success includes the soil bacterial community. It also highlights further avenues of study for successful soil inoculation of restored ecosystems using whole-soil medium.

4.2 Introduction

Ecosystem restoration has become a policy tool, and the probability of success or failure will shape planning for land uses and development that affect biodiversity and the viability of rare and endangered species (Drayton and Primack 2012); this is particularly true for rare and fragmented ecosystems, such as tallgrass prairie. In the last 250 years, anthropogenic disturbances have reduced tallgrass prairies to a fragmented series of habitat parcels that represent less than 1% of the original area occupied (Szeicz and MacDonald 1991, Bakowsky and Riley 1994, Packard and Ross 1997). Prairie ecosystems sequester soil carbon, provide pollinator habitat, and prevent soil erosion. Restoration of tallgrass prairie has typically focused on seeding native plant species into retired agricultural fields (Kindscher and Tieszen 1998, Rowe 2010). If assessed by floristic quality, the outcomes of tallgrass prairie restoration have been disappointing (Kindscher and Tieszen 1998). The underwhelming outcomes may be partially explained by the failure to assess and restore the high diversity, large biomass, and unique assemblages of soil microbes (Barber et al. 2017; see also Barroetavena et al. 1998, Bailey et al. 2002, Fierer et al. 2007). Restoration ecology has traditionally focused on the plant community (Murphy 2018), and despite the importance of the soil microbiome (including fungi and bacteria) having been long established, the effects of restoration on the soil microbial community has remained largely unknown due to historic difficulties in cost- and time-effective methods of assessment.

Soil microorganisms exert an influence on the soil net carbon balance, respiration, nitrogen mineralization, and plant nutrient availability (Liski et al. 2003, Zhang et al. 2005), playing a key role in determining the productivity, diversity and composition of plant communities (van der Heijden et al. 2008, Kulmatiski et al. 2008). The soil microbiome may also inhibit exotic species while

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supporting native and rare species specialized to the target ecosystem (Rúa et al. 2016, House and Bever 2018, Bauer et al. 2018). Soil microorganisms are in turn affected by climate, soil physical and chemical properties, vegetation, and substrate quantity and quality (Gholz et al. 2000). Plant-microbe mutualisms, including those aiding plant adaptation to climate change, may be especially important targets for conservation and restoration in order to help maintain or re-establish diverse grassland plant communities (House and Bever 2018, Hawkins and Crawford 2018). Although an implicit or explicit goal of tallgrass prairie restoration projects and the measure of restoration success is to establish conditions similar to high quality remnant sites, restoration studies have rarely compared the soil microbial community post-restoration to high quality target sites.

Although decades of agriculture and urbanization have significantly altered prairie microbial communities (Fierer et al. 2013, Barber et al. 2017), the process of tallgrass prairie restoration has been shown to shift the soil quality, microbial community biomass, and microbial community composition in the direction of remnant prairie sites. However, with typical restoration methods (seeding) it may take many decades for the microbial community to converge with that found in remnant prairie throughout the soil profile (McKinley et al. 2005) and key elements of diversity may be lost, particularly if restorations are geographically isolated from high-quality remnant sites, as is typically the case. Local soil inoculum has also been shown to hold greater promise than current commercially available products for grassland restoration (Emam 2016), but the success of inoculum and the reasons for its success and failure are not well understood.

Prior to agricultural and urban intensification, tallgrass prairie was the dominant land cover in Windsor-Essex County of southern Ontario, Canada, and the region contains some of the largest tallgrass prairie remnants in Ontario (Bakowsky and Riley 1994). As part of a major infrastructure project in the city of Windsor (42˚17'N and 83˚00'W), Ontario, Canada, ecosystem restoration of

tallgrass prairie was initiated using three methods: typical seeding, seeding and individual plant transplants, and sod mat translocation. In contrast to seeding, sod mat transplants have the potential to retain the seed bank, plant community, and soil microbial community intact, although the few previous attempts at sod mat transplantation have met with mixed success (Weber 1982, Kearns 1986). Reasons to expect failure with this method include previous failures of the sod mat transplant method; the dearth of experimental evidence to support best practices, including the optimal soil depth and season for transplant; the significant disturbance required to uproot, transplant, and re-assemble a sod mat mosaic, including severing of plant roots (Weber 1982); water loss from sod mats; a mismatch of transplant and recipient soil type; and weather conditions on the operation day (Kearns 1986). Even if the plant and microbial community is successfully transplanted in the initial operational stage of restoration, whether these communities will be stable and self-sustaining into future years is unknown, particularly if the soil conditions in the host site vary considerably from the source site.

On a smaller scale, it has been suggested that the single-plant transplants collected from remnant prairie sites, used in conjunction with seeding, may act as soil microbial inoculation for the restoration site and thereby help to re-establish the soil microbiome of high quality remnant sites (B. Macdonell, pers. comm., 2015). Although recent work has suggested that application soil inocula as part of restoration is critical to establishing target plant communities and that it drives grassland plant community composition (Middleton and Bever 2012, Wubs et al. 2016), the volume of soil, optimal timing, and degree of edaphic similarity required for successful establishment is unknown. Different methods of restoration for microbial inoculation within one project have rarely been compared, and previous sod mat transplants in grassland ecosystems have typically not investigated the microbial community (Revel 1993). This construction project presented an opportunity to assess these different

restoration methods using both a traditional metric of restoration success, above-ground vegetation monitoring, and a more recent method: analysis of the soil microbiome.

This chapter investigates the success of three methods of tallgrass prairie restoration in establishing a vegetation and soil bacterial community that converges with target remnant prairie sites five years post-restoration. The specific research questions addressed in this work are:

1. Do different methods of tallgrass prairie restoration show differential outcomes and 'success' with respect to soil bacterial communities?

2. Is the sod mat transplant method an effective technique for tallgrass prairie restoration?

3. Do aboveground (vegetation) and below-ground (soil bacteria) metrics of tallgrass prairie indicate consistent interpretations of community restoration success?

4.3 Methods

4.3.1 Experimental design

Sampling sites were delineated based on mapping imagery provided by the construction project leads, and site reconnaissance was undertaken in 2015 to confirm site boundaries. Restoration sites had been established for five years at the time of sampling. Sampling plots were evenly distributed within the sampling site area and not within 10 m of site boundaries. Sampling was completed between July 27th and 29th 2016. For this study, I selected eight sites representing five actively restored areas of the construction project and three nearby remnant areas of tallgrass prairie (Table 4.1). All restoration sites selected are characterized as tallgrass prairie and are within a 4 km radius of one another.

To allow for comparisons which speak to the success of different methods of restoration in this study, two local reference tallgrass prairie sites were selected. These sites are considered high

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quality tallgrass prairie and so can be considered the "target state" for restoration outcomes. The high quality designation is based on ecosystem attributes which are considered to typify southern Ontario tallgrass prairie in this area including abundance of tallgrass prairie indicator species and presence of rare vascular plant species associated with tallgrass prairie habitat. High quality remnant sites included the Ojibway Prairie Provincial Nature Reserve (OPPNR, "remnant highQ A") and a prairie remnant managed by the Ontario Ministry of Natural Resources and Forestry (MNRF, "remnant highQ B"). Both are part of the Ojibway Prairie Complex which supports 116 prairie indicator plant species (Pratt 1989) and is considered a remnant tallgrass prairie habitat based on historic records (Bakowsky and Riley 1994, Faber-Langendoen and Maycock 1994); the OPPNR also provides habitat for over 30 nationally rare vascular plants specific to tallgrass prairies in Ontario (Crins 1997). The MNRF prairie remnant is classified as a Fresh-Moist Tallgrass Prairie, with portions of Gray Dogwood Thicket Swamp and Savannah (S. Snyder, pers. comm., 2016; Hay 2016). The site has been actively managed through prescribed burns, brush-cutting and herbicide control for *Cornus* L. and *Fraxinus* L. species. My sampling was limited to the Fresh-Moist Tallgrass Prairie. The OPPNR is a 100 ha reserve which consists mostly of remnant tallgrass prairie and oak savannah, although micro-landscape variations exist, including shrubby zones and wet fern dominated areas (Ojibway Nature Centre 2015). An 8 ha area of representative open grassland was selected for sampling at the OPPNR. The site has been managed through prescribed burning and the removal of select invasive species.

A low-quality remnant prairie site ("remnant lowQ") was also included for reference, with the quality designation applied by the author based on site reconnaissance. The low quality designation was applied due to the absence of many rare species which typify the tallgrass prairie ecosystem in this area, the heavy clay and gravel texture of the site soil, and the relative abundance of weedy and invasive species compared to native tallgrass prairie indicator species. Considered a

remnant tallgrass prairie site by the construction project assessment, this site has undergone extensive undesired species controls, including manual removal and/or herbicide control for invasive *Phragmites australis* (Cav.) Trin. ex Steud., *Alliaria petiolata* (M. Bieb.) Cavara & Grande, *Cirsium arvense* (L.) Scop., *Robinia pseudoacacia* L., *Ailanthus altissima* (Mill.) Swingle, in addition to prescribed burns (S. Snyder, pers. comm., 2016).

Five actively restored sites, representing three different restoration methods from the infrastructure project, were also included in my research. Two sites were restored in 2013 via no-till drill seeding with a 20 species prairie mix (Appendix 1; "seeded"). Another two sites were seeded and additionally amended via transplanting ("seeded+transplant") individual plants of two species at risk (*Liatris spicata* [L.] Willd. and *Symphyotrichum praealtum* [Poir.] G.L. Nesom clumps or corms) collected from remnant prairie which was within in the construction zone. All four of these areas were used as agricultural land until 2011, at which point invasive species were intensively managed through herbicide application and manual removal, and the sites were allowed to succeed naturally into a Dry-Fresh Old Field Meadow (S. Snyder, pers. comm., 2016). Subsets of these sites shared agricultural crop cover, and sites have been labelled to represent this group identity (groups C and D; Table 4.1). In addition to seeding and individual plant transplants, the restoration project also transplanted parcels ("sod mat") of intact remnant prairie (1 m x 1 m x 20-25 cm deep). These were placed on a cleared area of soil, adjoined against one another, and infilled with soil from the source site where necessary to form a continuous sod mat. Permission for sodmat sampling was limited to one sodmat site; the sod mat site shares an agricultural history with the group C seeded and seeded+transplant sites.

Table 4.1 Research sites by type, including site area and the number of sampling plots per site as well as the total site area and total sampling plots by site type.

Site type	Site area (ha)	Sampling plots (no.)	Total area of site type (ha)
sod mat C	0.11	4	0.11
seeded C	0.91	5	1.94
seeded D	1.03	5	
seeded+transplant C	1.13	5	2.19
seeded+transplant D	1.06	5	
remnant highQ B	0.86	5	8.82
remnant highQ A	7.96	10	
remnant lowQ	1.10	5	1.10

4.3.2 Sampling procedures

Percent cover of each plant species (following Voss and Reznicek 2012), litter and bare ground was assessed using a 1 x 1 m quadrat at each sampling plot. When field determination of plant species was not possible, physical and photo plant vouchers were collected and verified by regional experts. Soil samples were collected using a 3 cm diameter soil corer to a depth of 10 cm. Three subsamples were collected at each plot and homogenized for analysis. Samples were stored at -20˚C until processing.

4.3.3 Soil analysis

Soil chemical and physical characteristics were measured by the Agriculture and Food Laboratory at the University of Guelph, and at the University of Waterloo. Briefly, soil pH was measured using a 1:2 ratio of soil to H_2O (McKeague 1978); organic and inorganic carbon content was measured by ashing the sample prior to catalytic combustion and thermal conductivity detection; ammonium ($NH4^+$) and nitrate ($NO3^-$) were measured using the KCl^- extractable method (Hood-Nowotny et al. 2010) using the Seal AQ2 analyzer (USEPA 600/4-79-020:Method 350.1 and 600/R93/100 Method 353.2); gravimetric soil moisture was determined by oven drying soils at 105°C for 24 h (Gardner 1965); and soil texture (% gravel, sand, fine sand, coarse sand, silt, clay) was determined using the hydrometer method (Kroetsch and Wang 2008) with one aggregate sample per site.

4.3.4 Genomic DNA extraction, amplification and sequencing

DNA was extracted from randomized soil samples (0.35 g) using the PowerSoil DNA Isolation Kit (MO BIO Laboratories, CA, USA). After addition of the lysis solution, samples were incubated at 70ºC for 10 min, followed by bead beating for 45 sec at 5.5 m/sec using a FastPrep instrument (MP Biomedicals, OH, USA). The remainder of the extraction was carried out following the

manufacturer's instruction. The V4-V5 region of the 16S rRNA gene was amplified using universal prokaryotic primer 515F-Y and 926R (Quince et al. 2011, Parada et al. 2016). Each primer contained a unique six base index sequence for sample multiplexing as well as Illumina flow cell binding and sequencing sites (Bartram et al. 2011, Kennedy et al. 2014). Each PCR was prepared in triplicate and contained 1X ThermoPol Buffer buffer, 0.2 µM forward primer, 0.2 µM reverse primer, 200 µM dNTPs, 15 µg BSA, 0.625 U Taq DNA polymerase (New England Biolabs, MA, USA), 1 µl of template (1 to 20 ng) in a total volume of 25 µl. The PCR was performed as follows: 95°C for 3 min, 35 cycles of 95°C for 30 sec, 50°C for 30 sec, 68°C for 1 min, and a final extension of 68°C for 7 min. Indexed PCR amplicons were quantified in a 1% agarose gel containing GelRed (Biotium, CA, USA) and equal quantities of each amplicon were pooled. The pooled 16S rRNA amplicons were excised from an agarose gel and purified using Wizard SV Gel and PCR Clean-Up System (Promega, WI, USA). A 5 pM library containing 15% PhiX was sequenced on a MiSeq instrument (Illumina Inc., San Diego, USA) using a 2 x 250 cycle MiSeq Reagent Kit v2 (Illumina Canada Inc, NB, Canada).

4.3.5 Sequence data processing

Paired-end reads were assembled using the paired-end assembler for Illumina sequences (PANDAseq version 2.8, Masella et al. 2012) and a total of 2,282,648 assembled sequences were obtained for all samples combined. Assembled reads were analyzed using Quantitative Insights Into Microbial Ecology (QIIME, Caporaso et al. 2010b), managed by automated exploration of microbial diversity v. 1.5 (AXIOME, Lynch et al. 2013). Sequences were clustered with UPARSE (Edgar 2013) at 97% identity and aligned with the Python Nearest Alignment Space Termination tool (PyNAST version 1.2.2, (Caporaso et al. 2010a). Representative sequences were classified using the Ribosomal

Database Project (RDP version 2.2, Wang et al. 2007) with a stringent confidence threshold (0.8) and the Greengenes database (McDonald et al. 2012) was used to assign taxonomy. The output of this process is data assigned with OTUs, operational taxonomic units, which are the most commonly used units in microbial diversity research. OTUs are considered pragmatic proxies for microbial "species" at different taxonomic levels due to the current absence of traditional systems of biological classification for microbes (Edgar 2018).

4.3.6 Data analysis

Desired species were defined as native plants which are typical of southern Ontario tallgrass prairies (Bakowsky and Riley 1994, Quinlan 2005) and/or included in seed mixes used for tallgrass prairie restoration, such as the mix used to seed this restoration project (St. Williams Nursery, stwilliamsnursery.com; Appendix 1). Desired species included common tallgrass prairie species, species at risk, and species with a high coefficients of conservation. Coefficients of conservation and native versus non-native status were determined using the Universal Floristic Quality Assessment (FQA) Calculator (Freyman et al. 2015) with the southern Ontario region database (Oldham et al. 1995). Species at risk rankings (S1 to S5) of plants were assigned based on the Natural Heritage Information Centre database (https://www.ontario.ca/page/natural-heritage-information-centre). Undesired plant species included non-native species that behave invasively in tallgrass prairie, including non-native cool season grasses. Trees and shrubs which are typically targeted for removal from tallgrass prairie during management activities were also included (Briggs et al. 2002).

The effects of site on soil pH, carbon, ammonium, nitrate, and moisture as well as desired vegetation species richness and bacterial taxonomic richness were each assessed using one-way ANOVAs. Statistical assumptions were tested in Minitab (Minitab 2017) using Levene's Test for

equal variance assumption and a visual assessment of residual plots for residual normality. Post hoc methods included Tukey's HSD Test for One-Way ANOVAs and Games-Howell for Welch's Tests. Indicator species analysis used the package indicspecies (Cáceres and Legendre 2009) with multipatt, and principal component analysis (PCoA) used the ecodist (Goslee and Urban 2007), vegan (Oksanen et al. 2018), and ggplot2 (Wickham 2009) packages in R (R Core Team 2017). The Functional Annotation of Prokaryotic Taxa (FAPROTAX) database (Louca et al. 2016) and BLASTN 2.8.0+ (Zhang et al. 2000) were used to investigate for established metabolic or other ecologically relevant functions of sampled bacteria taxa.

4.4 Results

4.4.1 Bacterial community

The 16S rRNA gene sequencing showed that the microbial taxa in the samples is represented by 16,355 OTUs, of which 131 OTUs are at or above 0.5% abundance and 46 are at or above 1% abundance. The three most abundant phyla represented are Proteobacteria, Acidobacteria, and Verrucomicrobia; these phyla are present in all samples, and no single phylum or group of phyla are present in obviously higher of lower average abundances based on site identity (Figure 4.1). Taxonomic richness, measured by number of OTUs (bacteria "species"), varied by site (One-Way ANOVA, $F_{7,36}$ = 5.33, p = < 0.001, R^2 = 50.88 %), where the sod mat samples had the highest richness, followed by the two high quality remnant sites, and the low quality and seeded/seeded+transplant sites (Figure 4.2).

Metric multidimensional scaling was performed to group the samples by associating the site and the OTU frequencies. The PCoA plot based on the Bray-Curtis metric (Figure 4.3) showed sites

restored using seeded/seeded+transplant methods do not group based on restoration method but based the agricultural history group (C or D). However, the sod mat site also belongs to agricultural history group C but sod mat samples group closely with both high-quality remnant sites. Statistical analysis (Multi-response Permutation Procedures, vegan package, Bray-Curtis distances, $A = 0.3141$ p= 0.001) showed that the "sod mat + high quality" and "all other sites" (lowQ, seeded, seeded+transplant) are distinct and separable. The indicator species analysis showed that the highest number of OTUs (taxa) were associated with the "high quality remnant + sod mat" site grouping (Table 4.3). The majority of the bacteria sequenced have no known metabolic or other ecologically relevant functions based on FAPROTAX or BLASTN, and there were no ecologically meaningful patterns of indicator taxa across the samples.
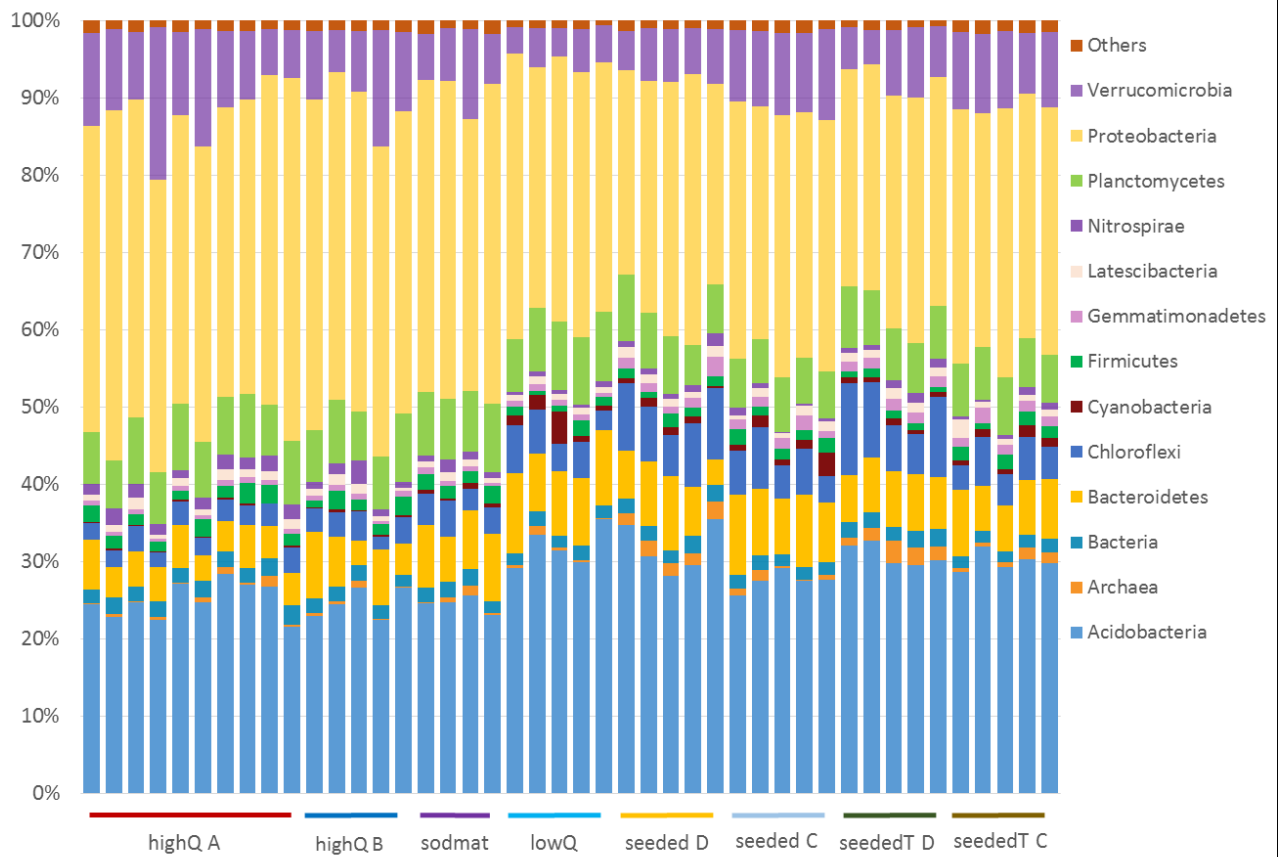
Figure 4.1 Taxonomic composition of bacterial communities in relation to site type. Relative abundances of bacterial phyla based on high-throughput 16S rRNA gene sequencing of 44 soil samples. Site identity along x axis. Others: relative abundances <1% in all samples.
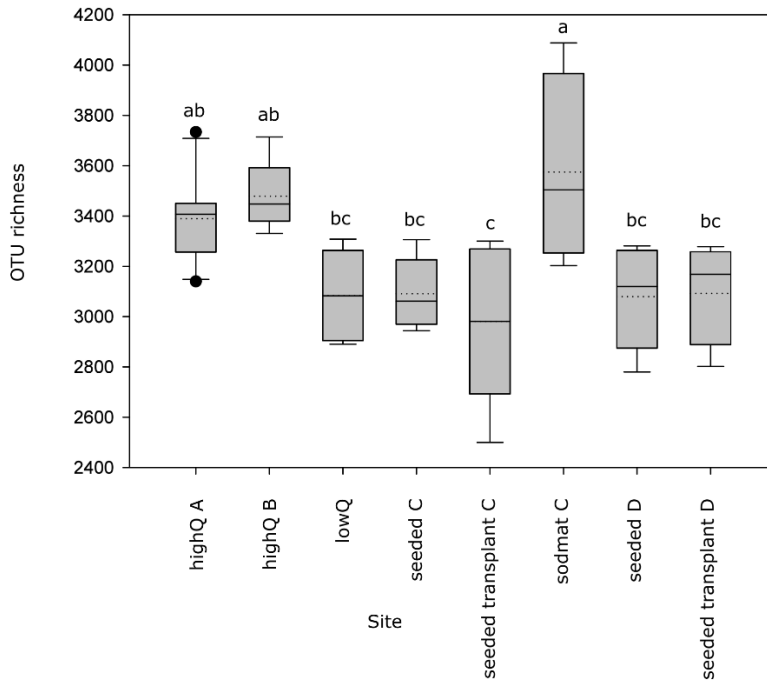
Figure 4.2 Number of operational taxonomic units (OTU) at each sampling site. Grey boxes represent the interquartile range; bottom and top whiskers extend to the 10th and 90th percentiles, respectively; black dots represent outliers. Dotted horizontal lines within boxes are mean values, solid horizontal lines are median values. Lowercase letters represent the groupings assigned by Tukey's HSD test.

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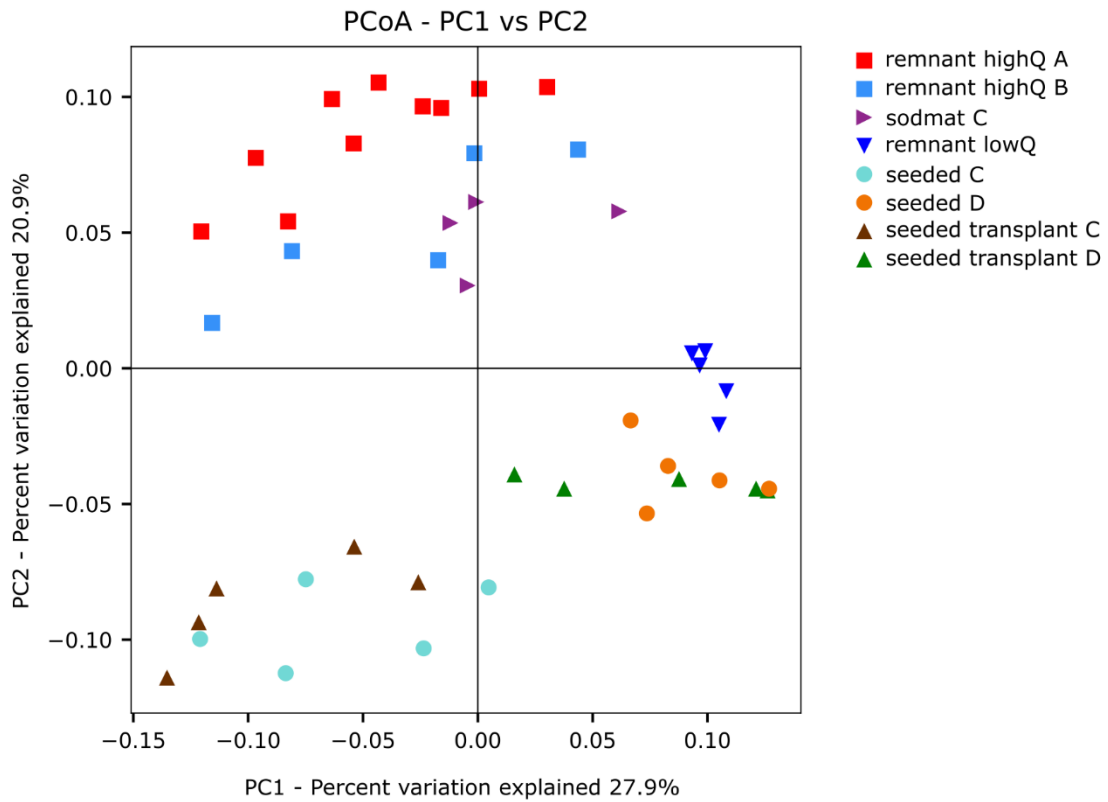
Figure 4.3 Principal coordinate analysis plot using Bray-Curtis dissimilarity of the soil microbial community illustrating the grouping of the sod mat samples with that of high quality remnant plots.

Table 4.3 The total number of bacteria "species" (OTU: Operational Taxonomic Units) associated with site types based on indicator species analysis. Dots indicate site groupings. Associated OTU groupings less than 50 are not shown.

# of indicator OTUs associated	highQ	sod mat	seeded	seededT	lowQ
352	•	•			
216	•	•			•
123	•	•	•	•	
243		•	•	•	•
226		•			•
61		•	•	•	
178			•	•	•
138			•	•	

4.4.2 Soil characteristics

Soil samples spanned a pH range of 4.89–7.59 across all sites. Sampling site identity had a statistically significant effect on soil pH (One-Way ANOVA, $F_{7,36} = 24.27$, p = < 0.001, $R^2 = 82.51$ %). Site identity had no significant effects on soil organic carbon, nitrate, ammonium, or soil moisture. Soil pH was highest in the low quality plots, lowest in the seeded and seeded+transplant plots (group C only), and intermediate in the remaining site types (Figure 4.4). Soil texture also varied strongly by sampling site; the low quality site classified as silt loam; the sod mat and both high quality sites as sandy loam; and all seeded and seeded+transplant sites as clay loam. Total soil carbon, inorganic carbon, and organic carbon were lowest in the seeded and seeded+transplant sites and highest in the remnant and sodmat sites (Table 4.4). Ammonium and nitrate both varied across the sites, and soil moisture was lowest in the low quality remnant site (Table 4.4).
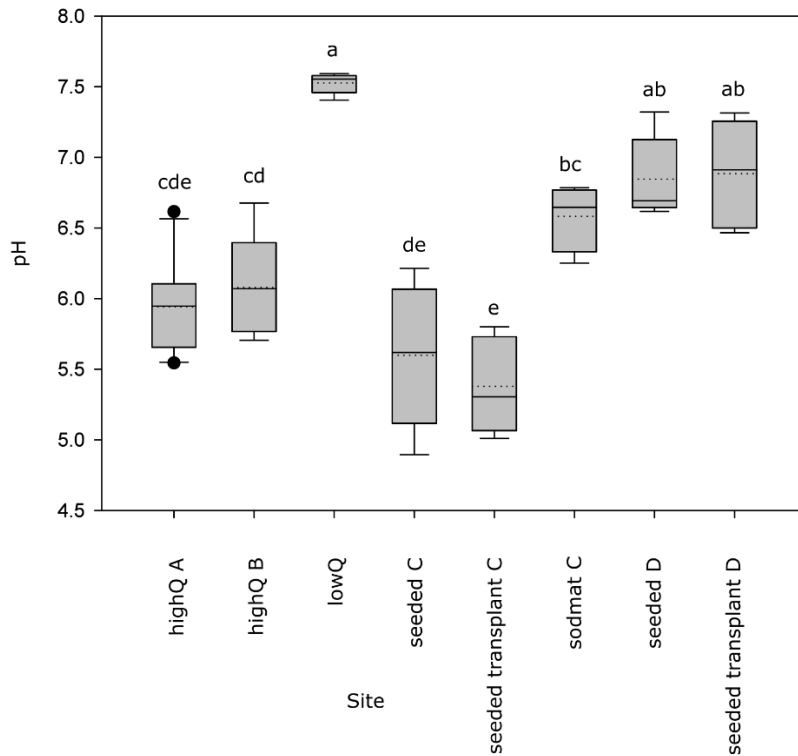
Figure 4.4 Soil pH by site, illustrating the distinctions between the low quality, high quality, and restored sites. Grey boxes represent the interquartile range; bottom and top whiskers extend to the 10th and 90th percentiles, respectively; black dots represent outliers. Dotted horizontal lines within boxes are mean values, solid horizontal lines are median values. Lowercase letters represent the groupings assigned by Tukey's HSD test.

Table 4.4 Mean soil characteristics by site. Total carbon (Total C), inorganic carbon (Inorganic C),

ammonium (NH4+), nitrate (NO3−), and gravimetric soil moisture (Moisture).

Site	Total C	Inorganic C	Organic C	NH4+	NO3-	Moisture
remnant HighQ A	3.73	0.23	3.51	20.48	1.79	14.08
remnant HighQ B	3.07	0.20	2.87	13.33	2.15	13.88
sodmat C	2.27	0.10	2.17	16.66	1.43	12.53
remnant low Q	3.77	1.88	1.89	9.43	3.49	7.64
seeded C	1.68	0.07	1.61	17.52	2.40	16.89
seeded D	1.70	0.09	1.60	10.43	0.97	12.54
seeded transplant C	1.89	0.07	1.82	20.62	1.20	17.04
seeded transplant D	1.84	0.10	1.75	10.88	1.39	17.24

4.4.3 Vegetation community

Site identity had a statistically significant effect on desired vegetation species richness (One-Way

ANOVA, $F_{7,36} = 4.72$, $p = 0.001$, $R^2 = 47.87$ %). Desired species richness was highest in the sod mat

plots, high quality A plots, and seeded (D) and seeded+transplant (C and D) plots; lowest in the low

quality plots; and intermediate in the remaining site types (Figure 4.5). Species at risk richness

showed a similar pattern (data not shown).

Pattern analysis of the dataset, which considered the above-ground vegetation community

using Bray-Curtis dissimilarity, shows no distinct groupings based on site types, although the high

quality sites are the most distinct (Figure 4.6). These results are in accordance with the statistical

analysis (Multi-response Permutation Procedures, vegan package, Bray-Curtis distances, $A = 0.07667$

$p = 0.001$) which showed a very weak separation between the five site types (sod mat, highQ, lowQ,

seeded, seeded+transplant).

Indicator species analysis of the vegetation community by site yielded 17 indicator species, of which nine were considered non-target species (neither undesired nor desired species; not included in Table 4.4), four were desired species (restored sites), and four were undesired species (low quality site; Table 4.5).
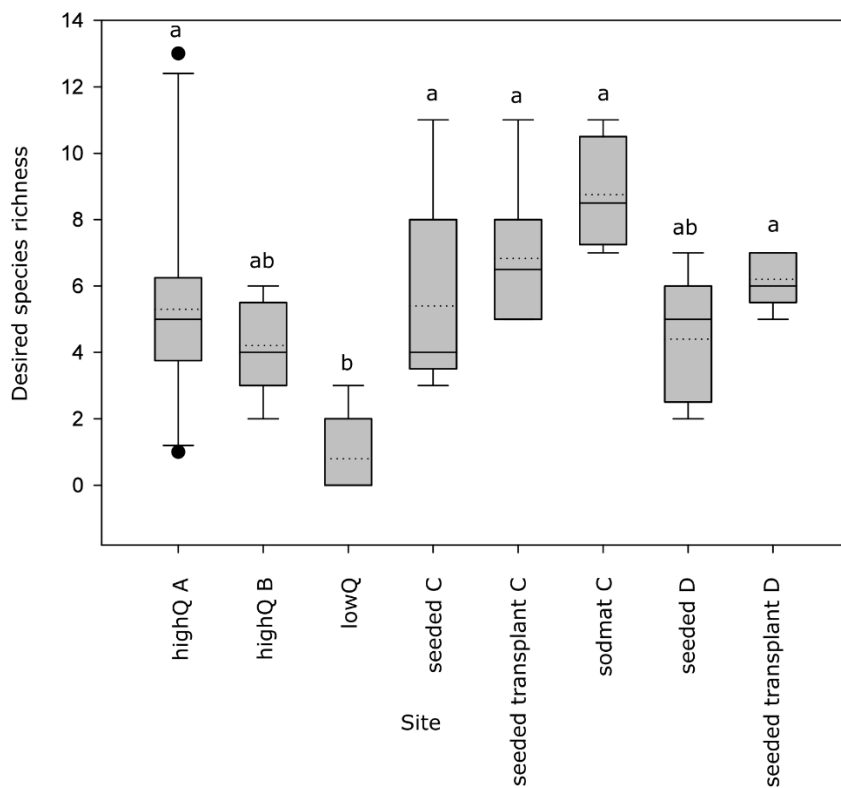
Figure 4.5 Vegetation species richness of desired plant species by site. Grey boxes represent the interquartile range; bottom and top whiskers extend to the 10th and 90th percentiles, respectively; black dots represent outliers. Dotted horizontal lines within boxes are mean values, solid horizontal lines are median values. Lowercase letters represent the groupings assigned by Tukey's HSD test.
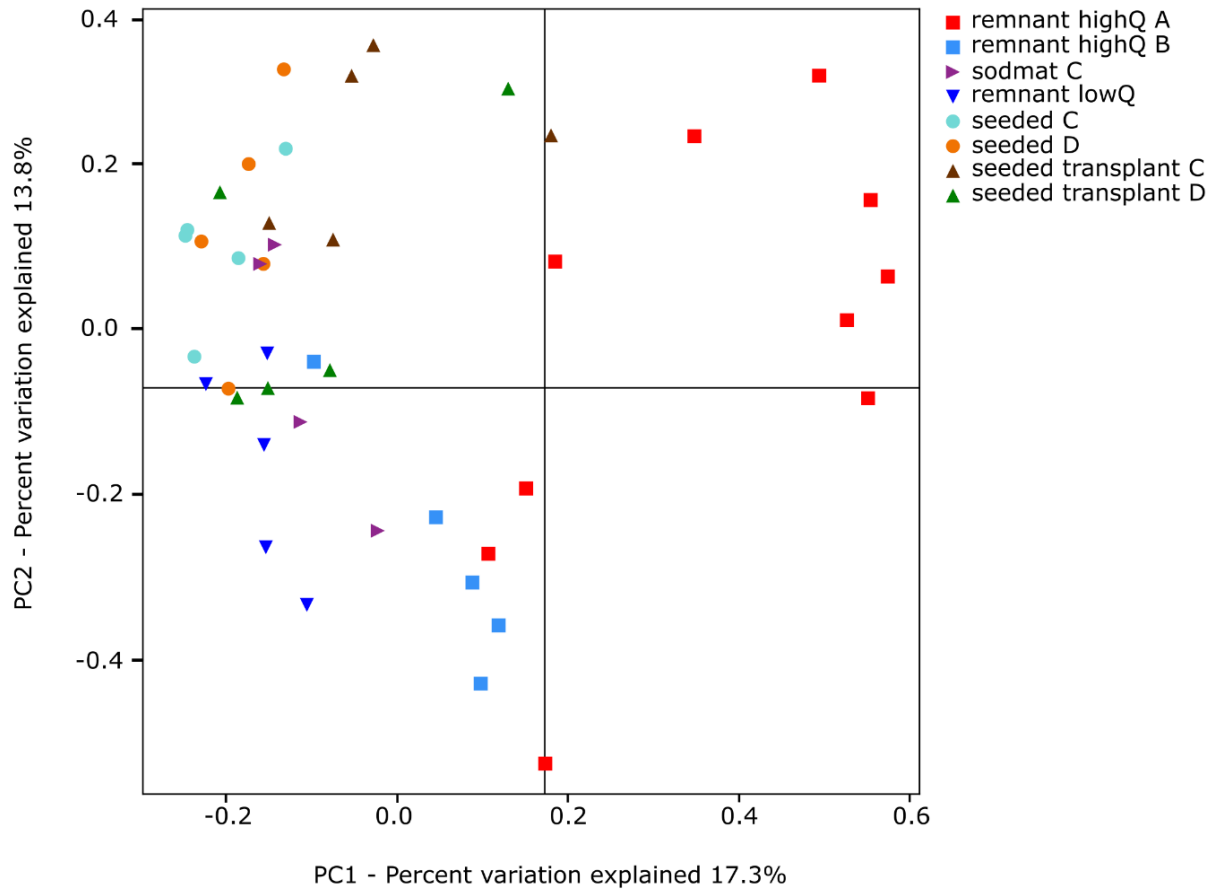
Figure 4.6 Principal Coordinate Analysis plot using Bray-Curtis dissimilarity distances of the above-ground vegetation community showing no strong distinctions between the restoration methods.

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Table 4.5 Indicator species analysis by site types showing undesired species as indicators of low quality sites. Obs. = observations; IV = calculated indicator value; %C = percent cover; species at risk ranking (S value) is shown where applicable.

Site	Indicator species	IV	p-value	Obs. in class	Total obs.	Mean %C in class	Status
remnant lowQ	*Lotus corniculatus* L.	0.89	0.005	4	4	11.0	Undesired (exotic)
	Cirsium vulgare	0.78	0.005	3	3	3.4	Undesired (exotic)
	Phragmites australis	0.78	0.01	3	3	1.1	Undesired (exotic)
	Medicago lupulina L.	0.63	0.01	2	2	2.5	Undesired (exotic)
seeded+transplant	*Panicum virgatum* L.	0.69	0.01	5	7	2.2	Desired (S4)
seeded+transplant, remnant highQ	*Andropogon gerardi* Vitman	0.73	0.045	14	19	22.9	Desired (S4)
sod mat, seeded	*Symphyotrichum novae-angliae* (L.) G.L. Nesom	0.65	0.045	7	8	0.8	Desired (S5)
sod mat, remnant highQ	*Pycnanthemum virginianum* (L.) B.L. Rob. & Fernald	0.81	0.025	17	22	4.1	Desired (S4)

4.5 Discussion

4.5.1 Soil bacteria communities vary by restoration method

In answer to the first question, only sod mat soils showed a similar soil bacterial community composition and taxonomic richness (OTU richness) to the target high quality remnant sites. The soil bacterial communities of the seeded and seeded+transplant sites do not reflect that of high quality remnant sites in the short-medium term (five years post-restoration), and there is no evidence to

suggest that the individual plant transplants sourced from high quality remnants and used in the seeded+transplant site restoration method have successfully inoculated the restoration sites with the source community in a widespread way. The lower OTU richness at the seeded and seeded+transplant sites is consistent with previous research which found that the soil microbial communities of disturbed grassland sites are less diverse than undisturbed sites (House and Bever 2018), and that cultivated soils previously covered by tallgrass prairie harbored bacterial communities that were distinct in composition from those found in the corresponding native prairie soils (Fierer et al. 2013).

While applying soil inocula can drive soil community composition towards that of the donor sites (Wubs et al. 2016), this response is likely dependent on the amount of donor soil added (Carbajo et al. 2011) and the transplants encountering favourable abiotic conditions at the restoration site (Kardol et al. 2009). In this experiment, both the amount or donor soil attached to the individual plant transplants and the similarity of the restoration site soil conditions to the source sites at the seeded+transplant sites were likely insufficient to successfully establish self-perpetuating soil microbial communities similar to the remnant prairie. While soil bacterial communities of restorations initiated by seeding (plant-focused restoration) can converge with local prairie remnants over time (Hay 2016, Barber et al. 2017, Allan 2017), evidence for this was not observed within the timeframe (five years post-restoration) captured by this study. Reasons for this could include a mismatch of abiotic soil conditions persisting at the restoration sites due to their agricultural legacies, and lack of adjacent high quality sites to act as a source of this microbial community.

Although current understanding of key microbial taxa and functional activities in both natural and restored ecosystems is limited, some previous research has suggested that the relative abundance of bacterial phyla Verrucomicrobia, Acidobacteria, Gemmatimonadetes, and Proteobacteria are important indicators of convergence with high quality remnant prairie soil communities (Fierer et al.

2013, Barber et al. 2017). However, this was not supported by the results of this study. Although Verrucomicrobia, Acidobacteria, and Proteobacteria were found in high relative abundances, they were found in each site sampled and there were no strong patterns of higher or lower abundances of these phyla in remnant versus restored sites. Similarly, bacteria in the phylum Gemmatimonadetes were found in low abundances in each sample and no pattern mapping to restoration method or site type was detected. With so few studies investigating the bacterial community of the tallgrass prairie ecosystem, this result likely reflects the dearth of comparative information and highlights the need for additional research sourced from different geographies, land use histories, plant assemblages, microclimates, etc.

4.5.2 Sod mat transplants as a promising method of tallgrass prairie restoration

Sod mat transplants do show promise as a tallgrass restoration technique within the five year timespan of this study. This success is despite the disturbance caused by the transplant operation, the mismatch of original source and target soil pH and texture, and the proportionately shallow root depth retained as compared to an established tallgrass prairie rhizosphere. The common principle of all translocation projects is the transfer of a varying portion of all inhabiting organisms at a site from the original site (the donor site) to the target area (the receiving or receptor site), and as measured by both desired plant species richness and soil bacterial community the sod mat method has been successful.

The seeded and seeded+transplant samples share a high number of associated OTUs with sod mat samples, likely due to the shared agricultural history of the destination fields in which the restorations were established. Despite the shared site histories, however, after five years following restoration only the sod mat site has soil characteristics (pH and texture) and a bacterial community similar to the high quality remnant sites. This surprising success may be related to the depth of the

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sod mats transplanted; whereas previous studies which observed species die-off within a few years were harvested at depths of 5-10 cm (Revel 1993), the sod mats in this study were harvested at 20-25 cm deep, maintaining more of the soil profile, root biomass, and a greater bulk of soil. The resemblance of the sod mat bacterial community to the high quality remnants was likely influenced by the maintenance of the abiotic soil conditions. Observations of higher mean pH in sod mat plots (6.58) than in the seeded (5.60) or seeded transplant plots (5.38) in the same area and sharing the same agricultural history (group C) are suggestive of the sod mats having retained pH more similar to the source location than to the recipient. Observations of a distinct soil texture in the sod mat plots (sandy loam) compared to the seeded and seeded transplant plots (clay loam) further support this supposition. As soil pH is a main factor controlling the bacterial community structure in some ecosystems (Fierer and Jackson 2006, Lauber et al. 2009, Rousk et al. 2010, Mandakovic et al. 2018) and soil physical and chemical properties are principal factors affecting the soil microbes (Gholz et al. 2000), the potential of sod mats to create and maintain 'islands' of suitable habitat for soil microbial communities by retaining abiotic conditions more closely resembling high quality remnant sites than the restoration sites is encouraging.

Although there have been few documented incidences of it being attempted and few long-term monitoring studies, some previous sod mat transplants have been successful in restoring/preserving the vegetation community of other North American grasslands (Revel 1993), and have shown promise in retaining viable seed banks of rare species (Park 1989). An absence of late successional species from the seed bank and poor dispersal and colonization possibilities due to habitat fragmentation can clearly impede restoration of target plant communities (Lindborg and Eriksson 2004, Ozinga et al. 2009); both of these problems are addressed at least in part with sod mat transplants in lieu of seeding-only restoration. In further support of sod mat transplants as a method of

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retaining populations of desired plant species, my research showed successful retention of desired species at five years following restoration, which is longer than many previous studies where steep drop-offs were observed after only 1-4 years (Allen 1994, Fahselt 2007).

Over the long-term, there is concern that unfavourable soil conditions at the restoration site may preclude successful establishment of soil biota outside of the sod mats (Kardol et al. 2009), thereby limiting their ability to act as inoculation areas for restored prairie. There is also concern that both above-ground and below-ground biodiversity within the sod mats may be lost over time (Revel 1993), especially given the small scale of the transplant area compared to remnant habitat. As sod mat transplants typically provide a means for preserving native sod that would otherwise be destroyed, however, the similarity of the soil microbial community to the target remnant habitat observed in this study suggests that some of this biodiversity has been preserved. This is particularly significant since while we suspect climactic warming may influence the soil microbial community structure and processes (e.g. respiration and nitrogen mineralization) (Ruess et al. 1999, Jonasson et al. 1999, Zhang et al. 2005), little is known as to the intensity or directionality of these effects. As we also don't know the ecological significance of a vast array of the soil microbial community, the precautionary approach is to aim to preserve microbial biodiversity during ecosystem restoration insomuch as possible.

Soil microbial communities also contain plant pathogens, and plant-specific pathogen loads are maximized under high plant densities, especially under dense monocultures, eventually incurring negative feedback on abundant plants (Bever 1994, Olff et al. 2000, Klironomos 2002). Because seeding-led prairie restorations often include only a small subset of the plant species found in high quality remnant prairie, there could be long-term issues with pathogen build-up if restoration sites become monocultures of a few successfully propagated species. Soil inoculation may therefore be

most beneficial in diverse systems where microbial diversity can influence plant community and ecosystem processes in a way that promotes convergence with target states and supports plant species with high coefficient of conservation values which are responsive to microbial mutualisms (Lau and Lennon 2011, Stover et al. 2012, Bauer et al. 2018). Local adaptation of soil microbes may also be important for describing the effect of microbial inoculation, as microbes adapt to the soil environment in such a way as to be less mutualistic to novel (exotic) host plants (Rúa et al. 2016); soil microbial communities may in this way inhibit exotic species while supporting native and rare species specialized to the ecosystem (House and Bever 2018, Bauer et al. 2018). Missing microbiome elements may be the driving force behind the lack of convergence observed between restorations initiated with a seeding- or individual plant-transplant-led approach (Fahselt 2007).

Although not perfectly replicating the high quality remnant community, the sod mat transplants in this study support bacterial communities significantly more similar than individual-plant transplants or seeded restoration sites five years after restoration. As the soil community, including invertebrates, fungi, archea, and bacteria, may enhance local plant species diversity and support significant shifts in the plant communities towards the dominance of the plant species from the remnant community (Deyn et al. 2003, Middleton and Bever 2012), whole soil inoculation offers a promising avenue for retaining and propagating soil biodiversity.

4.5.3 Sod mat transplants can work despite previous concerns

Several issues have been raised concerning sod mat transplants as restoration tools, which I will address here. Intact high quality remnant communities, although few and not necessarily representative, are crucial as benchmarks against which restoration can be measured. These habitats serve as genetic reservoirs, house rare species, and offer examples of the vegetation communities best

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suited for survival in particular locations and microclimates. As human land use expands, there has been considerable interest in transplanting these natural communities to permit for development of the original location (Fahselt 2007). Many concerns have been voiced around this option, including the disturbance associated with the process of transplantation, the fear that transplanting will pave the way for habitat destruction of high quality sites (Fahselt 2007), the compatibility of source and target sites (Allen 1994), and the poorly understood requirements of the species involved, which often includes rare species and taxa such as soil microbes about which very little ecologically-linked information is available (Fahselt 2007). No potential receptor locations are identical to the original site; they differ in topography, hydrology, exposure, soil, etc. (Allen 1994, Harris and Palmer 1996), and sod mat translocation in grassland ecosystems has met with mixed success both in the short and long-term (Worthington and Helliwell 1987, Park 1989, Good et al. 1999, Bruelheide and Flintrop 2000) with plant species diversity generally diverging to varying degrees from source sites within 3-6 years.

To adequately monitor the long-term success of sod mat transplants, the criteria of success would need to include creating a self-sustaining community that retains the pre-disturbance diversity, composition, and function including productivity, nutrient recycling, plant-animal mutualisms, pollination, allelopathic interactions, and food chain relationships. The success of this method would be best measured against the source community over multiple decades following restoration. Unfortunately, with infrastructure projects including the site used for this research, the source community is often eliminated and so long-term comparative monitoring must rely on adjacent natural communities. Habitat translocations are also typically the most ambitious and expensive option in restoration or construction projects, and are thus usually attempted only rarely to preserve components of ecosystems which otherwise would have been destroyed by human engineering

projects, such as the construction of roads, quarries and buildings (Good et al. 1999, Bruelheide and Flintrop 2000). Indeed, many transplant projects are performed under time pressure and do not allow for proper assessment or preservation of the initial state (Bruelheide and Flintrop 2000).

As the functional importance of inconspicuous species, such as soil microbes and other fauna, becomes more obvious (Fahselt 2007), we are fortunate to increasingly have the tools to address these unknowns of restoration head on. I argue that it is precisely because of these unknowns that we should be including sod mat transplants when possible in restoration, for example when infrastructure projects will be approved due to their human benefit outweighing the habitat quality concerns. As shown in this and other work, sod mat transplantation does not recreate a perfect remnant habitat equivalent, and so transplants in general are best viewed as less effective than conservation, but with potential to improve restoration. It is within the power of permitting agencies to require developers to incorporate sod mat transplants into their remediation and site preparation alongside existing seeding and individual plant transplants. On the subject of policy, as we may have to wait decades to determine the success of the restoration, it behooves policy makers to include the financial costs of long-term multi-taxa monitoring into the permits and licenses granted to developers, and developers to account for these costs into their long-term budgets for infrastructure projects.

4.5.4 Above-ground and below-ground measures tell a different story

For ecosystem restoration, above-ground vegetation measures may not be sufficient to determine if a restoration has been successful or whether a particular restoration method is the most appropriate. As the results of this study demonstrate, investigating aboveground vegetation alone will not necessarily indicate if all components of the ecosystem have been restored, and the story that a restoration and method tells can depend on the taxa included in the analysis and monitoring.

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Transplants of at-risk plant species are usually done to save them from condemned sites, enrich gene pools, increase the size of declining populations, or to establish new populations. With respect to above-ground vegetation, sod mat transplants, seeding, and seeding+transplant methods were successful in preserving rare and desired species in this study; while the sod mat method had the highest mean desired species richness (assigned based on coefficients of conservation based on FQA), it was not demonstrably more successful than the other restoration methods at establishing desired or at-risk plant species. Because a comprehensive site herbicide pre-treatment is not viable for sod mats as it is for the other restoration methods, the sod mat method likely transplanted both desired and undesired plant species in this study. The apparent equivalency of sod mats with seeding and seeding+transplant methods with respect to desired species establishment coupled with the higher cost and the inability to comprehensively pre-treat for undesired species using the sod mat restoration method could lead developers and policy makers to eschew sod mat transplants in favour of less costly and time-intensive methods if aboveground vegetation cover is the only metric of restoration success considered. Assigning this valuation would be an error, however. Although the sod mat method appears approximately equivalent to seeding and seeding+transplant methods based on above-ground vegetation metrics (Figures 4.5, 4.6) including several species at risk indicator species (Table 4.5), investigating restoration success through the lens of bacterial community composition leads to a very different conclusion; i.e., that sod mat transplants were the most successful method of sustaining a soil bacteria community composition similar to that of high quality remnants and thereby potentially conserving the biodiversity of the tallgrass prairie soil microbiome (Figure 4.3). If the goal of restoration is to establish a complete, self-sustaining ecosystem, above-ground vegetation metrics such as desired species richness (including target and rare species) should not be used in isolation; although sod mats were equivalent to the other restoration methods in above-ground vegetation

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measures, they were significantly more effective in establishing target microbial communities. Anecdotally, the sod mat site also had a low percent cover of bare ground, in contrast to the seeded and seeded+transplant sites. Individual plants were larger and appeared more mature and established, including herbaceous perennials such as *Pycnanthemum virginianum* and *Symphyotrichum novae-angliae* as well as woody perennials such as *Rosa setigera* (Michx.). The sod mat site was also the only place where *Asclepias sullivantii* (Engelm. ex Gray; Ontario rank S2/S3) was observed. Although not captured by the study design, these supplementary observations suggest that a high proportion of the plant community from the sod mat source site survived the transplant process. As the rhizosphere of the sod mat provides habitat and plant hosts for the target soil bacteria community, the successful retention of this living root mass may help to explain why the microbial diversity of sod mat sites is more similar to target remnant prairie.

Although transplanting individual plant species at risk has been increasingly both recommended and successful in establishing long-term plant communities of those target species in other, recent experiments (e.g. Clements 2013), in this study it was not successful in inoculating the soil bacterial community in a widespread way within five years post-restoration. The lack of successful inoculation suggests that while individual plant transplants may have the potential to carry target soil bacteria with them and inoculate the target site, additional research beyond what works well for the plants will need to be conducted so as to leverage this opportunity for multi-taxa transplant and ensure the viability of not only the plant species but the accompanying transplanted soil microbial community. For example, successful inoculation may require that an associated plant community become established prior to site inoculation.

4.6 Conclusions and Recommendations

The results of this study highlight the limitations of assessing the ecosystem based solely on above-ground vegetation. Success or failure of a restoration is usually determined by studies examining the successful establishment of target above-ground vegetation communities. This is not so much 'ecosystem restoration' as it is vegetation restoration, since greenhouse-grown plugs or seed mixes may not re-establish the soil microbial community, and even when convergence occurs it is over the course of decades and with biodiversity loss. This experiment reinforces the idea that aboveground vegetation assessment alone can be misleading – looking at only the vegetation community, the seeding and seeding+transplant method appear to perform just as well as the sod mat transplants as a method of tallgrass restoration, and at considerably less cost and effort. In the new era of decreasing financial cost for microbial analyses and in light of our developing understanding of the critical role that the soil microbiome plays in successful community functioning, a key recommendation from this study is that for gauging ecosystem restoration success, below-ground metrics should be included in restoration assessment.

Restoring beneficial biotic soil conditions remains a challenge in ecosystem restoration, and as species-rich grasslands contain unique microbial communities (French et al. 2017), successfully establishing these communities through restoration is a priority. The success of the intact sod mat translocation in retaining a similar soil bacterial community to the target high quality remnant prairie opens exciting new avenues for research. Although the individual plant transplants were not successful in inoculating the seeded prairie at either study site in a widespread way within the timeframe of this study, the sod mats demonstrate that the potential exists to establish soil microbiomes similar to target remnant prairie at restoration sites in a shorter timespan than by passive

recolonization (Barber et al. 2017). Of course, monitoring this experiment over an extended period of time would be necessary to evaluate the long-term success of the transplants (Drayton and Primack 2012), but the similarity of the sod mat soils and high quality remnants compared to the other methods employed for restoration is encouraging. Like other authors highlighting the potential of whole-soil inoculation, I recognize that soil from high quality remnant sites is a precious and limited resource and it is not my intention to advocate for degrading the few remaining sites which exist, especially for uncertain long-term gains. Developing ways to maximize the effectiveness of soil inoculations will be key to leveraging its potential for restoration success; future avenues for investigation include investigating the factors which could preserve microbial community, and I suggest soil texture and pH as good places to start based on this research. Although it was not successful at the scale of individual plant transplants in this study, the creation of hot spots as suggested by Carbajo et al. (2011) is an interesting experimental approach. The long-term investigation of the soil surrounding sod mat transplants would be an excellent way to utilize existing research sites, particularly the experimental modification of surrounding soils to match specific abiotic conditions conducive to microbial establishment.

Chapter 5

Translational ecology in the field of restoration

5.1 Problem context

"Today's environmental scientists have a powerful array of tools and techniques to measure and monitor the environment and to interpret vast and diverse data. Yet despite producing an enormous amount of new information, ecologists are often unable to convey knowledge effectively to the public and to policy-makers. Unless the discoveries of ecological science are rapidly translated into meaningful actions, they will remain quietly archived while the biosphere degrades"

(Schlesinger 2010 p. 609).

"People matter as much as data" (Murphy 2001)

Ecological research has a vital role to play in conserving and restoring the biosphere. Through careful study, measurement, and theory, scientists gain the information necessary to understand and mitigate or reverse ecosystem degradation. With new measurement tools available and a cadre of scientists dedicated to their research, one hopes that many of these environmental challenges would have solutions, that those solutions would be implemented, and that we would be living in an environmentally sustainable socioeconomic system. In many cases, however, we seem to be losing ground to developers, losing political will for 'environmental issues', and losing legal battles for robust ecological protections and mitigation. In the face of the sheer scale, growth, and complexity of problems that the environmental sciences face, producing good science has not been

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enough (Balmford and Cowling 2006). Ecosystems are losing ground not for a lack of focused and committed researchers but because ecosystem management is not just about biology, ecology, geography, earth sciences, etc., but about people and the choices they make (Balmford and Cowling 2006, Swart et al. 2018). The traditional separation of ecology and the social sciences and humanities has sometimes resulted in a disconnect between natural science and the application of its findings (Enquist et al. 2017). As Higgs (2005, p. 162) states, "the mere presence of scientific information does not provide a sufficient basis for appropriate action. Negotiating an appropriate outcome depends on defensible policy, cultural values, political process, economic practicality, and a host of things that natural and physical scientific knowledge contributes to but does not determine". Given the scale of current environmental challenges (e.g. climate change, biodiversity loss, habitat degradation) and the linked social justice issues related to them (e.g. air pollution, drought, soil salinization), society cannot afford business-as-usual science that values the discovery of new knowledge without clear pathways to translate this knowledge to policy and practice (Chapin 2017).

Although the push for useable natural science is not new, academic researchers in the last two decades are under increasing pressure to produce societally relevant knowledge, to demonstrate the 'value' and impact of their work, and to engage with non-academic audiences in meaningful ways (Arlettaz et al. 2010, Rau et al. 2018). Engagement and demonstrating research impact, it is argued, should not begin and end at making of a list of stakeholders (e.g. as a tick box for a grant application or report); these stakeholders should help shape the research in some way (Rau et al. 2018). The integration of stakeholder and end-user perspectives is especially appropriate since most environmental research, whether publically or privately funded, is intended to support, advance, or achieve a goal which requires policy and human systems to implement (Sarewitz and Pielke 2007). For research to cross the knowledge-practice boundary it must be salient (relevant to decision-making

bodies and provided when it is needed), credible (authoritative, believable, and trusted) and legitimate (developed via a process that considers the values and perspectives of all actors) to scientists, practitioners, and decision-makers (Cook et al. 2013; Cash et al. 2003).

Information that is generated and disseminated without the meaningful involvement of the intended users generally fails meet these criteria and thus fails to be incorporated into policy or action (Dilling 2007), and we must develop additional ways to better connect supply and demand and create "usable science" (Dilling 2007; Sarewitz and Pielke 2007; Evans 2019). This is especially true for the field of restoration ecology, where successful application is often a target outcome of research. Connection in research means an end to working in isolation; this can involve not only close involvement with diverse stakeholders from the outset of a project, but integration with other researchers and research areas to incorporate the economic and social consequences of environmental policies and the institutional landscape for implementation (Balmford and Cowling 2006). Although generating awareness (e.g. through public talks) has been a key strategy in previous public engagement by researchers, the next step, generating actions, has proven more difficult both in practice and in conception (van Kerkhoff and Lebel 2006). This "knowing-doing gap" (Pfeffer and Sutton 1999) is not unique to any one field of science (Pfeffer and Sutton 1999, Higgs 2005, McNie 2007, Knight et al. 2008), but addressing it is particularly important if we wish restoration science to have a tangible impact. Although tremendous strides have been made in recent decades to prioritize stakeholder engagement and actionable science, there is still a perceived disconnect on the part of some practitioners, communities, government agencies, and other stakeholders (Schlesinger 2010, Jackson et al. 2017, Lawson et al. 2017, Conservation Halton employee pers. comm. 2016, Ontario Parks employee pers. comm. 2016, Nature Conservancy Canada employee 2016, Evans 2019). The goal of this chapter is therefore to highlight the potential causes of this disconnect and explore the

emerging field of translational ecology as one approach to bridge science and practice. The intended audience for this paper are scientists who are looking for a new framework in which to situate and grow their relationships and connections to practitioner and stakeholder communities.

5.2 Causes of the research-practice disconnect

5.2.1 Outdated models of knowledge transfer and decision-making

5.2.1.1 Outdated models of knowledge transfer

Traditionally, the pursuit of knowledge—rather than knowledge applicability—has been the core driver of knowledge production in science research (Dunn et al. 2018), and the application and dissemination of science has tended to follow a "knowledge deficit" model. In this model, end-users of research are either seen as 'adopters' or 'rejectors' of new information, and the main barriers to improved policy and practice are the ignorance of practitioners, fuelled by poor access to high-quality research results (van Kerkhoff and Lebel 2006). In this model, end-users are seen as rational decision makers who will adopt and integrate new information generated by science (van Kerkhoff and Lebel 2006, Dilling 2007, Simis et al. 2016), and it is the end-users' responsibility to uptake and integrate new information (van Kerkhoff and Lebel 2006, Dilling 2007). In this "trickle down" approach, researchers publish for academic peers only, expecting relevant knowledge to trickle down eventually by unspecified means to decision makers without additional effort required by the research community (van Kerkhoff and Lebel 2006, Dilling 2007, Enquist et al. 2017, Dunn et al. 2018). This model of knowledge production assumes that the information produced will be useful to or needed by society, and that the form of knowledge produced (i.e. peer-reviewed journal papers) is accessible and will be used in the appropriate manner (Dilling 2007). Unfortunately, this information often fails to

trickle down in a timely or efficient way; many practitioners do not consult scientific journals due to a lack of time, access, and locally-relevant information (Cook et al. 2013). Research is also not the only source of information used for policy making, but is one of many inputs (e.g. public opinion polls) (Gluckman 2016).

Some of the previous attempts to address the failure of the knowledge deficit model to influence policy have remained mostly rooted in one-way knowledge transfer, focusing on making the research results easier to access and understand (van Kerkhoff and Lebel 2006). The appeal of this approach is that it doesn't require any fundamental change on the part of the research community—it doesn't conflict with traditional academic incentives for publication, it may offload responsivity for "science communication" to those not directly involved in knowledge production, and it doesn't challenge the business-as-usual view that technical solutions will eventually provide answers to environmental challenges (van Kerkhoff and Lebel 2006, Poliakoff and Webb 2007). This highlights an important question of responsibility for environmental change: under many conventional models, researchers are not responsible for the uses to which their research is put (van Kerkhoff and Lebel 2006) and power structures are maintained.

This is not to say that curiosity-driven research, or "science for science's sake" does not have a place in the research community, that every scientist needs to be doing applied research, that knowledge produced today will not have future uses, or that there is a single, fixed idea of what is "useful" and "useable" (Rau et al. 2018). Instead, addressing problems with previous models acknowledges that past ways of doing research have likely limited the successful implementation of science into practice, and that failure to translate good science into successful restoration outcomes is a challenge that we must continue to find ways to overcome moving forward.

5.2.1.2 Outdated decision-making

The ways in which some restoration decisions have been made has also contributed to the science-practice disconnect. Fundamentally, setting priorities for restoration is about weighing the alternatives and choosing among trade-offs. Regardless of how decisions are made and who is given the power to make them, there will usually be some disagreement about the desired outcomes of a restoration action, as well as whose interests it should serve and the goods and services it should ultimately provide (Wyant et al. 1995). Assessing these interests and the values and motivations that drive them is time consuming, complex, and uncertain (Cipollini et al. 2005, Clewell and Aronson 2006, Williams and Brown 2014), and conflict may result in subpar or unsuccessful restoration and management (White et al. 2009).

These risks have sometime been avoided entirely by employing a top-down decision-making approach which skirts pluralism of values in favour of an authoritative model. This model is associated with what Clewell and Aronson (2006) call a "technocratic" approach. In the technocratic approach, decisions are made by small groups of experts and the public and practitioners are often excluded from restoration planning or implementation for reasons of liability, quality control, timeliness, and budget (Clewell and Aronson 2006). Although this type of decision-making is considered necessary for the establishment of large projects such as national parks or preserves (Rosenzweig 2003), there are several potential problems with this approach including division and lack of public investment.

Division refers both to the division of opinions between researchers and planners and on-the-ground practitioners as well as between the researchers themselves. Division can occur when researchers have different priorities and experiences than the practitioners implementing the project,

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where practitioners see practical problems with the implementation of plan components, if practitioners feel that they should have been included earlier in the process, and, chiefly, when inter-group and intra-group communication has been sparse and the "why" of a restoration has not been discussed or agreed upon (Poff et al. 2003, Cipollini et al. 2005, Clewell and Aronson 2006). Division can lead to inter- and intra-agency conflict and a breakdown in trust and working relationships as well as project delays (for a discussion of how to reconcile expert division, see Cipollini et al. 2005).

A related issue which affects top-down decision-making by government agencies in particular is jurisdiction. Due to the nature of many large environmental agencies, the time and resources of any one jurisdiction is limited, and job descriptions are tightly-woven union contracts which preclude the involvement of outside personnel. While projects may be tendered to one particular organizational unit, jurisdictions of government or agencies may not be capable of addressing the full range of interconnected socioeconomic and environmental issues of a project due to their own mandates and fragmented responsibilities (Kozak and Piazza 2014). This can limit the scope of projects and preclude the possibility that the most qualified personnel are brought on (anonymous Ontario Parks employee, pers. comm., 2016). With relatively flexible mandates and opportunities for collaboration, academic researchers and ecological research programs can sometimes ameliorate problems of jurisdiction in agencies by acting as an outsider bringing 'free' labour and expertise to a project and asking relatively little from the agency in return (e.g. site access). As will be discussed in a later section, however, this form of collaboration can be more or less useful to the agencies involved with it, and if poorly managed the results of the research may never be used.

Lack of investment in project outcomes is another issue which can plague top-down decision-making processes. Simply put, if practitioners are excluded from the opportunity to set the priorities for restoration projects, they tend not to feel invested in the outcomes of said projects and their

potential benefits (Clewell and Aronson 2006). Top-down approaches to complex environmental

challenges are not only potentially counterproductive, they also risk wasting local competencies and

placed-based knowledge (Kaplan 2002). There may be significant local variants in how best to

achieve a particular goal, and if stakeholders are consulted only superficially they may not support the

outcome and view their involvement as a waste of time (Kaplan 2002, Kozak and Piazza 2014).

Issues of investment will be further explored in the following sections.

5.2.2 Disconnect between practitioners and researchers

In addition to traditional models of knowledge production and decision-making, there are

several other key reasons for the disconnect that can occur between practitioners and researchers:

culture, relevance, and accessibility. With respect to organizational culture, there can be a "great

divide" between practitioners and environmental researchers based on disparities in organizational

culture and values (Finch and Patton-Mallory 1993). This is also known as the "implementation gap"

and is the distance between restoration scientists and 'practitioners', who generally do the on-the-

ground work of conservation (purchase land, do prescribed burns, lobby politicians, pull invasive

weeds) (Anonymous 2007).

Despite the scope and quality of information available in peer-reviewed journals, the

activities of conservation and restoration organizations rarely appear to be informed by published

research; instead, many organizations and their personnel develop their own (often unpublished)

assessment and implementation techniques (Prendergast, Quinn, and Lawton 1999; Hopkinson,

Evans, and Gregory 2000; Knight et al. 2008) based on personal experience and intuition

(Anonymous 2007). In contrast to the one-way knowledge transfer described in the above section,

this represents a zero-way transfer – practitioners seldom read academic journal articles, and

practitioners, in turn, rarely document their actions or collate their data in forms accessible to researchers (Anonymous 2007).

A lack of relevance may also create a disconnect between restoration science and action. To achieve maximum relevancy and effectiveness, policy-makers often prefer research with rapid turnaround and quick delivery of results; this is often in conflict with the multi-year research cycles associated with academia (Cook et al. 2013, Rau et al. 2018). Due to this time lag, even targeted research may be out of sync with management needs (Cook et al. 2013). When not targeted at a specific project or context, the communication gap between researchers and policymakers or practitioners can be even worse; to use research results, a comprehensive literature review is often be required, since relevant results may be scattered and fragmented throughout the literature (Finch and Patton-Mallory 1993). Because they have limited access to the scientific literature and/or little time to devote to reading scientific articles (Arlettaz et al. 2010), practitioners may not get the relevant information they need to enact result-led conservation or restoration actions.

Exacerbating access issues, policymakers and practitioners may find academic research results too complex and too contingent for their specific case (Haas 2004, Rau et al. 2018), and may hold a general aversion toward a prescriptive approach (Prendergast et al. 1999). If consultation with practitioners is absent, it can reduce the applicability of environmental science, as stakeholder trust in science and research agencies is related to how fair the process is perceived to be (Arlettaz et al. 2010, Riley et al. 2018). Research agendas may also focus on issues that are not immediately relevant or easily translatable to practice (Arlettaz et al. 2010, Gluckman 2016). Reasons for the lack of utility include an absence of the social and economic contexts, cost-effectiveness of management options, and management prescriptions not being quantitative or spatially explicit (Prendergast et al. 1999).

While it is generally agreed that good science it vital to inform environmental decision-making, ensuring that this science gets put into practice requires a move beyond the trickle-down model of knowledge translation (van Kerkhoff and Lebel 2006). Despite previous attempts that bridge the research-practice divide, producing science that informs policy and practice is an enduring challenge (McNie 2007, Knight et al. 2008, Cook et al. 2013). Simple fixes based in the knowledge-deficit model such as facilitating practitioner access to the primary literature have not been wholly effective (Pfeffer and Sutton 1999, van Kerkhoff and Lebel 2006). In order to produce useful scientific information that improves ecological decision-making and outcomes (McNie 2007), we require additional efforts and frameworks which foster closer links between those who produce scientific knowledge and those who are expected to use it, and which recraft traditional science-society linkages in the process (Rau et al. 2018). Translational ecology represents one such effort.

5.3 Translational ecology

Translational ecology (TE) has been defined in a variety of related ways by different authors, with a main theme of producing actionable science to address complex environmental problems (Haas 2004). TE is "action oriented research" (Chapin 2017) and "user-inspired research" where end-users are involved in the research process and "scientists, practitioners, and stakeholders work together to develop ideas and products that are accessible, actionable, and shaped by all participating parties" (Enquist et al. 2017 p. 541). Similar terms describing science that contributes to both scientific understanding and policy decisions have been coined by other disciplines, including "use-inspired science", "actionable science", and "translational science" (Cook et al. 2013). As TE has been the term most used and developed in the ecological field, it is the term chosen for this thesis.

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TE differs from basic or applied ecology in its fundamental goals and approach. In contrast to applied ecology, TE requires direct, deliberate engagement of end-users (Enquist et al. 2017); although applied ecology is also focused on producing and applying ecological knowledge to solve human problems, TE scholars distinguish TE from applied ecology based on its necessary involvement of the end knowledge user, policy concerns, and an explicit science-practitioner partnership that lasts through the knowledge-to-action process (Seifert 2017, Tucker 2018). This distinction is certainly not black and white – many applied ecologists are likely following many of the tenants of a translational ecology approach, and the approach itself is flexible. The main goals of the TE approach are to link scientific discovery with practical application, and produce data, analyses, projections, or tools that can support decisions in natural resource management; it includes not only information but also guidance on the appropriate use of that information that, ideally, results in improved decision making (Haseltine 2006, Beier et al. 2015, Enquist et al. 2017). TE is use-oriented and aims to connect end-users of environmental science to the field research carried out by scientists who study the basis of environmental problems (Schlesinger 2010, Enquist et al. 2017, Wall et al. 2017).

Knowledge transfer using TE is intentionally bilateral; biophysical and social science inform management actions (i.e., evidence-based policy) and management needs inform scientific research (i.e., policy-relevant science) (Cook et al. 2013). Using the TE approach, an early step of the research program is to identify which users are to be served, with which specific problems, and at what spatial and temporal scales (Dilling 2007, Enquist et al. 2017). TE also places an emphasis on continuous dialogue between stakeholders and scientists, where stakeholders alert scientists to areas in need of study and the partnership ensures that all stakeholders are aware of the implications of scientific discoveries and understand their potential impacts (Schlesinger 2010). TE fits within 'Mode 2'

knowledge production, which is expected to be transdisciplinary, heterogeneous, reflexive and socially accountable, subject to novel forms of quality control, and generated in a context of application (Hessels and van Lente 2008, Cook et al. 2013, Rau et al. 2018). This represents efforts to enhance and redirect science-society exchanges (Rau et al. 2018).

Although the TE approach always involves stakeholder engagement, not all TE takes the form of engagement-intensive coproduction of knowledge – when used with intention, consultative or contractual approaches can produce usable science that supports management decisions (Wall et al. 2017). For stakeholders whose position and time constraints may prohibit involvement in every part of the research process, engaging with them at various key points throughout the TE process will help to ensure that outputs are tailored to their needs (Enquist et al. 2017). Outputs of TE research can include papers, reports, datasets, workshops, and new relationships; selecting the most effective format to communicate and share the research results is an important consideration, and more than one type of output may be necessary to meet multiple needs (Wall et al. 2017).

5.4 4. Key advantages of the translational approach

5.4.1 Produce more effective science

One of the primary advantages to the TE approach is that it produces actionable science by addressing the researcher-practitioner disconnect. By involving practitioners from the outset, TE intentionally creates and fosters relationships and communication between researchers and practitioners. TE is designed to help achieve adaptive management outcomes (Wall et al. 2017); by partnering with resource managers, research outcomes address specific decision contexts and provide directly actionable knowledge which reduces delays in implementation of new knowledge into

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practice (Enquist et al. 2017). Although there are many variations on the definition of Adaptive Management (AM), a core tenet is the feedback process between learning and decision-making, with each informing and adapting the other (Williams and Brown 2014). In contrast to technocratic decision-making which may be rigid once planned or implemented, AM embodies the ideal of "learning by doing" (Walters and Holling 1990) and recognizes the need to act immediately while also planning to learn for the future through iterative feedback, monitoring, and continuation of processes (Clewell and Aronson 2006, Westgate et al. 2013, Rist et al. 2013). Because TE is an interdisciplinary approach, research and outputs can integrate biological analyses with examination of the economic and social consequences of actions and on the institutional landscape for implementation (Pierce et al. 2005, Balmford and Cowling 2006); this can further facilitate the results of ecological research being useful and adapted to local contexts.

The often case-specific nature of TE is especially appropriate for restoration ecology, as conditions and appropriate solutions vary based on place-dependent physical, biological, and socioeconomic factors. As research in the field of restoration ecology often aims to elucidate not only issues of theoretical ecology but also practical, hands-on ecosystem management, the TE approach is well suited to ensuring that research that is meant to be helpful and applicable in real-world contexts achieves its goals and is actually put into practice. By ensuring that the science is informed by end-user needs and with their involvement, TE also facilitates the dissemination of science to society, the serious consideration of science by decision makers, the promotion of dialogue with stakeholders, rapid use of relevant scientific research (Enquist et al. 2017, Safford et al. 2017), and increased local stakeholder capacity (Arlettaz et al. 2010, Rau et al. 2018).

5.4.2 Reinvigorate the social contract between science and society

In addition to helping bridge the science-practice divide, the TE approach has the potential to reinvigorate the social contract between science and society (Wall et al. 2017). TE can build trust in the scientific process and results, address society's "extinction of experience" with natural systems, and increase support for environmental research.

5.4.2.1 Building trust in the scientific process

Engaging stakeholders in the process of scientific inquiry can create transparency and build trust, but only if it is mindfully done. In addition to the "science phobia" that some members of the public may have developed during their schooling, scientists are often seen as an "other" existing outside of the community. TE's focus on meaningful participation and dialogue can bridge this divide and also help address the recognized "diversity gap" in the environmental sciences, i.e. that those producing research do not necessarily represent the diversity of the end-users or collaborators from different fields. The many forms of diversity include, but are not limited to, socioeconomic status; gender; race and ethnicity; worldview; and, for researchers, epistemological orientation. When employees of environmental NGOs, research institutions, and public agencies are predominantly representative of a narrow set of people (race, socioeconomic status, gender, religious affiliation, political orientation), it can create a feedback cycle where "environmentalists" are perceived as a distinct group of "others" (White 1996, Mock 2014, Blackburn 2017, Walker 2018, Gould et al. 2018, Murray et al. 2018). The environmental protection narrative itself also has a problematic past, as it is rooted in settler-colonial traditions, which include some cultures dominating others (Gould et al. 2018).

The real and perceived lack of diversity in the ecological sciences is problematic for many reasons, including that restoration action and decision-making (both in its current and historical

manifestations) can be exclusionary to the very groups who are disproportionately affected by environmental degradation (Morrison and Dunlap 1986, Lodwick 1994, Bullard et al. 2008, Gould et al. 2018) and more willing to make compromises to achieve sustainable practices (Macias 2016). The diversity gap also limits the perspectives and experiences for generating effective restoration policies – diverse research teams can enhance creativity and innovation (Milliken et al. 2003), and different perspectives on the nature-human relationship can create policies that work for, engage, and represent more than just a narrow subset of the population (Medin and Bang 2014, Artelle et al. 2018).

Despite the perceived homogeneity of environmental science, great diversity exists among populations who care deeply about environmental issues (Gould et al. 2018) and embracing diverse narratives and conceptualizations is critical to understanding why certain practices, approaches, and actions may be more appealing to some individuals and communities than others (Lanham 2016, Swart et al. 2018). The way that people engage with and enjoy nature can also vary, and may not resemble dominant (European-American, capitalist) notions of preservation, conservation, and leisure time (Tuck et al. 2014). Addressing the "diversity gap" by bridging and creating experiences and connection is a key advantage of the TE approach (Gould et al. 2018, Motta 2018). With a core tenant of inclusion, the TE approach also offers the opportunity to address "historical, nuanced, and often sensitive sociocultural considerations that may underlie the perceived lack of engagement among minority populations" (Gould et al. 2018 p. 3).

The authority of science can also be undermined when the public expects concrete solutions and researchers are unable to offer them due to incomplete information or scientific understanding (Udo et al. 2004, Mallow et al. 2010, Makri 2017, Winslow et al. 2018). Unfortunately, the issues for which scientific input is most needed are often those for which the science is the most complex, multidisciplinary, and incomplete (Gluckman 2016). The absence of trust in the scientist and the

scientific process can be a barrier to accepting the outcomes of academic research. Since restoration ecology involves addressing issues that are politically and socially charged (e.g., climate change, land use), TE's commitment to respectful listening and dialogue is a valuable approach to bridging preconceptions of science and scientists (Chapin 2017, Winslow et al. 2018). Greater involvement in the research process may help stakeholders to realistically assess what science can and cannot currently answer, and being involved in research question design creates opportunities for research to address priority questions. In addition, TE holds promise as one way to reverse the "extinction of experience" that characterizes our increasingly urbanized world.

5.4.2.2 Address society's "extinction of experience"

"Extinction of experience" and "nature deficit disorder" are terms used to describe the phenomenon where increasingly fewer people, and especially children, have daily contact with nature (Pyle 1978, Louv 2008, Soga and Gaston 2016). This loss of engagement is not only with remote wilderness environments, but also involves a decrease in a diversity of activities and experiences, including time spent in urban greenspaces (Soga and Gaston 2016). A loss of interaction with natural areas is problematic for a variety of reasons. In addition to being linked to deteriorating public health and well-being (Nutsford et al. 2013, Soga and Gaston 2016, van den Bosch and Ode Sang 2017), separation from the natural world is correlated with a decline in pro-environmental attitudes and behaviours (Miller 2005, Soga and Gaston 2016). Nature-connected people not only derive more cultural ecosystem services for the environment, they have also been shown to care more about biodiversity loss, including of the loss of non-charismatic species essential for ecosystem functioning (McGinlay et al. 2018).

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This disaffection with nature can also progressively ratchet down expectations of quality and ecological function over time, since baselines for environmental degradation will be based on progressively less exposure to diverse and well-functioning ecosystems (Miller 2005). Extinction of experience is therefore a fundamental obstacle to reversing global environmental degradation (Miller 2005, Balmford and Cowling 2006, Soga and Gaston 2016), as investment in restoration and conservation depends in large part on public opinion. By inviting public participation and a diversity of values into the process, the TE approach can produce science and outcomes that are more meaningful to members of the public, and thereby facilitate increased use of natural areas. For example, a park co-designed by local residents in partnership with city councillors and ecologists will likely be used more and cared for with an increased sense of pride and ownership than a park created in the absence of this collaboration.

Essentially, when people see the value of environmental actions, they are more likely to invest in and support these measures, and in turn receive the benefits of them. While some worry that designing restorations to develop mutually beneficial relationships between stakeholders and ecosystems will conflict with the aim of creating "wild spaces" (Throop and Purdom 2006), others consider the experience of involvement one of restoration's most important benefits for participants (Jordan 1986, 1989, Clewell and Aronson 2006). To develop restoration techniques and projects that are ecologically, economically, and socially viable, it is wise to dedicate some of our limited resources towards innovative collaborative relationships between scientists, local communities and practitioners (Jones et al. 2018).

5.4.2.3 Increase support for environmental research

Our current context of complex environmental challenges combined with reduced research funding and a complex political climate means that there is a pressing need for restoration ecologists to effectively communicate the value of their science to a diverse range of stakeholders (Enquist et al. 2017). In the past, filling the knowledge deficit of public audiences has tended to be a one-size-fits-all solution to the issue of public support for science (Simis et al. 2016, Winslow et al. 2018). Unfortunately, the 'educate-the-public' approach appears to have been insufficient to foster investment in ecosystem restoration and conservation (Hawken 1993). In the knowledge-deficit model, the interpretation of facts is assumed to be based on rational reasoning that is identical for all members of the public, such that if researchers present information in a rational and objective manner, the public will be supportive of science (Simis et al. 2016).

Science communication based exclusively on fact dissemination may fail or even initiate long-term barriers between scientists and the public due to cognitive biases and the pushback effect (Nyhan and Reifler 2010, Zaval and Cornwell 2016, Shermer 2017, Wood and Porter 2017). Additionally, recent work has shown that it is *interest* in science, not knowledge, that drives public support for scientific research (Motta 2018). While both science knowledge and science interest are associated with increased support for science funding, only gains in science interest are linked to increasing federal funding for science over time, even when controlling for other known correlates of science funding attitudes (e.g. political ideology, religiosity) (Motta 2018). This had led some proponents to suggest that parts of academia may need to re-evaluate their approach we want to continue to receive funding from an increasingly skeptical public and private investment sector (Ecklund et al. 2012).

If presented without concrete actionable steps, some forms of environmental education can also have the undesirable effect of making the listener feel helpless, and the fear of a future characterized by degraded ecosystems has rarely been an effective motivator for lasting changes towards sustainable behaviours (Hawken 1993, Kaplan 2002). The scale and complexity of environmental challenges being what they are, people who feel helpless, that their behaviours do not make any positive contribution, are likely to avoid considering environmental issues because doing so causes stress, discomfort, and feels overwhelming (Roberts and Bacon 1997, Kaplan 2002). Disinterest or disaffection towards addressing environmental challenges is compounded by the altruism-centered approach currently popular in the academic and social discourse; that is, making sacrifices and behaving counter to one's self interest because of guilt or fear for the consequences (Kaplan 2002).

Although the public is willing to make concessions both in principle and in practice for positive environmental change (McCune et al. 2017), the tendency for avoidance and hopelessness combined with the extinction of experience creates a feedback cycle where disaffection reinforces avoidance, and people are less likely to consider altruistic motivations for behavioural change worth their effort or consideration. Fortunately, fostering a sense of value and investment in natural systems coupled with meaningful participation in restoration activities can break this loop. People often find participation both satisfying and empowering, and if the process generates multiply desirable choices, individuals may feel it is possible to behave responsibly without worrying about having to make undue sacrifice in the process (Wandersman 1979, Kaplan and Kaplan 1989, Kaplan 2002, Higgs 2003). By becoming involved in the process of restoration research from conception to application, members of the public gain interest and investment in the process, replacing fear and helplessness and leading to better, more effective outcomes tailored to specific contexts. The TE approach, with its

focus on multi-way dialogues and use-driven research, represents a way to produce tangible outcomes in which members of society are invested (Enquist et al. 2017), and offers opportunities for bridging a divide between scientists and the broader public.

5.5 Challenges to doing Translational Ecology

Despite the clear advantages of a TE approach for restoration ecology, moving forward with this framework is not a straightforward process. Current barriers to adopting a translational approach include a deficit of skills and training opportunities, and a potential mismatch in academic incentives, including advancement and prestige.

5.5.1 Training and skill deficit

It is important to recognize that implementing a TE approach within the current system is not a simple process. Co-production of knowledge with diverse stakeholders requires careful moderation of considerable conceptual, epistemic and practical challenges, and many academics have not received training to facilitate potentially conflict-laden group processes (Ecklund et al. 2012, Rau et al. 2018, Winslow et al. 2018). Developing relationships between researchers and stakeholders also takes time and resources, which may not fit into traditional grant and research timeline structures assigned by University administrators (Dunn et al. 2018). Even in an inherently transdisciplinary field such as restoration ecology, there is often a separation between those trained in the social sciences and those trained in natural sciences (Higgs 2005), and these institutional level disconnects can perpetuate negative biases. For example, scientists who have less positive attitudes toward the social sciences are more likely to adhere to the knowledge deficit model of science communication where the public is assumed to be a homogeneous, rational-decision-making group eager to access and adopt

new information (Simis et al. 2016). Translational restoration ecologists would also require training to navigate sub-optimal or unexpected situations, when the project outcome is unexpected or does not align with the anticipated plan or impact (Rau et al. 2018).

5.5.2 Academic incentive mismatch

A fundamental challenge to adopting a more translational approach to ecological research is that research scientists may not be specifically rewarded, from an academic or advancement standpoint, for a commitment to the practical application of their research (Chapron and Arlettaz 2008, Shanley and López 2009, Arlettaz et al. 2010). As shown in the literature, many researchers face significant institutional disincentives to engage in production of diverse outputs or training (Dilling 2007, Shanley and López 2009), especially in the tenure process (Ecklund et al. 2012). A lack of institutional support can also be present for the most basic form of translation, science outreach. Engaging in outreach activities can have a negative, or at best, have no effect (positive or negative) on a scientist's career (Jensen et al. 2008). The work involved in TE, including science outreach, can also suffer from a perceived lack of prestige, especially since it necessitates alternate communication and dissemination measures that can be perceived as less valuable to those in charge of management and promotions (Brunson and Baker 2016, Rau et al. 2018). Research by Ecklund et al. (2012) has suggested that outreach and engagement are also seen as a more feminine, care-oriented task, which may further decrease the legitimacy of this type of work under some current academic cultures. Indeed, women are markedly more involved in outreach work than men, a finding that holds true in each discipline (Ecklund et al. 2012). As a result of this prestige issue, early career scientists (graduate students and post-doctoral fellows) who express an interest in pursuing a career in science

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outreach report facing significant disapproval of this choice from peers and mentors (Ecklund et al. 2012, University of Waterloo graduate students pers. comm. 2017).

To advance their careers under the current metrics of success, some researchers feel that they must conform to existing structures and processes of advancement (Starbuck 2006, Knight et al. 2008). Overall, peer-reviewed journal articles remain the primary output of research and are generally perceived to have greater intellectual credibility than other forms of scientific outputs (Shanley and López 2009). Public engagement is therefore often considered as an "optional extra" which is a good idea but does not necessarily contribute to career advancement (Poliakoff and Webb 2007). With limited time and resources, a lack of reward system for scientists to engage in applied, policy-relevant research disincentivizes translational science in favour of academic publications in top-tier journals (van Kerkhoff and Lebel 2006, Shanley and López 2009, Rau et al. 2018), despite dissemination to local people being a more effective translational tool than peer-reviewed paper publication (Shanley and López 2009).

The bias against action-oriented research and products is multiplied by the current publishing climate favouring theoretical advances, synthesis and global patterns over field-work and case-based manuscripts. To attract funding and facilitate publication in reputable journals, ecologists may feel pressure to design highly (academically) impactful research that generates high citation rates (Cook et al. 2013, Ríos-Saldaña et al. 2018). Currently, fieldwork-based and case-based studies receive fewer citations than other types of research and are published in lower-impact journals (Ríos-Saldaña et al. 2018). In the 'publish or perish' culture of academia, scientists under pressure to publish in high-ranking journals may eschew application-based research in favour of better job opportunities, funding, and career advancement (Reich 2013). Beyond the advantages of translational restoration ecology for enacting science-driven positive outcomes, this publication system overlooks the reality

that observations and experiments provide essential data for modelling and meta-analyses (Ríos-Saldaña et al. 2018).

I do not suggest that all restoration science needs to follow the TE approach, nor that basic or 'science for science's sake' research has no place in our field. In many cases, an expectation for deep involvement of each researcher in the application of their work is an unrealistic and unfair expectation given the limited time and resources available to them, and may be an inefficient use of their specific training and expertise (Arlettaz et al. 2010, Rau et al. 2018). Instead, I join other scholars (Higgs 2005, Arlettaz et al. 2010, Simis et al. 2016, Rau et al. 2018) who call for an increased recognition that simply providing information will not necessarily change behaviour in ways that benefit the continuation of restoration science. This process should include, but not be limited to, the academic community spreading norms that support user-oriented and participatory approaches to research (Arlettaz et al. 2010). To ease the burden of tailored communication and dissemination of research to policy-makers and wider communities, the field should also further promote dedicated outreach roles and well-supported training systems. Efforts to increase the legitimacy of translational work will not only improve the utility of research results, it will address the current disadvantages that some scientists engaged in these activities face and support those already engaged in them (Ecklund et al. 2012).

Current initiatives to promote the TE approach include the Ecological Society of America (ESA) Student Section's horizon scanning exercise (Winslow et al. 2018), ESA's December 2017 Special Issue on TE, and organizational meetings for the formation of an ESA section of TE. Propositions raised at these events include that: ecology departments should provide science communication curricula in graduate training, ecology conferences should host TE workshops, ecology journals should publish translational science, and ecology departments should hire at least

one individual within each department responsible for studying and conducting TE (Brunson and Baker 2016, Winslow et al. 2018). There have also been calls for mid-career training of both researchers and practitioners, but the short-term feasibility of these solutions has been questioned in the absence of changes to institutional incentive and funding structures (Anonymous 2007, Brunson and Baker 2016). Changing both top-down (institutional) and bottom-up (perceived prestige, incentives) pressures inhibiting TE in restoration science will require increased buy-in not only from large ecology-focused organizations like the Ecological Society of America, but also from prominent field-specific societies and journals. I suggest that the Society for Ecological Restoration as an entity should engage with translational approaches at both the Chapter and international level. I feel that the translational approach, and in particular engaging end-users and practitioners in the setting of research agendas, will produce useful and immediately applicable restoration science.

5.6 Reflecting on my dissertation

This thesis advances knowledge on questions directly related to restoration practice in the southern Ontario tallgrass prairie ecosystem as requested by practitioners and land managers. Accordingly, the research questions addressed in each manuscript (chapters 2 through 4) were created with input from local land managers and restoration practitioners in addition to a detailed assessment of the available literature. Each manuscript also includes a summary of key findings and specific recommendations, which are summarized below. While the findings of this research can and should be used to inform broader theoretical work and future meta analyses, the research presented here also informs the specific context relevant to stakeholders and thus the results and recommendations of the data chapters are largely targeted to the southern Ontario context. This is local versus universal applicability is a recognized trade-off of using the TE approach, and represents both a strength

(applicability, utility to specific stakeholders) and a limitation (generalizability) of translational science.

This dissertation advances our scientific understanding of belowground elements of tallgrass prairie restoration (exotic earthworms and soil microbial communities), and contributes to the larger narrative of how we can measure and achieve restoration success in tallgrass prairie. The core significant original contributions of this dissertation, namely that 1) invasive earthworms are present and abundant in all remnant and restored tallgrass prairies in southern Ontario; 2) the largest and most widespread invasive earthworm, *Lumbricus terrestris*, can play an important role in seed granivory and burial in tallgrass prairie habitats, and these effects are uneven across the target and weed species investigated; and 3) above- and below-ground measures of restoration success can tell different stories, and conventional methods do not maintain microbial communities similar to high quality remnant prairie in the short term, whereas sod mat transplants do; inform the broader literature on invasive species, methods of restoration ecology, and tallgrass prairie habitat management.

The primary message related to experimental outcomes for restoration practitioners are: 1) since invasive earthworm are impractical or impossible to eliminate, their presence and high abundance documented in this research suggests that land managers should consider them as a permanent factor in restoration and conservation planning for all southern Ontario tallgrass prairie; depending on the specific goals of restoration, this may include a consideration of their roles as detritivores, granivores, seedling herbivores, and/or soil transformers (water infiltration, homogenization, organic layer burial). 2) The documented preference of *L. terrestris* for invasive *M. albus* and *C. arvense* may increase the unpredictability of populations of these plant species through time, which may subsequently change the frequency or follow-up required for herbicide treatment of these plants. I recommend periodic (every 3-5 years) follow-up monitoring of these plants in the years

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after treatment application to assure that populations do not arise unexpectedly and reoccur from the seed bank as a result of previous burial activity by earthworms. In addition, of the native seed mix species tested, digestion (destruction) of over 50% of the target restoration seeds ingested suggests that earthworms, particularly at the densities observed in the field, may be important seed predators following seeding for restoration. The common practice of seeding in early or mid-fall, when earthworms are most active, is therefore not recommended; instead, seeding could be done in early winter/late fall following several hard frosts to minimize the granivory pressures from earthworms. With respect to seeding practices, certain seed trichomes do appear to have a protective effect against earthworm granivory. I therefore recommend that whenever possible, pre-sorting and seed application activities be designed to minimize physical abrasion of the seeds to retain these protective structures intact. Finally, *L. spicata* seeds were ingested and destroyed by *L. terrestris* in this study. With a baseline viability of around 1% and a high cost by weight of seed, this species can already be difficult to establish at restoration sites. Unless abundant, cost-effective seed sources are available (e.g. from neighbouring established restoration projects), the earthworm granivory may compound the low viability of this species in such a way that planting plugs or established plants may be a more effective method of establishing *L. spicata* populations for tallgrass prairie sites. 3) Although not a perfect solution by any means, transplanting sod mats of established tallgrass prairie to restoration sites as implemented in the Windsor study does maintain elements of the soil bacterial community and the desired plant community similar to target communities five years after transplanting. If there is an option to use this method in an area that is unavoidably slated for destruction (e.g. due to development), sod mat transplants are a worthwhile option if the restoration goal is to conserve as many elements of the ecosystem (above and belowground) as possible.

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The core findings of this thesis also offer several avenues for future research. On the subject of invasive earthworms, investigating the driving factors behind earthworm distribution and colonization patterns in different ecosystem contexts could support effective management of new invasive annelid species (including new species arriving from Asia, such as the jumping worm, *Amynthas agrestis* Goto & Hatai, 1899) and help to predict and perhaps prevent their invasion into new habitats (e.g. northern boreal forests). In order to further quantify the effects of earthworm species on restoration activities, it would also be interesting to identify the granivory preferences of additional, less widespread earthworm species, and investigate effects of earthworm seed burial and granivory in diverse field conditions and with different earthworm communities. Laboratory studies using chemical analyses with additional species and genetic source populations of seed could also build the results of this and previous work (e.g. Clause et al. 2016b) to establish stronger predictive relationships between seed characteristics and susceptibility to predation and burial by earthworm species. This type of overarching research will likely require a broad array of case studies based in different habitats, each of which could be designed to benefit local land managers and restoration practitioners while also informing broader theory (the TE approach).

With respect to methods of restoration and above- and below-ground relationships, the next logical step would be to investigate the effectiveness of sod mats and other methods of bulk-soil transplant at conserving additional elements of the soil microbiome beyond soil bacteria (e.g. mycorrhizal fungi, nematodes, arthropods). Given the promise of whole-soil inoculation for conserving and restoring microbiome biodiversity, investigating the ecological role of specific microbial taxa will inform future restoration methods based in microbial biology; this represents an ideal opportunity for collaboration between restoration ecologists and molecular biologists. Since the results of this dissertation also reinforce the idea that aboveground vegetation assessment alone can

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be misleading, a key recommendation from this work is that restoration ecologists should incorporate below-ground measures of success into their restoration designs. These directions for future work are synergistic with a TE approach as well, since practitioners and land managers are often those most willing to try novel approaches, and incorporating above- and below-ground metrics will require collaboration between different areas of research and expertise. Finally, comparisons of all research findings with tallgrass prairie habitats in the Midwestern United States are interesting areas for future work; areas of difference and convergence in these comparisons could elucidate driving ecological factors and open up additional avenues of investigation.

By deliberately engaging the end-users of my research in question development and producing and communicating context-specific results and recommendations that can guide future management decisions, this dissertation is in line with the core tenants of TE. However, this thesis does not meet the expanded definition of TE, which involves an interactive process of knowledge production. To achieve this would require long-term relationships (beyond the time scale of a typical graduate program) between the researcher/research group and stakeholders, where the recommendations provided would be implemented, re-assessed for effectiveness and feasibility, and adjusted using an Adaptive Management approach. Long-term collaboration with other researchers working in the target habitat would also be beneficial for producing management recommendations that integrate multiple layered considerations.

In the spirit of the TE approach, the findings of my research have been and will be shared in both traditional (peer-reviewed journal publications, PhD dissertation) and alternate forms. To date, these alternate forms have included plain-language reports for partner agencies (Ontario Parks, Grand River Conservation Agency, rare Charitable Research Reserve, private land owners, Conservation Halton, the Ministry of Natural Resources and Forestry, the Ministry of Transportation, the Nature

Conservancy of Canada), public presentations (rare Charitable Research Reserve, REEP Green Solutions), 'lunch-and-learn' events for agency staff (Conservation Halton), stakeholder agency meetings (Centre for Applied Science in Ontario Protected Areas, Invasive Species Summit for Young Professionals, Ontario Invasive Plant Council), and blog posts (Nature Conservancy Canada Land Lines Blog). To facilitate access to the peer-reviewed literature itself, the academic papers resulting from this dissertation will be made available to the stakeholders involved.

References

Addison, J. A. 2009. Distribution and impacts of invasive earthworms in Canadian forest ecosystems. Biological Invasions 11:59–79.

Ahrenhoerster, R., and T. Wilson. 1981. Prairie restoration for the beginner. Prairie Seed Source, North Lake, WI.

Aira, M., F. Monroy, and J. Domínguez. 2005. Ageing effects on nitrogen dynamics and enzyme activities in casts of Aporrectodea caliginosa (Lumbricidae). Pedobiologia 49:467–473.

Aira, M., and T. G. Piearce. 2009. The earthworm Lumbricus terrestris favours the establishment of Lolium perenne over Agrostis capillaris seedlings through seed consumption and burial. Applied Soil Ecology 41:360–363.

Allan, S. N. 2017, July 20. Disturbance and the community composition of arbuscular mycorrhizal fungi in Ontario tallgrass prairies. Western University, London, ON.

Allen, T. R., T. Millar, S. M. Berch, and M. L. Berbee. 2003. Culturing and direct DNA extraction find different fungi from the same ericoid mycorrhizal roots. New Phytologist 160:255–272.

Allen, W. H. 1994. Reintroduction of endangered plants. BioScience 44:65–68.

Allison, S. K. 2002. When Is a Restoration Successful? Results from a 45-Year-Old Tallgrass Prairie Restoration. Ecological Restoration 20:10–17.

Almquist, T. L., and R. G. Lym. 2010. Effect of aminopyralid on Canada Thistle Cirsium arvense and the native plant community in a restored tallgrass prairie. Invasive Plant Science and Management 3:155–168.

Anderson, H. 2013. Invasive White Sweet Clover (Melilotus albus) best management practices in Ontario. Ontario Invasive Plant Council, Peterborough, ON.

Anderson, R. C. 2006. Evolution and origin of the central grassland of North America: climate, fire, and mammalian grazers. The Journal of the Torrey Botanical Society 133:626–647.

Anderson, R. H., S. D. Fuhlendorf, and D. M. Engle. 2006. Soil Nitrogen Availability in Tallgrass Prairie Under the Fire–Grazing Interaction. Rangeland Ecology & Management 59:625–631.

Anonymous. 2007. The great divide. Nature 450:135–136.

Arlettaz, R., M. Schaub, J. Fournier, T. S. Reichlin, A. Sierro, J. E. M. Watson, and V. Braunisch. 2010. From publications to public actions: when conservation biologists bridge the gap between research and implementation. BioScience 60:835–842.

Arnold, A. E., D. A. Henk, R. L. Eells, F. Lutzoni, and R. Vilgalys. 2007. Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental PCR. Mycologia 99:185–206.

Artelle, K., J. Stephenson, C. Bragg, J. Housty, W. Housty, M. Kawharu, and N. Turner. 2018. Values-led management: the guidance of place-based values in environmental relationships of the past, present, and future. Ecology and Society 23.

Ayanlaja, S. A., S. O. Owa, M. O. Adigun, B. A. Senjobi, and A. O. Olaleye. 2001. Leachate from earthworm castings breaks seed dormancy and preferentially promotes radicle growth in Jute. HortScience 36:143–144.

Bailey, V. L., J. L. Smith, and H. Bolton. 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology and Biochemistry 34:997–1007.

Bakowsky, W., and J. Riley. 1994. A survey of the prairies and savannas of southern Ontario. Pages 7–16 *in* P. D. Lewis, A. Woodliffe, and P. Pratt, editors. Proceedings of the Thirteenth North American Prairie Conference: Spirit of the Land, Our Prairie Legacy. August 6-9, 1992. Corporation of the City of Windsor, Windsor, ON.

Balmford, A., and R. M. Cowling. 2006. Fusion or failure? The future of conservation biology. Conservation Biology 20:692–695.

Barber, N. A., K. M. Chantos-Davidson, R. Amel Peralta, J. P. Sherwood, and W. D. Swingley. 2017. Soil microbial community composition in tallgrass prairie restorations converge with remnants across a 27-year chronosequence. Environmental Microbiology.

Barroetavena, C., S. D. Gisler, D. L. Luoma, and R. J. Meinke. 1998. Mycorrhizal status of the endangered species Astragalus applegatei Peck as determined from a soil bioassay. Mycorrhiza 8:117–119.

Bartram, A. K., M. D. J. Lynch, J. C. Stearns, G. Moreno-Hagelsieb, and J. D. Neufeld. 2011. Generation of multimillion-sequence 16S rRNA gene libraries from complex microbial communities by assembling paired-end illumina reads. Applied and Environmental Microbiology 77:3846–3852.

Bauer, J. T., L. Koziol, and J. D. Bever. 2018. Ecology of Floristic Quality Assessment: testing for correlations between coefficients of conservatism, species traits and mycorrhizal responsiveness. AoB PLANTS 10.

Beier, P., D. Behar, L. Helbrecht, and A. Science WorkGroup of ACCCNRS. 2015. How-to guide for the co-production of actionable science.

Benninghoff, W. S. 1964. The prairie peninsula as a filter barrier to postglacial plant migration. Proceedings of the Indiana Academy of Science 73:116–124.

Berger, J. 1990. Environmental restoration: science and strategies for restoring the earth. Island Press, Washington D.C.

Best, L. B. 1986. Conservation Tillage: Ecological Traps for Nesting Birds? Wildlife Society Bulletin (1973-2006) 14:308–317.

Bever, J. D., I. A. Dickie, E. Facelli, J. M. Facelli, J. Klironomos, M. Moora, M. C. Rillig, W. D. Stock, M. Tibbett, and M. Zobel. 2010. Rooting theories of plant community ecology in microbial interactions. Trends in Ecology & Evolution 25:468–478.

Bever, J. D., P. A. Schultz, A. Pringle, and J. B. Morton. 2001. Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. BioScience 51:923–931.

Bies, L. 2006. The Biofuels Explosion: Is Green Energy Good for Wildlife? Wildlife Society Bulletin 34:1203–1205.

Blackburn, H. 2017. The status of women in STEM in Higher Education: a review of the literature 2007–2017. Science & Technology Libraries 36:235–273.

Blois, J. L., J. W. (Jack) Williams, E. C. Grimm, S. T. Jackson, and R. W. Graham. 2011. A methodological framework for assessing and reducing temporal uncertainty in paleovegetation mapping from late-Quaternary pollen records. Quaternary Science Reviews 30:1926–1939.

Bohlen, P. J., P. M. Groffman, T. J. Fahey, M. C. Fisk, E. Suarez, D. M. Pelletier, and R. T. Fahey. 2004. Ecosystem consequences of exotic earthworm invasion of north temperate forests. Ecosystems 7:1–12.

Borer, E. T., E. W. Seabloom, D. S. Gruner, W. S. Harpole, H. Hillebrand, E. M. Lind, P. B. Adler, J. Alberti, T. M. Anderson, J. D. Bakker, L. Biederman, D. Blumenthal, C. S. Brown, L. A. Brudvig, Y. M. Buckley, M. Cadotte, C. Chu, E. E. Cleland, M. J. Crawley, P. Daleo, E. I. Damschen, K. F. Davies, N. M. DeCrappeo, G. Du, J. Firn, Y. Hautier, R. W. Heckman, A. Hector, J. HilleRisLambers, O. Iribarne, J. A. Klein, J. M. H. Knops, K. J. L. Pierre, A. D. B. Leakey, W. Li, A. S. MacDougall, R. L. McCulley, B. A. Melbourne, C. E. Mitchell, J. L. Moore, B. Mortensen, L. R. O'Halloran, J. L. Orrock, J. Pascual, S. M. Prober, D. A. Pyke,

A. C. Risch, M. Schuetz, M. D. Smith, C. J. Stevens, L. L. Sullivan, R. J. Williams, P. D. Wragg, J. P. Wright, and L. H. Yang. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. Nature 508:517.

van den Bosch, M., and Å. Ode Sang. 2017. Urban natural environments as nature-based solutions for improved public health – A systematic review of reviews. Environmental Research 158:373–384.

Briggs, J. M., A. K. Knapp, and B. L. Brock. 2002. Expansion of woody plants in tallgrass prairie: a fifteen-year study of fire and fire-grazing interactions. The American Midland Naturalist 147:287–294.

Briones, M. J. I., and O. Schmidt. 2017. Conventional tillage decreases the abundance and biomass of earthworms and alters their community structure in a global meta-analysis. Global Change Biology 23:4396–4419.

Brown, G. G. 1995. How do earthworms affect microfloral and faunal community diversity? Plant and Soil 170:209–231.

Bruelheide, H., and T. Flintrop. 2000. Evaluating the transplantation of a meadow in the Harz Mountains, Germany. Biological Conservation 92:109–120.

Brunson, M. W., and M. A. Baker. 2016. Translational training for tomorrow's environmental scientists. Journal of Environmental Studies and Sciences 6:295–299.

Bullard, R. D., P. Mohai, R. Saha, and B. Wright. 2008. Toxic wastes and race at twenty: why race still matters after all these years. Environmental Law 38:371–411.

Burmeier, S., T. W. Donath, A. Otte, and R. L. Eckstein. 2010. Rapid burial has differential effects on germination and emergence of small- and large-seeded herbaceous plant species. Seed Science Research 20:189–200.

Butt, K. R., and N. Grigoropoulou. 2010. Basic research tools for earthworm ecology. Research article. https://www.hindawi.com/journals/aess/2010/562816/.

Butt, K. R., and C. N. Lowe. 2007. Presence of earthworm species within and beneath Lumbricus terrestris (L.) middens. European Journal of Soil Biology 43:S57–S60.

Butt, K. R., and V. Nuutinen. 2005. The dawn of the dew worm. Biologist 52:218–223.

Cabin, R. J. 2011. Intelligent tinkering. Island Press, Washington D.C.

Cáceres, M. D., and P. Legendre. 2009. Associations between species and groups of sites: indices and statistical inference. Ecology 90:3566–3574.

Callaham, M. A., J. M. Blair, and P. F. Hendrix. 2001. Different behavioral patterns of the earthworms Octolasion tyrtaeum and Diplocardia spp. in tallgrass prairie soils: potential influences on plant growth. Biology and Fertility of Soils 34:49–56.

Callaham, M. A., J. M. Blair, T. C. Todd, D. J. Kitchen, and M. R. Whiles. 2003. Macroinvertebrates in North American tallgrass prairie soils: effects of fire, mowing, and fertilization on density and biomass. Soil Biology and Biochemistry 35:1079–1093.

Callaham, M. A., G. González, C. M. Hale, L. Heneghan, S. L. Lachnicht, and X. Zou. 2006. Policy and management responses to earthworm invasions in North America. Pages 117–129 Biological Invasions Belowground: Earthworms as Invasive Species. Springer, Dordrecht.

Camill, P., M. J. McKone, S. T. Sturges, W. J. Severud, E. Ellis, J. Limmer, C. B. Martin, R. T. Navratil, A. J. Purdie, B. S. Sandel, S. Talukder, and A. Trout. 2004. Community- and ecosystem-level changes in a species-rich tallgrass prairie restoration. Ecological Applications 14:1680–1694.

Caporaso, J. G., K. Bittinger, F. D. Bushman, T. Z. DeSantis, G. L. Andersen, and R. Knight. 2010a. PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26:266–267.

Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, G. A. Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D. McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J. Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld, and R. Knight. 2010b. QIIME allows analysis of high-throughput community sequencing data. Nature Methods 7:335–336.

Carbajo, V., B. den Braber, W. H. van der Putten, and G. B. D. Deyn. 2011. Enhancement of late successional plants on ex-arable land by soil inoculations. PLOS ONE 6:e21943.

Cardinale, B. J., J. E. Duffy, A. Gonzalez, D. U. Hooper, C. Perrings, P. Venail, A. Narwani, G. M. Mace, D. Tilman, D. A. Wardle, A. P. Kinzig, G. C. Daily, M. Loreau, J. B. Grace, A. Larigauderie, D. S. Srivastava, and S. Naeem. 2012. Biodiversity loss and its impact on humanity. Nature 486:59–67.

Cassin, C. M., and P. M. Kotanen. 2016. Invasive earthworms as seed predators of temperate forest plants. Biological Invasions 18:1567–1580.

Cervantes, E., J. J. Martín, and E. Saadaoui. 2016. Updated methods for seed shape analysis. Research article. https://www.hindawi.com/journals/scientifica/2016/5691825/.

Chambers, J. C., and J. A. MacMahon. 1994. A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. Annual Review of Ecology and Systematics 25:263–292.

Chapin, S. 2017. Now is the time for translational ecology. Frontiers in Ecology and the Environment 15:539–539.

Chapron, G., and R. Arlettaz. 2008, January 9. Conservation: academics should "conserve or perish." Comments and Opinion. https://www.nature.com/articles/451127b.

Choi, A., T. Sackett, S. Smith, and M. Bellocq. 2017. Exotic earthworm (Oligochaeta: Lumbricidae) assemblages on a landscape-scale in central Canadian woodlands: Importance of region and vegetation type. Canadian Journal of Forest Research 47.

Cipollini, K. A., A. L. Maruyama, and C. L. Zimmerman. 2005. Planning for restoration: a decision analysis approach to prioritization. Restoration Ecology 13:460–470.

Clause, J., S. Barot, and E. Forey. 2016a. Earthworms promote greater richness and abundance in the emergence of plant species across a grassland-forest ecotone. Journal of Plant Ecology 9:703–711.

Clause, J., E. Forey, N. Eisenhauer, C. E. Seal, A. Soudey, L. Colville, and S. Barot. 2016b. Seed selection by earthworms: chemical seed properties matter more than morphological traits. Plant and Soil.

Clause, J., E. Forey, C. J. Lortie, A. M. Lambert, and S. Barot. 2015. Non-native earthworms promote plant invasion by ingesting seeds and modifying soil properties. Acta Oecologica 64:10–20.

Clause, J., P. Margerie, E. Langlois, T. Decaëns, and E. Forey. 2011a. Fat but slim: criteria of seed attractiveness for earthworms. Pedobiologia 54:S159–S165.

Clause, J., P. Margerie, E. Langlois, T. Decaëns, and E. Forey. 2011b. Fat but slim: Criteria of seed attractiveness for earthworms. Pedobiologia 54:S159–S165.

Clements, D. R. 2013. Translocation of rare plant species to restore Garry oak ecosystems in western Canada: challenges and opportunities. Botany 91:283–291.

Clewell, A. F., and J. Aronson. 2006. Motivations for the restoration of ecosystems. Conservation

> Biology 20:420–428.

Coleman, D. C., and D. H. Wall. 2015. Chapter 5 - Soil fauna: Occurrence, biodiversity, and roles in

> ecosystem function. Pages 111–149 *in* E. A. Paul, editor. Soil Microbiology, Ecology and

> Biochemistry. 4th edition. Academic Press, Boston, MA.

Collins, S. L. 2000. Disturbance Frequency and Community Stability in Native Tallgrass Prairie. The

> American Naturalist 155:311–325.

Comin, F. A. 2010. The challenges of humanity in the twenty-first century and the role of ecological

> restoration. Pages 3–20 *in* F. A. Comin, editor. Ecological Restoration: A Global Challenge.

> Cambridge University Press, Cambridge.

Cook, C. N., M. B. Mascia, M. W. Schwartz, H. P. Possingham, and R. A. Fuller. 2013. Achieving

> conservation science that bridges the knowledge-action boundary. Conservation Biology: The

> Journal of the Society for Conservation Biology 27:669–678.

COSEWIC. 2000. COSEWIC assessment and update status report on the Greater Prairie-Chicken in

> Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, ON.

COSEWIC. 2010. COSEWIC assessment and status report on the Dense Blazing Star Liatris spicata

> in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, ON.

Craven, D., M. P. Thakur, E. K. Cameron, L. E. Frelich, R. Beauséjour, R. B. Blair, B. Blossey, J.

> Burtis, A. Choi, A. Dávalos, T. J. Fahey, N. A. Fisichelli, K. Gibson, I. T. Handa, K.

> Hopfensperger, S. R. Loss, V. Nuzzo, J. C. Maerz, T. Sackett, B. C. Scharenbroch, S. M.

> Smith, M. Vellend, L. G. Umek, and N. Eisenhauer. 2016. The unseen invaders: introduced

> earthworms as drivers of change in plant communities in North American forests (a meta-

> analysis). Global Change Biology.

Crins, W. J. 1997. Rare and endangered plants and their habitats in Canada. The Canadian Field Naturalist 111:506–519.

Curry, J. P., and O. Schmidt. 2007. The feeding ecology of earthworms – A review. Pedobiologia 50:463–477.

Danz, N. P., P. B. Reich, L. E. Frelich, and G. J. Niemi. 2011. Vegetation controls vary across space and spatial scale in a historic grassland-forest biome boundary. Ecography 34:402–414.

Dávalos, A., V. Nuzzo, and B. Blossey. 2014. Demographic responses of rare forest plants to multiple stressors: the role of deer, invasive species and nutrients. Journal of Ecology 102:1222–1233.

Decaëns, T., L. Mariani, N. Betancourt, and J. J. Jiménez. 2003. Seed dispersion by surface casting activities of earthworms in Colombian grasslands. Acta Oecologica 24:175–185.

Deyn, G. B. D., C. E. Raaijmakers, H. R. Zoomer, M. P. Berg, P. C. de Ruiter, H. A. Verhoef, T. M. Bezemer, and W. H. van der Putten. 2003. Soil invertebrate fauna enhances grassland succession and diversity. Nature 422:711–713.

Diboll, N. 1997. Designing seed mixes. Pages 135–149 *in* S. Packard and C. F. Mutel, editors. The tallgrass restoration handbook. Island Press, Washington D.C.

Dilling, L. 2007. Towards science in support of decision making: characterizing the supply of carbon cycle science. Environmental Science & Policy 10:48–61.

Donath, T. W., and R. L. Eckstein. 2012. Litter effects on seedling establishment interact with seed position and earthworm activity. Plant Biology 14:163–170.

Drayton, B., and R. B. Primack. 2012. Success rates for reintroductions of eight perennial plant species after 15 years. Restoration Ecology 20:299–303.

Drouin, M., R. Bradley, L. Lapointe, and J. Whalen. 2014. Non-native anecic earthworms (Lumbricus terrestris L.) reduce seed germination and seedling survival of temperate and boreal trees species. Applied Soil Ecology 75:145–149.

Dunn, G., J. J. Bos, and R. R. Brown. 2018. Mediating the science-policy interface: Insights from the urban water sector in Melbourne, Australia. Environmental Science & Policy 82:143–150.

Ecklund, E. H., S. A. James, and A. E. Lincoln. 2012. How academic biologists and physicists view science outreach. PLOS ONE 7:e36240.

Edgar, R. C. 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10:996–998.

Edgar, R. C. 2018. Updating the 97% identity threshold for 16S ribosomal RNA OTUs. Bioinformatics (Oxford, England) 34:2371–2375.

Edwards, C. A., and P. J. Bohlen. 1996. Biology and ecology of earthworms. 3rd edition. Chapman and Hall, London.

Eisenhauer, N., O. Butenschoen, S. Radsick, and S. Scheu. 2010. Earthworms as seedling predators: importance of seeds and seedlings for earthworm nutrition. Soil Biology and Biochemistry 42:1245–1252.

Eisenhauer, N., A. Milcu, N. Nitschke, A. C. W. Sabais, C. Scherber, and S. Scheu. 2009a. Earthworm and belowground competition effects on plant productivity in a plant diversity gradient. Oecologia 161:291–301.

Eisenhauer, N., and S. Scheu. 2008. Invasibility of experimental grassland communities: the role of earthworms, plant functional group identity and seed size. Oikos 117:1026–1036.

Eisenhauer, N., M. Schuy, O. Butenschoen, and S. Scheu. 2009b. Direct and indirect effects of endogeic earthworms on plant seeds. Pedobiologia 52:151–162.

Eisenhauer, N., D. Straube, E. A. Johnson, D. Parkinson, and S. Scheu. 2009c. Exotic ecosystem
engineers change the emergence of plants from the seed bank of a deciduous forest.
Ecosystems 12:1008–1016.

Emam, T. 2016. Local soil, but not commercial AMF inoculum, increases native and non-native grass
growth at a mine restoration site. Restoration Ecology 24:35–44.

Enquist, C. A. F., S. T. Jackson, G. M. Garfin, F. W. Davis, L. R. Gerber, J. A. Littell, J. L. Tank, A.
J. Terando, T. U. Wall, B. Halpern, J. K. Hiers, T. L. Morelli, E. McNie, N. L. Stephenson,
M. A. Williamson, C. A. Woodhouse, L. Yung, M. W. Brunson, K. R. Hall, L. M. Hallett, D.
M. Lawson, M. A. Moritz, K. Nydick, A. Pairis, A. J. Ray, C. Regan, H. D. Safford, M. W.
Schwartz, and M. R. Shaw. 2017. Foundations of translational ecology. Frontiers in Ecology
and the Environment 15:541–550.

Environment Canada. 2002. A guide to establishing prairie and meadow communities in southern
Ontario. Government of Canada.

Evans, M. 2019, January 22. Communities still not at the core of restoration, experts say.
https://news.globallandscapesforum.org/32153/communities-still-not-at-the-core-of-
restoration-experts-say/.

Faber-Langendoen, D., and P. F. Maycock. 1994. A vegetation analysis of tallgrass prairie in southern
Ontario. Pages 17–32 *in* R. Wickett, L. P. Dolan, A. Woodliffe, and P. Pratt, editors.
Proceedings of the Thirteenth North American Prairie Conference: Spirit of the Land, Our
Prairie Legacy. Corporation of the City of Windsor, Windsor, Ontario.

Fahselt, D. 2007. Is transplanting an effective means of preserving vegetation? Canadian Journal of
Botany 85:1007–1017.

Fay, P. A., S. M. Prober, W. S. Harpole, J. M. H. Knops, J. D. Bakker, E. T. Borer, E. M. Lind, A. S. MacDougall, E. W. Seabloom, P. D. Wragg, P. B. Adler, D. M. Blumenthal, Y. M. Buckley, C. Chu, E. E. Cleland, S. L. Collins, K. F. Davies, G. Du, X. Feng, J. Firn, D. S. Gruner, N. Hagenah, Y. Hautier, R. W. Heckman, V. L. Jin, K. P. Kirkman, J. Klein, L. M. Ladwig, Q. Li, R. L. McCulley, B. A. Melbourne, C. E. Mitchell, J. L. Moore, J. W. Morgan, A. C. Risch, M. Schütz, C. J. Stevens, D. A. Wedin, and L. H. Yang. 2015. Grassland productivity limited by multiple nutrients. Nature Plants 1:15080.

Fenner, M., and K. Thompson. 2005. The ecology of seeds. Cambridge University Press, Cambridge.

Fierer, N., M. Breitbart, J. Nulton, P. Salamon, C. Lozupone, R. Jones, M. Robeson, R. A. Edwards, B. Felts, S. Rayhawk, R. Knight, F. Rohwer, and R. B. Jackson. 2007. Metagenomic and small-subunit rRNA analyses reveal the genetic diversity of Bacteria, Archaea, Fungi, and Viruses in soil. Appl. Environ. Microbiol. 73:7059–7066.

Fierer, N., and R. B. Jackson. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences 103:626–631.

Fierer, N., J. Ladau, J. C. Clemente, J. W. Leff, S. M. Owens, K. S. Pollard, R. Knight, J. A. Gilbert, and R. L. McCulley. 2013. Reconstructing the microbial diversity and function of pre-agricultural tallgrass prairie soils in the United States. Science 342:621–624.

Fike, J. H., D. J. Parrish, D. D. Wolf, J. A. Balasko, J. T. Green, M. Rasnake, and J. H. Reynolds. 2006. Long-term yield potential of switchgrass-for-biofuel systems. Biomass and Bioenergy 30:198–206.

Finch, D. M., and M. Patton-Mallory. 1993. Closing the gap between research and management. In: Finch, Deborah M.; Stangel, Peter W. (eds.). Status and management of neotropical migratory birds: September 21-25, 1992, Estes Park, Colorado. Gen. Tech. Rep. RM-229.

Fort Collins, Colo.: Rocky Mountain Forest and Range Experiment Station, U.S. Dept. of Agriculture, Forest Service: 12-16 229.

Fletcher, R. J., and R. R. Koford. 2002. Habitat and Landscape Associations of Breeding Birds in Native and Restored Grasslands. The Journal of Wildlife Management 66:1011–1022.

Fletcher, R. J., B. A. Robertson, J. Evans, P. J. Doran, J. R. Alavalapati, and D. W. Schemske. 2011. Biodiversity conservation in the era of biofuels: risks and opportunities. Frontiers in Ecology and the Environment 9:161–168.

Forey, E., S. Barot, T. Decaëns, E. Langlois, K.-R. Laossi, P. Margerie, S. Scheu, and N. Eisenhauer. 2011. Importance of earthworm–seed interactions for the composition and structure of plant communities: a review. Acta Oecologica 37:594–603.

Freyman, W. A., L. A. Masters, and S. Packard. 2015. The Universal Floristic Quality Assessment (FQA) Calculator: an online tool for ecological assessment and monitoring. Methods in Ecology and Evolution 7:380–383.

Gadd, G. M. 1993. Interactions of fungip with toxic metals. New Phytologist 124:25–60.

Gange, A. C., V. K. Brown, and L. M. Farmer. 1990. A test of mycorrhizal benefit in an early successional plant community. New Phytologist 115:85–91.

Gardner, W. H. 1965. Water content. Pages 82–127 *in* C. A. Black, editor. Methods of Soil Analysis. Part 1. Physical and Mineralogical Properties, Including Statistics of Measurement and Sampling. American Society of Agronomy.

Gates, G. E. 1982. Farewell to North American megadriles. Megadrilogica 4:12–77.

Gholz, H. L., D. A. Wedin, S. M. Smitherman, M. E. Harmon, and W. J. Parton. 2000. Long-term dynamics of pine and hardwood litter in contrasting environments: toward a global model of decomposition. Global Change Biology 6:751–765.

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Gill, J. L., J. W. Williams, S. T. Jackson, J. P. Donnelly, and G. C. Schellinger. 2012. Climatic and megaherbivory controls on late-glacial vegetation dynamics: a new, high-resolution, multi-proxy record from Silver Lake, Ohio. Quaternary Science Reviews 34:66–80.

Gluckman, P. 2016. The science–policy interface. Science 353:969–969.

Good, J. E. G., H. L. Wallace, P. A. Stevens, and G. L. Radford. 1999. Translocation of herb-rich grassland from a site in wales prior to opencast coal extraction. Restoration Ecology 7:336–347.

Goslee, S. C., and D. L. Urban. 2007. The ecodist package for dissimilarity-based analysis of ecological data. Journal of   Statistical Software 22:1–19.

Gould, R. K., I. Phukan, M. E. Mendoza, N. M. Ardoin, and B. Panikkar. 2018. Seizing opportunities to diversify conservation. Conservation Letters 11:e12431.

Government of Canada. 2002. Species at Risk Act, SC 2002 c. 29.

Government of Ontario. 2007. Endangered Species Act, S.O. 2007, c. 6.

Grant, J. D. 1983. The activities of earthworms and the fates of seeds. Pages 107–122 *in* J. E. Satchell, editor. Earthworm Ecology from Darwin to Vermiculture. Chapman and Hall, London.

Grime, J. P., J. M. Macky, S. H. Hillier, and D. J. Read. 1987. Mechanisms of floristic diversity: Evidence from microcosms. Nature 328:420–422.

Grman, E., T. Bassett, and L. A. Brudvig. 2013. Confronting contingency in restoration: management and site history determine outcomes of assembling prairies, but site characteristics and landscape context have little effect. Journal of Applied Ecology 50:1234–1243.

Grman, E., T. Bassett, C. R. Zirbel, and L. A. Brudvig. 2015. Dispersal and establishment filters influence the assembly of restored prairie plant communities. Restoration Ecology 23:892–899.

Grman, E., and L. A. Brudvig. 2014. Beta diversity among prairie restorations increases with species pool size, but not through enhanced species sorting. Journal of Ecology 102:1017–1024.

Gucker, C. L. 2009. Melilotus alba, M. officinalis. Page Fire Effects Information System. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory.

Guild, W. J. 1952. Variation in earthworm numbers within field populations. Journal of Animal Ecology 21:169–183.

Haas, P. 2004. When does power listen to truth? A constructivist approach to the policy process. Journal of European Public Policy 11:569–592.

Hale, C. 2007. Earthworms of the Great Lakes. Kollath and Stensaas, Duluth, MN.

Hamilton, L. S. 1990. Restoration of degraded tropical forests. Pages 113–123 *in* J. J. Berger, editor. Environmental restoration: Science for restoring the earth. Island Press, Washington D.C.

Hamilton, W. E., and D. Y. Sillman. 1989. Influence of earthworm middens on the distribution of soil microarthropods. Biology and Fertility of Soils 8:279–284.

Haney, R. L., J. R. Kiniry, and M.-V. V. Johnson. 2010. Soil microbial activity under different grass species: Underground impacts of biofuel cropping. Agriculture, Ecosystems & Environment 139:754–758.

Harris, J. A., P. Birch, and J. Palmer. 1996. Land restoration and reclamation. Addison Wesley Longman, Essex, England.

Harris, J. A., and J. P. Palmer, editors. 1996. Land restoration and reclamation: principles and practice. Addison Wesley Longman Limited, Essex, England.

Hartenstein, R., and L. Amico. 1983. Production and carrying capacity for the earthworm Lumbricus terrestris in culture. Soil Biology and Biochemistry 15:51–54.

Haseltine, S. D. 2006. Scientists should help frame the discussion. BioScience 56:289–290.

Hautier, Y., E. W. Seabloom, E. T. Borer, P. B. Adler, W. S. Harpole, H. Hillebrand, E. M. Lind, A. S. MacDougall, C. J. Stevens, J. D. Bakker, Y. M. Buckley, C. Chu, S. L. Collins, P. Daleo, E. I. Damschen, K. F. Davies, P. A. Fay, J. Firn, D. S. Gruner, V. L. Jin, J. A. Klein, J. M. H. Knops, K. J. L. Pierre, W. Li, R. L. McCulley, B. A. Melbourne, J. L. Moore, L. R. O'Halloran, S. M. Prober, A. C. Risch, M. Sankaran, M. Schuetz, and A. Hector. 2014. Eutrophication weakens stabilizing effects of diversity in natural grasslands. Nature 508:521.

Hawken, P. 1993. The ecology of commerce : a declaration of sustainability. HarperCollins, New York, NY.

Hawkins, A. P., and K. M. Crawford. 2018. Interactions between plants and soil microbes may alter the relative importance of intraspecific and interspecific plant competition in a changing climate. AoB PLANTS 10.

Hay, C. R. 2016. Agaricomycetes of Ontario Tallgrass Prairies. Master of Science, Western University, London Ontario.

van der Heijden, M. G. A., R. D. Bardgett, and N. M. V. Straalen. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters 11:296–310.

Hessels, L. K., and H. van Lente. 2008. Re-thinking new knowledge production: a literature review and a research agenda. Research Policy 37:740–760.

Higgins, K. F. 1986. Interpretation and compendium of historical fire accounts in the Northern Great Plains. Federal Government Series, U.S. Fish and Wildlife Service, Washington, D.C.

Higgs, E. 2003. Nature by design. MIT Press, Cambridge, MA.

Higgs, E. 2005. The two-culture problem: ecological restoration and the integration of knowledge. Restoration Ecology 13:159–164.

Higgs, E., J. Harris, S. Murphy, K. Bowers, R. Hobbs, W. Jenkins, J. Kidwell, N. Lopoukhine, B. Sollereder, K. Suding, A. Thompson, and S. Whisenant. 2018. On principles and standards in ecological restoration. Restoration Ecology 26:399–403.

Hobbs, R. J., E. Higgs, and J. A. Harris. 2009. Novel ecosystems: implications for conservation and restoration. Trends in Ecology & Evolution 24:599–605.

Hobbs, R. J., E. S. Higgs, and C. M. Hall. 2013. Defining novel ecosystems. Pages 58–60 Novel Ecosystems. Wiley-Blackwell.

Hood-Nowotny, R., N. H.-N. Umana, E. Inselbacher, P. Oswald- Lachouani, and W. Wanek. 2010. Alternative methods for measuring inorganic, organic, and total dissolved nitrogen in soil. Soil Science Society of America Journal 74:1018–1027.

Hopwood, J. L. 2008. The contribution of roadside grassland restorations to native bee conservation. Biological Conservation 141:2632–2640.

House, G. L., and J. D. Bever. 2018. Disturbance reduces the differentiation of mycorrhizal fungal communities in grasslands along a precipitation gradient. Ecological Applications.

Hulme, P. E. 1998. Post-dispersal seed predation: consequences for plant demography and evolution. Perspectives in Plant Ecology, Evolution and Systematics 1:32–46.

Irving, W. 1956. A Tour on the Prairies. University of Oklahoma Press.

IUCN. 2008, January 1. Platanthera praeclara: Goedeke, T., Sharma, J., Delphey, P. & Marshall Mattson, K.: The IUCN Red List of Threatened Species 2008: e.T132834A3464336. International Union for Conservation of Nature.

Jackson, M., C. Myrholm, C. Shaw, and T. Ramsfield. 2017a. Using nested PCR to improve detection of earthworm eDNA in Canada. Soil Biology and Biochemistry 113:215–218.

Jackson, S. T., G. M. Garfin, and C. A. F. Enquist. 2017b. Toward an effective practice of translational ecology. Frontiers in Ecology and the Environment 15:540–540.

Jangid, K., M. A. Williams, A. J. Franzluebbers, T. M. Schmidt, D. C. Coleman, and W. B. Whitman. 2011. Land-use history has a stronger impact on soil microbial community composition than aboveground vegetation and soil properties. Soil Biology and Biochemistry 43:2184–2193.

Jangid, K., M. Williams, A. Franzluebbers, J. Blair, D. Coleman, and W. Whitman. 2010. Development of soil microbial communities during tallgrass prairie restoration. Soil Biology and Biochemistry 42:302.

Jennings, B. W., and S. A. Watmough. 2016. The impact of invasive earthworms on soil respiration and soil carbon within temperate hardwood forests. Ecosystems 19.

Jensen, P., J.-B. Rouquier, P. Kreimer, and Y. Croissant. 2008. Scientists who engage with society perform better academically. Science and Public Policy 35:527–541.

Jill Clapperton, M., J. J. Miller, F. J. Larney, and C. Wayne Lindwall. 1997. Earthworm populations as affected by long-term tillage practices in southern Alberta, Canada. Soil Biology and Biochemistry 29:631–633.

Jonasson, S., A. Michelsen, I. K. Schmidt, and E. V. Nielsen. 1999. Responses in microbes and plants to changed temperature, nutrient, and light regimes in the Arctic. Ecology 80:1828–1843.

Jones, H. P., P. C. Jones, E. B. Barbier, R. C. Blackburn, J. M. R. Benayas, K. D. Holl, M.

McCrackin, P. Meli, D. Montoya, and D. M. Mateos. 2018. Restoration and repair of Earth's

damaged ecosystems. Proceedings of the Royal Society B 285:20172577.

Jones, R. A., J. W. Williams, and S. T. Jackson. 2017. Vegetation history since the last glacial

maximum in the Ozark highlands (USA): A new record from Cupola Pond, Missouri.

Quaternary Science Reviews 170:174–187.

Jordan, W. R. I. 1986. Restoration and the reentry of nature. Restoration and Management Notes 4:2.

Jordan, W. R. I. 1989. Restoring the restorationist. Restoration and Management Notes 7:55.

Jordan, W. R., R. L. Peters, and E. B. Allen. 1988. Ecological restoration as a strategy for conserving

biological diversity. Environmental Management 12:55–72.

Kaplan, S. 2002. New ways to promote proenvironmental behavior: human nature and

environmentally responsible behavior. Journal of Social Issues 56:491–508.

Kaplan, S., and R. Kaplan. 1989. The visual environment: public participation in design and planning.

Journal of Social Issues 45:59–86.

Kardol, P., T. M. Bezemer, and W. H. V. D. Putten. 2009. Soil organism and plant introductions in

restoration of species-rich grassland communities. Restoration Ecology 17:258–269.

Kardol, P., N. J. Cornips, M. M. L. van Kempen, J. M. T. Bakx-Schotman, and W. H. van der Putten.

2007. Microbe-mediated plant–soil feedback causes historical contingency effects in plant

community assembly. Ecological Monographs 77:147–162.

Kearns, K. S. 1986. A comparison of transplanting times and methods for salvaging prairie forbs and

grasses. Pages 197–200 The prairie: past, present, and future. Moorhead, Minnesota.

Kennedy, K., M. W. Hall, M. D. J. Lynch, G. Moreno-Hagelsieb, and J. D. Neufeld. 2014. Evaluating bias of illumina-based bacterial 16S rRNA gene profiles. Applied and Environmental Microbiology 80:5717–5722.

van Kerkhoff, L., and L. Lebel. 2006. Linking knowledge and action for sustainable development. Annual Review of Environment and Resources 31:445–477.

Kindscher, K., and L. L. Tieszen. 1998. Floristic and soil organic matter changes after five and thirty-five years of native tallgrass prairie restoration. Restoration Ecology 6:181–196.

King, J. E. 1981. Late quarternary vegetational history of Illinois. Ecological Monographs 51:43–62.

Klironomos, J. N., J. McCune, M. Hart, and J. Neville. 2000. The influence of arbuscular mycorrhizae on the relationship between plant diversity and productivity. Ecology Letters 3:137–141.

Knight, A. T., R. M. Cowling, M. Rouget, A. Balmford, A. T. Lombard, and B. M. Campbell. 2008. Knowing but not doing: selecting priority conservation areas and the research–implementation gap. Conservation Biology 22:610–617.

Kozak, J. P., and B. P. Piazza. 2014. A proposed process for applying a structured decision-making framework to restoration planning in the Atchafalaya River Basin, Louisiana, U.S.A. Restoration Ecology 23:46–52.

Kroetsch, D., and C. Wang. 2008. Particle size distribution. Pages 713–725 *in* M. R. Carter and E. G. Gregorich, editors. Soil sampling and methods of analysis. 2nd edition. CRC Press, Boca Raton, FL.

Kubicek, C. P., and I. S. Druzhinina, editors. 2007. Fungal responses to disturbance: agriculture and forestry. Pages 47–68 Environmental and Microbial Relationships. Springer Berlin Heidelberg, Berlin, Heidelberg.

Kulmatiski, A., K. H. Beard, J. R. Stevens, and S. M. Cobbold. 2008. Plant–soil feedbacks: a meta-analytical review. Ecology Letters 11:980–992.

Lajeunesse, E. J. 1960. The Windsor border region. University of Toronto Press, Toronto, ON.

Lanham, D. J. 2016, September 22. Birding while black.

Laossi, K.-R., D.-C. Noguera, and S. Barot. 2010. Earthworm-mediated maternal effects on seed germination and seedling growth in three annual plants. Soil Biology and Biochemistry 42:319–323.

Larios, L., D. E. Pearson, and J. L. Maron. 2017. Incorporating the effects of generalist seed predators into plant community theory. Functional Ecology 31:1856–1867.

Lau, J. A., and J. T. Lennon. 2011. Evolutionary ecology of plant–microbe interactions: soil microbial structure alters selection on plant traits. New Phytologist 192:215–224.

Lauber, C. L., M. Hamady, R. Knight, and N. Fierer. 2009. Pyrosequencing-based assessment of soil pH as a predictor of soil bacterial community structure at the continental scale. Appl. Environ. Microbiol. 75:5111–5120.

Lawrence, A. P., and M. A. Bowers. 2002. A test of the 'hot' mustard extraction method of sampling earthworms. Soil Biology and Biochemistry 34:549–552.

Lawson, D. M., K. R. Hall, L. Yung, and C. A. F. Enquist. 2017. Building translational ecology communities of practice: insights from the field. Frontiers in Ecology and the Environment 15:569–577.

Leach, M. K., and T. J. Givnish. 1996. Ecological determinants of species loss in remnant prairies. Science 273:1555–1558.

Lee, K. E. 1985. Earthworms : their ecology and relationships with soils and land use. Academic Press, Sydney.

Lewis, R. R. I. 1990. Wetlands restoration/creation/enhancement terminology: suggestions for

    standardization. Pages 417–422 *in* J. A. Kusler and M. E. Kentula, editors. Wetland creation

    and restoration: The status of the science. Island Press, Washington D.C.

Leys, B., S. C. Brewer, S. McConaghy, J. Mueller, and K. K. McLauchlan. 2015. Fire history

    reconstruction in grassland ecosystems: amount of charcoal reflects local area burned.

    Environmental Research Letters 10:114009.

Lindborg, R., and O. Eriksson. 2004. Historical landscape connectivity affects present plant species

    diversity. Ecology 85:1840–1845.

Liski, J., A. Nissinen, M. Erhard, and O. Taskinen. 2003. Climatic effects on litter decomposition

    from arctic tundra to tropical rainforest. Global Change Biology 9:575–584.

Lodwick, D. G. 1994. Changing worldviews and landscape restoration. Pages 97–110 *in* D. A.

    Baldwin, J. De Luce, and C. Pletsch, editors. Beyond preservation: Restoring and inventing

    landscapes. University of Minnesota Press, Minneapolis, MI.

Loss, S. R., S. Paudel, C. M. Laughlin, and C. Zou. 2017. Local-scale correlates of native and non-

    native earthworm distributions in juniper-encroached tallgrass prairie. Biological Invasions

    19:1621–1635.

Louca, S., L. W. Parfrey, and M. Doebeli. 2016. Decoupling function and taxonomy in the global

    ocean microbiome. Science 353:1272–1277.

Louv, R. 2008. Last child in the woods: saving our children from Nature-Deficit Disorder. Algonquin

    Books, Chapel Hill, NC.

Lynch, M. D., A. P. Masella, M. W. Hall, A. K. Bartram, and J. D. Neufeld. 2013. AXIOME:

    automated exploration of microbial diversity. GigaScience 2:3.

MacDougall, A. S., and R. Turkington. 2007. Does the Type of Disturbance Matter When Restoring Disturbance-Dependent Grasslands? Restoration Ecology 15:263–272.

MacDougall, A. S., S. D. Wilson, and J. D. Bakker. 2008. Climatic variability alters the outcome of long-term community assembly. Journal of Ecology 96:346–354.

Macias, T. 2016. Ecological assimilation: race, ethnicity, and the inverted gap of environmental concern. Society & Natural Resources 29:3–19.

Makri, A. 2017. Give the public the tools to trust scientists. Nature News 541:261.

Mallow, J., H. Kastrup, F. B. Bryant, N. Hislop, R. Shefner, and M. Udo. 2010. Science anxiety, science attitudes, and gender: interviews from a binational study. Journal of Science Education and Technology 19:356–369.

Mandakovic, D., C. Rojas, J. Maldonado, M. Latorre, D. Travisany, E. Delage, A. Bihouée, G. Jean, F. P. Díaz, B. Fernández-Gómez, P. Cabrera, A. Gaete, C. Latorre, R. A. Gutiérrez, A. Maass, V. Cambiazo, S. A. Navarrete, D. Eveillard, and M. González. 2018. Structure and co-occurrence patterns in microbial communities under acute environmental stress reveal ecological factors fostering resilience. Scientific Reports 8:5875.

Marhan, S., and S. Scheu. 2005. Effects of sand and litter availability on organic matter decomposition in soil and in casts of Lumbricus terrestris L. Geoderma 128:155–166.

Marlon, J. R., P. J. Bartlein, A.-L. Daniau, S. P. Harrison, S. Y. Maezumi, M. J. Power, W. Tinner, and B. Vanniére. 2013. Global biomass burning: a synthesis and review of Holocene paleofire records and their controls. Quaternary Science Reviews 65:5–25.

Martin, L. M., K. A. Moloney, and B. J. Wilsey. 2005. An assessment of grassland restoration success using species diversity components. Journal of Applied Ecology 42:327–336.

Mascaro, J., J. A. Harris, L. Lach, A. Thompson, M. P. Perring, D. M. Richardson, and E. C. Ellis. 2013. Origins of the novel ecosystems concept. Pages 45–57 Novel Ecosystems. Wiley-Blackwell.

Masella, A. P., A. K. Bartram, J. M. Truszkowski, D. G. Brown, and J. D. Neufeld. 2012. PANDAseq: paired-end assembler for illumina sequences. BMC Bioinformatics 13:31.

McCracken, J., R. Reid, R. Renfrew, B. Frei, J. Jalava, A. Cowie, and A. Couturier. 2018. Bobolink and Eastern Meadowlark Recovery Strategy Executive Summary. Ministry of the Environment, Conservation and Parks, Ontario, Canada.

McCune, J. L., A. M. Carlsson, S. Colla, C. Davy, B. Favaro, A. T. Ford, K. C. Fraser, and E. G. Martins. 2017. Assessing public commitment to endangered species protection: a Canadian case study. FACETS.

McDonald, D., M. N. Price, J. Goodrich, E. P. Nawrocki, T. Z. DeSantis, A. Probst, G. L. Andersen, R. Knight, and P. Hugenholtz. 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. The ISME Journal 6:610–618.

McGinlay, J., D. J. Parsons, J. Morris, A. Graves, M. Hubatova, R. B. Bradbury, and J. M. Bullock. 2018. Leisure activities and social factors influence the generation of cultural ecosystem service benefits. Ecosystem Services 31:468–480.

McKeague, J. A. 1978. Manual on soil sampling and methods of analysis. 2nd edition. Canadian Society of Soil Science, Ottawa, ON.

McKinley, V. L. 2001. Microbial biomass and activity in soils from virgin prairies compared with prairie restoration, forest and agricultural sites in Illinois. Pages 107–117 *in* N. P. Bernstein

and L. J. Ostrander, editors. Proceedings of the Seventeenth North American Prairie Conference : seeds for the future, roots of the past. Mason City, IA.

McKinley, V. L., A. D. Peacock, and D. C. White. 2005. Microbial community PLFA and PHB responses to ecosystem restoration in tallgrass prairie soils. Soil Biology and Biochemistry 37:1946–1958.

McLachlan, S. M., and A. L. Knispel. 2005. Assessment of long-term tallgrass prairie restoration in Manitoba, Canada. Biological Conservation 124:75–88.

McLauchlan, K. K., J. M. Craine, J. B. Nippert, and T. W. Ocheltree. 2014. Lack of eutrophication in a tallgrass prairie ecosystem over 27 years. Ecology 95:1225–1235.

McLaughlin, S. B., D. G. De La Torre Ugarte, C. T. Garten Jr, L. R. Lynd, M. A. Sanderson, V. R. Tolbert, and D. D. Wolf. 2002. High-value renewable energy from prairie grasses. Environmental Science & Technology 36:2122–2129.

McLaughlin, S. B., and M. E. Walsh. 1998. Evaluating the environmental consequences of producing herbaceous crops for bioenergy. Biomass and Bioenergy 14:317–324.

McNie, E. C. 2007. Reconciling the supply of scientific information with user demands: an analysis of the problem and review of the literature. Environmental Science & Policy 10:17–38.

Mcrill, M., and G. R. Sagar. 1973. Earthworms and seeds. Nature 243:482.

Medin, D. L., and M. Bang. 2014. The cultural side of science communication. Proceedings of the National Academy of Sciences 111:13621–13626.

Medve, R. J. 1984. The mycorrhizae of pioneer species in disturbed ecosystems in western Pennsylvania. American Journal of Botany 71:787–794.

Middleton, E. L., and J. D. Bever. 2012. Inoculation with a native soil community advances succession in a grassland restoration. Restoration Ecology 20:218–226.

Milcu, A., J. Schumacher, and S. Scheu. 2006. Earthworms (Lumbricus terrestris) affect plant

      seedling recruitment and microhabitat heterogeneity. Functional Ecology 20:261–268.

Miller, J. R. 2005. Biodiversity conservation and the extinction of experience. Trends in Ecology &

      Evolution 20:430–434.

Miller, J. R., and R. J. Hobbs. 2007. Habitat restoration—do we know what we're doing? Restoration

      Ecology 15:382–390.

Milliken, F. J., C. A. Bartel, and T. R. Kurtzberg. 2003. Diversity and creativity in work groups: a

      dynamic perspective on the affective and cognitive processes that link diversity and

      performance. Oxford University Press.

Minitab 18.1 statistical software. 2017. . Minitab Inc.

Mock, B. 2014. Are there two different versions of environmentalism, one 'white,' one 'black'?

Moles, A. T., and M. Westoby. 2006. Seed size and plant strategy across the whole life cycle. Oikos

      113:91–105.

Moos, M. T., and B. F. Cumming. 2011. Changes in the parkland-boreal forest boundary in

      northwestern Ontario over the Holocene. Quaternary Science Reviews 30:1232–1242.

Morgan, J. P., D. R. Collicutt, and J. D. Thompson. 1995. Restoring Canada's native prairies: a

      practical manual. Prairie Habitats, Argyle, MB.

Morrison, D. E., and R. E. Dunlap. 1986. Environmentalism and elitism: a conceptual and empirical

      analysis. Environmental Management 10:581–589.

Motta, M. 2018. Explaining science funding attitudes in the United States: the case for science

      interest. Public Understanding of Science:0963662518795397.

Murchie, W. R. 1958. Biology of the oligochaete Eisenia rosea (Savigny) in an upland forest of

      Southern Michigan. The American Midland Naturalist 66:113–31.

Murphy, S. D. 2001. Address to the Society of Ecological Restoration conference. Address.

Murphy, S. D. 2018. Restoration ecology's silver jubilee: meeting the challenges and forging

opportunities. Restoration Ecology 26:3–4.

Murray, D., K. Siler, V. Lariviére, W. M. Chan, A. M. Collings, J. Raymond, and C. R. Sugimoto.

2018. Gender and international diversity improves equity in peer review. bioRxiv:400515.

Nelson, D. M., F. S. Hu, E. C. Grimm, B. B. Curry, and J. E. Slate. 2006. The Influence of Aridity

and Fire on Holocene Prairie Communities in the Eastern Prairie Peninsula. Ecology

87:2523–2536.

Nordström, S., and S. Rundgren. 1972. Methods of Sampling Lumbricids. Oikos 23:344–352.

Nutsford, D., A. L. Pearson, and S. Kingham. 2013. An ecological study investigating the association

between access to urban green space and mental health. Public Health 127:1005–1011.

Nuzzo, V., A. Dávalos, and B. Blossey. 2015. Invasive earthworms shape forest seed bank

composition. Diversity and Distributions 21:560–570.

Nyhan, B., and J. Reifler. 2010. When corrections fail: the persistence of political misperceptions.

Political Behavior 32:303–330.

Ojibway Nature Centre. 2015. Overview of Ojibway Prairie Complex. Department of Parks &

Recreation, Windsor, Ontario.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. Legendre, D. McGlinn, P. R. Minchin, R. B.

O'Hara, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2018.

vegan: Community Ecology Package.

Oldham, M. J., W. D. Backowsky, and D. A. Sutherland. 1995. Floristic quality assessment system

for southern Ontario. Natural Heritage Information Centre, Ontario Ministry of natural

Resources, Peterborough, Ontario.

Orrock, J. L., E. T. Borer, L. A. Brudvig, J. Firn, A. S. MacDougall, B. A. Melbourne, L. H. Yang, D. V. Baker, A. Bar-Massada, M. J. Crawley, E. I. Damschen, K. F. Davies, D. S. Gruner, A. D. Kay, E. Lind, R. L. McCulley, and E. W. Seabloom. 2015. A continent-wide study reveals clear relationships between regional abiotic conditions and post-dispersal seed predation. Journal of Biogeography 42:662–670.

Ozinga, W. A., C. Römermann, R. M. Bekker, A. Prinzing, W. L. M. Tamis, J. H. J. Schaminée, S. M. Hennekens, K. Thompson, P. Poschlod, M. Kleyer, J. P. Bakker, and J. M. V. Groenendael. 2009. Dispersal failure contributes to plant losses in NW Europe. Ecology Letters 12:66–74.

Packard, S., and C. F. Mutel, editors. 1997. The tallgrass restoration handbook. Island Press, Washington D.C.

Packard, S., and L. M. Ross. 1997. Restoring remnants. Pages 63–88 *in* S. Packard and C. F. Mutel, editors. The tallgrass restoration handbook. Washington D.C.

Paiero, S. M., S. A. Marshall, P. D. Pratt, and M. Buck. 2010. Insects of Ojibway prairie, a southern Ontario tallgrass prairie. Pages 199–225 *in* J. D. Shorthouse and K. D. Floate, editors. Arthropods of Canadian grasslands (Volume 1): Ecology and Interactions in Grassland Habitats. Biological Survey of Canada. Biological Survey of Canada.

Palmer, M. A., J. B. Zedler, and D. A. Falk. 2006. Ecological theory and restoration ecology. Pages 1–10 *in* P. A. Falk, M. A. Palmer, and J. B. Zedler, editors. Foundations of Restoration Ecology. Island Press, Washington D.C.

Parada, A. E., D. M. Needham, and J. A. Fuhrman. 2016. Every base matters: Assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environmental Microbiology 18:1403–1414.

Park, D. G. 1989. Soil transfer as a method of moving grassland and marsh vegetation. Pages 264–
280 *in* G. P. Buckley, editor. Biological habitat reconstruction. Belhaven Press, New York,
NY.

Peay, K. G., P. G. Kennedy, and T. D. Bruns. 2008. Fungal community ecology: a hybrid beast with a
molecular master. BioScience 58:799–810.

Pelosi, C., M. Bertrand, Y. Capowiez, H. Boizard, and J. Roger-Estrade. 2009. Earthworm collection
from agricultural fields: comparisons of selected expellants in presence/absence of hand-
sorting. European Journal of Soil Biology 45:176–183.

Pfeffer, J., and R. I. Sutton. 1999. Knowing "what" to do is not enough: turning knowledge into
action. California Management Review; Berkeley 42:83–108.

Pierce, S. M., R. M. Cowling, A. T. Knight, A. T. Lombard, M. Rouget, and T. Wolf. 2005.
Systematic conservation planning products for land-use planning: Interpretation for
implementation. Biological Conservation 125:441–458.

Poff, N. L., J. D. Allan, M. A. Palmer, D. D. Hart, B. D. Richter, A. H. Arthington, K. H. Rogers, J.
L. Meyer, and J. A. Stanford. 2003. River flows and water wars: emerging science for
environmental decision making. Frontiers in Ecology and the Environment 1:298–306.

Poliakoff, E., and T. L. Webb. 2007. What factors predict scientists' intentions to participate in public
engagement of science activities? Science Communication 29:242–263.

Poulsen, J. R., C. W. Osenberg, C. J. Clark, D. J. Levey, and B. M. Bolker. 2007. Plants as reef fish:
fitting the functional form of seedling recruitment. The American Naturalist 170:167–183.

Pratt, P. D. 1989. Ojibway Prairie—A disjunct prairie in southwestern Ontario. North American
Prairie Conference 11:251–252.

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Prendergast, J. R., R. M. Quinn, and J. H. Lawton. 1999. The gaps between theory and practice in selecting nature reserves. Conservation Biology 13:484–492.

Price, G. W., and A. M. Gordon. 1998. Spatial and temporal distribution of earthworms in a temperate intercropping system in southern Ontario, Canada. Agroforestry Systems 44:141–149.

Pyle, R. M. 1978. The extinction of experience. Horticulture 56:64–67.

Quackenbush, P. M., R. A. Butler, N. C. Emery, M. A. Jenkins, E. J. Kladivko, and K. D. Gibson. 2012. Lumbricus terrestris prefers to consume Garlic Mustard Alliaria petiolata seeds. Invasive Plant Science and Management 5:148–154.

Quince, C., A. Lanzen, R. J. Davenport, and P. J. Turnbaugh. 2011. Removing noise from pyrosequenced amplicons. BMC Bioinformatics 12:38.

Quinlan, K. 2005. A landowner's guide to tallgrass prairie and savanna management in Ontario. Tallgrass Ontario, Ridgetown, ON.

R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rau, H., G. Goggins, and F. Fahy. 2018. From invisibility to impact: recognising the scientific and societal relevance of interdisciplinary sustainability research. Research Policy 47:266–276.

Regnier, E., S. K. Harrison, J. Liu, J. T. Schmoll, C. A. Edwards, N. Arancon, and C. Holloman. 2008. Impact of an exotic earthworm on seed dispersal of an indigenous US weed. Journal of Applied Ecology 45:1621–1629.

Reich, E. S. 2013. Science publishing: the golden club. Nature News 502:291.

Revel, R. D. 1993. Canada's rough fescue grasslands: a trial restoration project in Alberta is yielding encouraging results. Restoration & Management Notes 11:117–124.

Reynolds, J. 1994. The distribution of the earthworms (Oligochaeta) of Indiana: a case for the post quaternary introduction theory for megadrile migration in North America. Megadrilogica 5:28–32.

Reynolds, J. 2011a. Earthworms of the University of Guelph Arboretum. Megadrilogica 15:15–20.

Reynolds, J. 2014. A checklist by counties of earthworms (Oligochaeta: Lumbricidae, Megascolecidae and Sparganophilidae) in Ontario, Canada. Megadrilogica 16:111–135.

Reynolds, J., and J. W. Reynolds. 1992. Additional Ontario earthworm county records (Lumbricidae). Megadrilogica 4:143–144.

Reynolds, J. W. 1977. The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Royal Ontario Museum, Toronto, ON.

Reynolds, J. W. 2011b. The earthworms (Oligochaeta: Lumbricidae) of Pelee Island, Ontario, Canada. Megadrilogica 15:23–33.

Rice, C., F. Garcia, C. O. Hampton, and C. Owensby. 1994. Soil microbial response in tallgrass prairie to elevated CO_2. Plant and Soil 165:67–74.

Riley, S. J., J. K. Ford, H. A. Triezenberg, and P. E. Lederle. 2018. Stakeholder trust in a state wildlife agency. The Journal of Wildlife Management 82:1528–1535.

Rillig, M. C. 2004. Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecology Letters 7:740–754.

Ríos-Saldaña, C. A., M. Delibes-Mateos, and C. C. Ferreira. 2018. Are fieldwork studies being relegated to second place in conservation science? Global Ecology and Conservation 14:e00389.

Rist, L., A. Felton, L. Samuelsson, C. Sandström, and O. Rosvall. 2013. A new paradigm for Adaptive Management. Ecology and Society 18.

Roberts, J. A., and D. R. Bacon. 1997. Exploring the subtle relationships between environmental concern and ecologically conscious consumer behavior. Journal of Business Research 40:79–89.

Rodger, L. 1998. Tallgrass communities of southern Ontario: a recovery plan. World Wildlife Fund Canada and the Ontario Ministry of Natural Resources, Toronto, Ontario.

Rodgers, C. S., and R. C. Anderson. 1979. Presettlement Vegetation of Two Prairie Peninsula Counties. Botanical Gazette 140:232–240.

Rosenzweig, M. L. 2003. Win-win ecology: how the earth's species can survive in the midst of human enterprise. Oxford University Press, New York, NY.

Rousk, J., E. Bååth, P. C. Brookes, C. L. Lauber, C. Lozupone, J. G. Caporaso, R. Knight, and N. Fierer. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. The ISME Journal 4:1340–1351.

Rowe, H. I. 2010. Tricks of the trade: techniques and opinions from 38 experts in tallgrass prairie restoration. Restoration Ecology 18:253–262.

Rúa, M. A., A. Antoninka, P. M. Antunes, V. B. Chaudhary, C. Gehring, L. J. Lamit, B. J. Piculell, J. D. Bever, C. Zabinski, J. F. Meadow, M. J. Lajeunesse, B. G. Milligan, J. Karst, and J. D. Hoeksema. 2016. Home-field advantage? evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. BMC Evolutionary Biology 16:122.

Ruess, L., A. Michelsen, I. K. Schmidt, and S. Jonasson. 1999. Simulated climate change affecting microorganisms, nematode density and biodiversity in subarctic soils. Plant and Soil 212:63–73.

Ruiz-Jaen, M. C., and T. M. Aide. 2018. Restoration Success: How Is It Being Measured? Restoration Ecology:569–577.

Russell, E. W. B. 1983. Indian-Set Fires in the Forests of the Northeastern United States. Ecology 64:78–88.

Safford, H. D., S. C. Sawyer, S. D. Kocher, K. J. Hiers, and M. Cross. 2017. Linking knowledge to action: the role of boundary spanners in translating ecology. Frontiers in Ecology and the Environment 15:560–568.

Samson, F., and F. Knopf. 1994. Prairie conservation in North America. BioScience 44:418–421.

Sarewitz, D., and R. A. Pielke. 2007. The neglected heart of science policy: reconciling supply of and demand for science. Environmental Science & Policy 10:5–16.

Schlesinger, W. H. 2010. Translational ecology. Science 329:609–609.

Schmiede, R., T. W. Donath, and A. Otte. 2009. Seed bank development after the restoration of alluvial grassland via transfer of seed-containing plant material. Biological Conservation 142:404–413.

Schramm, P. 1992. Prairie restoration: a twenty-five year perspective on establishment and management. Pages 169–178 *in* D. D. Smith and C. A. Jacobs, editors. Proceedings of the Twelfth North American Prairie Conference : recapturing a vanishing heritage. Cedar Falls, Iowa.

Seastedt, T. R., J. M. Briggs, and D. J. Gibson. 1991. Controls of nitrogen limitation in tallgrass prairie. Oecologia 87:72–79.

Seifert, J. 2017, December 20. Figure for thought: How translational ecology can help science make a difference. National Center for Ecological Analysis and Synthesis.

Shakir, S. H., and D. L. Dindal. 1997. Density and biomass of earthworms in forest and herbaceous microecosystems in central New York, North America. Soil Biology and Biochemistry 29:275–285.

Shanley, P., and C. López. 2009. Out of the loop: why research rarely reaches policy makers and the public and what can be done. Biotropica 41:535–544.

Shermer, M. 2017. How to convince someone when facts fail. https://www.scientificamerican.com/article/how-to-convince-someone-when-facts-fail/.

Shumway, D. L., and R. T. Koide. 1994. Seed preferences of Lumbricus terrestris L. Applied Soil Ecology 1:11–15.

Simis, M. J., H. Madden, M. A. Cacciatore, and S. K. Yeo. 2016. The lure of rationality: Why does the deficit model persist in science communication? Public Understanding of Science 25:400–414.

Simonsen, J., J. Posner, M. Rosemeyer, and J. Baldock. 2010. Endogeic and anecic earthworm abundance in six Midwestern cropping systems. Applied Soil Ecology 44:147–155.

Six, J., S. D. Frey, R. K. Thiet, and K. M. Batten. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal 70:555–569.

Sluis, W. J. 2002. Patterns of species richness and composition in re-created grassland. Restoration Ecology 10:677–684.

Soga, M., and K. J. Gaston. 2016. Extinction of experience: the loss of human–nature interactions. Frontiers in Ecology and the Environment 14:94–101.

Staddon, P. L., A. Heinemeyer, and A. H. Fitter. 2002. Mycorrhizas and global environmental change: research at different scales. Plant and Soil 244:253–261.

Starbuck, W. H. 2006. The production of knowledge: the challenge of social science research. Oxford University Press, New York, NY.

Stover, H. J., R. G. Thorn, J. M. Bowles, M. A. Bernards, and C. R. Jacobs. 2012. Arbuscular mycorrhizal fungi and vascular plant species abundance and community structure in tallgrass prairies with varying agricultural disturbance histories. Applied Soil Ecology 60:61–70.

Stroud, J. L., D. E. Irons, J. E. Carter, C. W. Watts, P. J. Murray, S. L. Norris, and A. P. Whitmore. 2016. Lumbricus terrestris middens are biological and chemical hotspots in a minimum tillage arable ecosystem. Applied Soil Ecology 105:31–35.

Stuckley, R. L. 1981. Origin and development of the concept of the prairie peninsula. Page *in* R. L. Stuckley and K. J. Reese, editors. In the shadow of Transeau: Proceedings of the sixth North American prairie conference, 1978. Ohio Biological Survey Biological Notes, OH.

Swart, J. A. A., J. Zevenberg, P. Ho, J. Cortina, M. Reed, M. Derak, S. Vella, H. Zhao, and H. J. van der Windt. 2018. Involving society in restoration and conservation. Restoration Ecology 26:S3–S6.

Swink, F., and G. Wilhelm. 1994. Plants of the Chicago region. Indiana Academy of Science.

Szeicz, J. M., and G. M. MacDonald. 1991. Postglacial vegetation history of oak savanna in southern Ontario. Canadian Journal of Botany 69:1507–1519.

Talbot, J. M., T. D. Bruns, J. W. Taylor, D. P. Smith, S. Branco, S. I. Glassman, S. Erlandson, R. Vilgalys, H.-L. Liao, M. E. Smith, and K. G. Peay. 2014. Endemism and functional convergence across the North American soil mycobiome. Proceedings of the National Academy of Sciences 111:6341–6346.

Thompson, K., S. R. Band, and J. G. Hodgson. 1993. Seed size and shape predict persistence in soil. Functional Ecology 7:236–241.

Thompson, K., A. Green, and A. M. Jewels. 1994. Seeds in soil and worm casts from a neutral grassland. Functional Ecology 8:29–35.

Thrall, P. H., D. A. Millsom, A. C. Jeavons, M. Waayers, G. R. Harvey, D. J. Bagnall, and J. Brockwell. 2005. Seed inoculation with effective root-nodule bacteria enhances revegetation success. Journal of Applied Ecology 42:740–751.

Throop, W., and R. Purdom. 2006. Wilderness restoration: the paradox of public participation. Restoration Ecology 14:493–499.

Traba, J., F. M. Azcárate, and B. Peco. 2004. From what depth do seeds emerge? A soil seed bank experiment with Mediterranean grassland species. Seed Science Research 14:297–303.

Traba, J., F. M. Azcárate, and B. Peco. 2006. The fate of seeds in Mediterranean soil seed banks in relation to their traits. Journal of Vegetation Science 17:5–10.

Transeau, E. N. 1935. The prairie peninsula. Ecology 16:423–437.

Tuck, E., M. McKenzie, and K. McCoy. 2014. Land education: Indigenous, post-colonial, and decolonizing perspectives on place and environmental education research. Environmental Education Research 20:1–23.

Tucker, C. 2018, March 12. The EEB & Flow: Gained in translation: translational ecology for the Anthropocene.

Turkington, R. A., P. B. Cavers, and E. Rempel. 1978. The biology of Canadian weeds: 29. Melilotus alba Desr. and M. officinalis (L.) Lam. Canadian Journal of Plant Science 58:523–537.

Udo, M. K., G. P. Ramsey, and J. V. Mallow. 2004. Science anxiety and gender in students taking general education science courses. Journal of Science Education and Technology 13:435–446.

Umbanhowar, C. E., P. Camill, C. E. Geiss, and R. Teed. 2006. Asymmetric vegetation responses to mid-Holocene aridity at the prairie–forest ecotone in south-central Minnesota. Quaternary Research 66:53–66.

USDA. 2017. Guide to texture by feel. Govenment. https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/edu/?cid=nrcs142p2_054311.

VandenBygaart, A. J., R. Protz, and A. D. Tomlin. 1999. Changes in pore structure in a no-till chronosequence of silt loam soils, southern Ontario. Canadian Journal of Soil Science 79:149–160.

Voss, E. G., and A. A. Reznicek. 2012. Field manual of Michigan flora. University of Michigan Press, Ann Arbor, MI.

Walker, K. A. 2018. Gender gap in professional entomology: women are underrepresented in academia and the U.S. government. Annals of the Entomological Society of America.

Wall, T. U., E. McNie, and G. Garfin. 2017. Use-inspired science: making science usable by and useful to decision makers. Frontiers in Ecology and the Environment 15:551–559.

Walters, C. J., and C. S. Holling. 1990. Large-scale management experiments and learning by doing. Ecology 71:2060–2068.

Wandersman, A. 1979. User Participation: a study of types of participation, effects, mediators, and individual differences. Environment and Behavior 11:185–208.

Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73:5261–5267.

Webb, J. A., S. C. de Little, K. A. Miller, M. J. Stewardson, I. D. Rutherfurd, A. K. Sharpe, L. Patulny, and N. L. Poff. 2014. A general approach to predicting ecological responses to environmental flows: making best use of the literature, expert knowledge, and monitoring data. River Research and Applications 31:505–514.

Weber, S. 1982. Prairie sod transplant results assessed (Minnesota). Restoration & Management Notes 1:11.

Westgate, M. J., G. E. Likens, and D. B. Lindenmayer. 2013. Adaptive management of biological systems: a review. Biological Conservation 158:128–139.

Westoby, M., E. Jurado, and M. Leishman. 1992. Comparative evolutionary ecology of seed size. Trends in Ecology & Evolution 7:368–372.

Whiles, M. R., and R. E. Charlton. 2006. The ecological significance of tallgrass prairie arthropods. Annual Review of Entomology 51:387–412.

White, R. 1996. "Are you an environmentalist or do you work for a living?": work and nature. Pages 171–185 *in* W. Cronon, editor. Uncommon ground. W.W. Norton & Company, New York, NY.

White, R. M., A. Fischer, K. Marshall, J. M. J. Travis, T. J. Webb, S. di Falco, S. M. Redpath, and R. van der Wal. 2009. Developing an integrated conceptual framework to understand biodiversity conflicts. Land Use Policy 26:242–253.

Wickham, H. 2009. ggplot2: elegant graphics for data analysis. Springer-Verlag, New York, NY.

Willems, J. H., and K. G. A. Huijsmans. 1994. Vertical seed dispersal by earthworms: a quantitative approach. Ecography 17:124–130.

Williams, B. K., and E. D. Brown. 2014. Adaptive management: from more talk to real action. Environmental Management 53:465–479.

Williams, M. A., C. W. Rice, and C. E. Owensby. 2001. Nitrogen Competition in a Tallgrass Prairie Ecosystem Exposed to Elevated Carbon Dioxide.

Wilson, G. W. T., and D. C. Hartnett. 1998. Interspecific variation in plant responses to mycorrhizal colonization in tallgrass prairie. American Journal of Botany 85:1732–1738.

Winslow, H. D., J. P. Scholl, A. E. Sorensen, K. E. Fisher, J. A. Klassen, Calle Leonardo, Kandlikar Gaurav S., Kortessis Nicholas, Kucera Dion C., Marias Danielle E., Narango Desiree L., O'Keeffe Kayleigh, Recart Wilnelia, Ridolfi Elizabeth, and Shea Monika E. 2018. How do we ensure the future of our discipline is vibrant? Student reflections on careers and culture of ecology. Ecosphere 9:e02099.

Wood, T., and E. Porter. 2017. The elusive backfire effect: mass attitudes' steadfast factual adherence. SSRN Scholarly Paper, Social Science Research Network, Rochester, NY.

Worthington, T. R., and D. R. Helliwell. 1987. Transference of semi-natural grassland and marshland onto newly created landfill. Biological Conservation 41:301–311.

Wortley, L., J.-M. Hero, and M. Howes. 2018. Evaluating Ecological Restoration Success: A Review of the Literature. Restoration Ecology:537–543.

Wright, H. E. J. 1968. History of the prairie peninsula. Pages 78–88 Quarternary of Illinois. University of Illinois, Urbana, IL.

Wubs, E. R. J., W. H. van der Putten, M. Bosch, and T. M. Bezemer. 2016. Soil inoculation steers restoration of terrestrial ecosystems. Nature Plants 2:16107.

Wyant, J. G., R. A. Meganck, and S. H. Ham. 1995. A planning and decision-making framework for ecological restoration. Environmental Management 19:789–796.

Xue, X., Y. Luo, X. Zhou, R. Sherry, and X. Jia. 2011. Climate warming increases soil erosion, carbon and nitrogen loss with biofuel feedstock harvest in tallgrass prairie. GCB Bioenergy 3:198–207.

Zaller, J. G., and N. Saxler. 2007. Selective vertical seed transport by earthworms: Implications for the diversity of grassland ecosystems. European Journal of Soil Biology 43:S86–S91.

Zaval, L., and J. F. M. Cornwell. 2016. Cognitive biases, non-rational judgments, and public perceptions of climate change. Oxford Research Encyclopedia of Climate Science.

Zhang, W., K. M. Parker, Y. Luo, S. Wan, L. L. Wallace, and S. Hu. 2005. Soil microbial responses to experimental warming and clipping in a tallgrass prairie. Global Change Biology 11:266–277.

Zhang, Z., S. Schwartz, L. Wagner, and W. Miller. 2000. A greedy algorithm for aligning DNA sequences. Journal of Computational Biology: A Journal of Computational Molecular Cell Biology 7:203–214.

Appendix A

Species compositions of the seed mix used by the Ontario Naturescape company contracted to seed the seeded and seeded+transplant sites using the no-till drill method

Common Name	Latin name
Big Bluestem	*Andropogon gerardi* Vitman
Little Bluestem	*Schizachyrium scoparium* (Michaux) Nash var. *scoparium*
Old Switch Panicgrass	*Panicum virgatum* Linnaeus
Yellow Indiangrass	*Sorghastrum nutans* (Linnaeus) Nash
Prairie Dropseed	*Sporobolus heterolepis* (A. Gray) A. Gray
Canada Wildrye	*Elymus canadensis* Linnaeus
Butterfly Milkweed	*Asclepias tuberosa* Linnaeus
Canada Tick-trefoil	*Desmodium canadense* (Linnaeus) de Candolle
Common Sneezeweed	*Helenium autumnale* Linnaeus
Round-headed Bush-clover	*Lespedeza capitata* Michaux
Wild Bergamot	*Monarda fistulosa* Linnaeus
Common Evening-primrose	*Oenothera biennis* Linnaeus
Virginia Mountain-mint	*Pycnanthemum virginianum* (Linnaeus) B.L. Robinson & Fernald
Grey-headed Prairie Coneflower	*Ratibida pinnata* (Ventenat) Barnhart
Black-eyed Susan	*Rudbeckia hirta* Linnaeus
Hairy Beardtongue	*Penstemon hirsutus* (Linnaeus) Willdenow
Great Blue Lobelia	*Lobelia siphilitica* Linnaeus
Long-headed Anemone	*Anemone cylindrica* A. Gray
Hoary Vervain	*Verbena stricta* Ventenat
Tall Tickseed	*Coreopsis tripteris* Linnaeus