Legacies of afforestation on prairie plant, seed bank, and nematode communities in a northern rough fescue prairie

By

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CHAPTER 1: GENERAL INTRODUCTION

Restoration above and below-ground

Species composition, relative to historical baselines is the most common benchmark of success in the restoration of grassland ecosystems (Samson & Knopf, 1996). However, in a changing climate, recent advances in restoration ecology have acknowledged that incorporating ecosystem function into restoration goals may be a more realistic approach (Higgs et al., 2014; Perring et al., 2015). As the functional success of restoration has increasingly been explored beyond the traditional above-ground components it is now also understood that below-ground communities have a greater role in ecosystem function than previously thought (Kardol and Wardle 2010; Sylvain and Wall 2011). Evidence that measurable differences do exist in soil communities of disturbed, restored, invaded and native ecosystems now indicate that restoration likely relies strongly on the functional composition of the microbiome (Kardol et al. 2005; Holtkamp et al. 2008; Biederman and Boutton 2009; Herzberger et al. 2015).

One of the main challenges to restoration is that its outcomes can often be unpredictable. In some cases, it may be because above and belowground processes and feedback mechanisms are rarely considered and poorly understood (Harris, 2003; Reynolds et al., 2003; Putten et al., 2013). Soil biota play an important role in driving the assembly of plant communities following disturbance and can in turn be transformed by different plant inputs into the soil (Bezemer et al., 2010; de la Peña et al., 2016). The feedback mechanisms between above and belowground communities demonstrate the need for functional bioindicators in restoration to increase the predictability and sustainability of ecosystem management (Pankhurst et al., 1995; De Deyn et al., 2003;

Koziol & Bever, 2017). Of the known feedback mechanisms, most can be separated into positive and negative interactions, with negative feedback acting as the driving force in maintaining species diversity whereas positive feedback promotes species abundance (Reynolds et al., 2003; Bever et al., 2012). These mechanisms can be species-specific and can determine the direction in which a community assembles following disturbance (Herrera-Paredes & Lebeis 2016). This is important within the context of restoration because in order to promote desired community assembly or the growth of target species, a balance between positive and negative feedback is required to maintain community structure, composition, and function (Reynolds et al. 2003).

The persistence of feedback effects between the plant and soil community after a disturbance event, stress or change in community composition presents a challenge for predicting the future of an ecosystem and evaluating the potential to restore it. Effects from these events or conditions that persist and continue to influence the structure of communities are termed ‘legacy effects’ (Cuddington 2011). An example of this can be found in chronological gradients of introduced vegetation. Li et al. (2007) showed that soil community composition in grasslands and shrublands converted to tea plantations at different times depended more on the original plant community than the current plantation’s vegetation. These legacies have also been observed with vegetation removal experiments, where soil community structure is able to persist despite the absence of the original vegetation (Elgersma et al. 2011). For restoration practices such as the removal of invasive species, this suggests that the long-term effects of unwanted vegetation on soil microorganisms could create persistent barriers to community regeneration (Ehrenfeld et al., 2005). Consequently, problems can arise for restoration efforts targeting current plant communities because these may actually be a result of feedbacks from a soil community of the past. Therefore, monitoring restored ecosystems needs to go beyond aboveground composition and consider the responses of belowground communities.

Objectives

The broad goal of my thesis is to explore the effects of afforestation, the establishment of trees in grasslands, on rough fescue prairie communities both above and below-ground to better understand the mechanisms underlying the success of restoration. To achieve this goal, my work looks at two rough fescue prairies converted to white spruce plantations within Riding Mountain National Park that have distinct chronosequences of tree removal. I tested the hypothesis that afforestation legacies can alter prairie plant and soil communities and act as barriers to restoration by examining the composition, diversity, and structure of the plant, seed bank, and soil nematode community along the chronosequence of tree removal. By using a chronosequence approach, I was able to examine restoration progress over time and provide comparisons between above and belowground communities at different stages of community assembly following tree removal.

In my second chapter I explore the effects of afforestation on prairie plant and seed bank communities to determine if restoration of prairie community composition, diversity and structure has been successful and if the seed bank has the potential to contribute to future restoration. I then pursue a more functional approach in my third chapter where I look at afforested prairie soil communities to determine if they have been impacted by the establishment of trees. In this chapter I use nematode communities as bioindicators of the soil food web structure and demonstrate that shifts in feeding groups are associated with afforestation legacies. Overall, my goal for this thesis is to evaluate the importance of monitoring restoration practices from both an above and belowground perspective to see if this improves our understanding of disturbances on declining ecosystems like the rough fescue prairie.

In both chapters 2 and 3 I chose to use redundancy analysis (RDA) to model the differences in community composition along the restoration chronosequence. RDA is a method of constrained ordination that uses eigenvector analysis to determine what proportion of variation observed in the response variable (species composition) can be explained by variation observed in the predictive variable (treatment) (Legendre & Legendre, 2012). This technique is commonly used when species composition is expected to have a linear relationship with the predictor variable (treatment) (2012). I selected this analysis over other methods such as canonical correspondence analysis (CCA) because of the way it can model the success of restoration by highlighting trends in the distribution of abundant species. RDA operates in Euclidean distance space and therefore considers the absolute abundances of all species when partitioning sampling units and would be applicable across the five independent treatments I surveyed. Another technique that is also common is CCA, which is commonly used for community analysis over continuous gradients where species distributions are expected to be unimodal (Kenkel, 2006) and instead operates in Chi-squared space (Legendre & Legendre, 2012). This causes sampling units to be separated and grouped on the basis of rare species. In my case, this does not reflect the goals of restoration well because rare species alone do not define success and that consideration of the whole community distribution and, in particular, abundant species is required. Overall, I found that RDA captured the story of restoration along the chronosequence best and was more appropriate for answering the questions I asked regarding restoration success.

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CHAPTER 2: LEGACY EFFECTS OF AFFORESTATION ON PRAIRIE PLANT AND SEED BANK COMMUNITIES IN A NORTHERN CANADIAN PRAIRIE

Abstract

Afforestation resulting from fire suppression, modified grazing, plantation establishment and climate change poses a threat to northern prairie ecosystems. Trees alter the composition and function of plant and soil communities and can compromise the subsequent restoration of afforested prairies. To evaluate the hypothesis that legacies of afforestation persist in restored prairie communities and decrease the potential for passive restoration, I examined the composition, structure, and diversity of plant and seedbank communities along a 20 year chronosequence of plantation tree removal from a northern rough fescue prairie in Riding Mountain National Park, Manitoba, Canada. Tree removal increased the abundance of weedy species in the plant and seed bank communities and the oldest restored prairies had the lowest diversity after a 20 year period of passive restoration. As a result, time since tree removal and the encroachment of invasive species were key in explaining the composition of restored prairie communities. Low correlation between the species composition of plant and seedbank communities, including the complete absence of *Festuca hallii* in restored treatments, demonstrated that legacies of afforestation eliminated the potential of seedbanks to facilitate passive prairie restoration. I conclude that tree removal alone is insufficient for the restoration of northern fescue prairies and that, in the absence of active management, the persistence of low-diversity plant and seedbank communities constitutes an important legacy of afforestation and a barrier to future restoration.

Introduction

Grasslands are declining globally, with approximately half of existing grassland ecosystems currently affected by degradation and a decline in net primary productivity (Gang *et al.*, 2014). As a result, the vital ecosystem services provided by grasslands, including climate and water regulation (Gordon *et al.*, 2010; Lal *et al.*, 2013), biodiversity conservation (Liebman *et al.*, 2013), and the production of biomass (Tilman & Downing, 1994) have made these ecosystems important targets for conservation and restoration. In addition to existing threats such as fire suppression (Valkó *et al.*, 2014) and the disruption of historic grazing and mowing (Burns *et al.*, 2009; Nagata *et al.*, 2016; Tälle *et al.*, 2016), climate change has recently been recognized as one of the underlying causes of global grassland decline (Van Auken, 2009). Grasslands degraded by the disruption of natural processes have decreased resilience to human disturbance and are more susceptible to ecosystem collapse (MacDougall *et al.*, 2013). Permanent changes in the structure and function of grassland communities have been observed globally (Knapp *et al.*, 2008; Carilla & Grau, 2010; Michielsen *et al.*, 2017) and have coincided with losses of biodiversity, wildlife habitat, carbon sequestration, and pollination (Veldman *et al.*, 2015; Isbell *et al.*, 2017).

Changes in land use and disrupted disturbance regimes have resulted in changes in the dominant vegetation of many grasslands worldwide (Ratajczack *et al.*, 2012). One of the most detrimental changes is the establishment of trees, or afforestation, which threatens grasslands globally and has been accelerated by a combination of forest encroachment and the conversion of grasslands to plantations (Veen et al. 2009, Veldman *et al.*, 2015). As a consequence of afforestation, many grasslands have decreased in their diversity of native grasses and forbs and have lost important plant functional groups including hemi-parasites, C3 and C4 grasses and legumes (Bisteau & Mahy, 2005; Taft & Kron, 2014).

Barriers to grassland recovery following afforestation may persist long after trees have been removed (Knapp *et al*., 2008). These ‘legacy effects’ can continue to impact the function of grasslands (Cuddington, 2012) and have been attributed to the depletion of grassland soil seed banks (Stahlheber *et al.*, 2015), changes in soil carbon sequestration (Thuille & Schulze, 2006; Pinno & Wilson, 2011), nutrient turnover (Chen *et al.*, 2008), pH (Rigueiro-Rodríguez *et al.*, 2012), moisture retention (Nosetto *et al.*, 2005), which can prevent the reestablishment of native grassland species (Briggs *et al.*, 2005). As a consequence, determining if grassland community re-assembly can occur passively following a legacy of afforestation has important implications for grassland conservation and restoration globally.

I aimed to examine the effects of afforestation on the structure, composition, and diversity of threatened Great Plains rough fescue prairies in north-central Manitoba, Canada. Rough fescue prairies are characterized by the climax species *Festuca hallii*. Rough fescue prairie currently occupies less than five percent of its original extent in North America and has experienced considerable declines as a result of tree encroachment and afforestation (Widenmaier & Strong, 2010; Thorpe *et al.*, 2015). The main research questions were: 1) Do afforestation legacies persist over time in both the plant and seed bank communities of passively restored prairies; and 2) What is the potential of soil seedbanks to contribute to prairie community reassembly following afforestation? To test these questions, I examined the structure, composition, diversity, and correlation of the plant and seed bank communities along a chronosequence representing time since tree removal in an afforested northern fescue prairie.

Methods

Study Region

Research was conducted in Riding Mountain National Park, Manitoba, Canada (50°65’ N, 99°97’W). This national park is located in the Boreal Forest Region of north-central Canada (Cody, 1988) and covers an area of 2978 km^2. Daily mean temperatures range from -17.5℃ in January to +17℃ in July, with an average annual precipitation of 488 mm (Environment Canada, 2013). The main soil types in the park belong to the Chernozemic soil order and the Grey-Wooded or Grey-Black Transitional Zone groups with the majority developing over surface deposits of glacial till (Ehrlich *et al.* 1958; Soil Classification Working Group, 1998). Most of the park supports mixed-forest, which includes white spruce (*Picea glauca*), trembling aspen (*Populus tremuloides*), balsam poplar (*Populus balsamifera*), black spruce (*Picea mariana*), and jack pine (*Pinus banksiana*) (Cody, 1988).

Riding Mountain National Park contains a large proportion of the remaining Great Plains rough fescue prairie in Manitoba and western Canada (Trottier, 1986). Fescue prairies throughout western Canada have declined significantly as a consequence of settlement, industrial development, exotic plant invasions, and afforestation (Widenmaier & Strong, 2010; Otfinowski *et al.*, 2007; Thorpe *et al.*, 2015). In Riding Mountain National Park, several fescue prairies were converted to white spruce plantations between 1928 and 1950 to supplement trees removed by logging prior to the establishment of the national park (Higgs, 1993). Plantations were established by centre-hole planting seedlings of white spruce, six feet apart on undisturbed clay-loam till soils covered by northern fescue prairies (Tucker *et al.*, 1968). Recently, several former plantations have been removed to initiate the restoration of historic prairies. At the two sites surveyed (Townsite plantation [22 ha; 50°66'N, 99°95'W]: 1948-2016, Lake Audy plantation [70 ha; 50°45'N, 100°13'W]: 1929-2016), trees were cut in the winter and removed without further management. I acknowledge that my inability to find a perfect replicate of given study at another site due to a lack of comparable restoration chronosequences is a limitation of the study and should be considered when interpreting the results presented. However, I selected two sites that have similar histories of white spruce afforestation and chronosequences of tree removal to evaluate the effects of afforestation legacies on prairie plant and seed bank communities. I observed similar trends in composition, structure and diversity in the plant community at both sites and selected the longer restoration chronosequence at the Lake Audy plantation for the detailed comparisons between the plant and seed bank communities.

The Lake Audy plantation was established on a historic rough fescue prairie in 1929 and is surrounded by patches of remnant fescue prairies, less than 60 ha to the east and south of the study site (Fig. 2.1). Fescue prairies in Riding Mountain National Park are often small (<100 m^2) and are bordered by groves of trees (*Populus tremuloides*, *Populus balsamifera*, *Picea glauca*) and shrubs (*Prunus virginiana, Rosa acicularis, Symphoricarpos occidentalis*), characteristic of the Aspen Parkland bioregion (Thorpe et al. 2015). A larger fescue prairie (approx. 100 ha) borders the study site from the north in a designated enclosure for plains bison (*Bison bison bison*). Most of the surrounding prairies have high concentrations of the exotic grass *Poa pratensis* likely due to the absence of historical grazing and fire which has been prevented by plantation establishment. At the Lake Audy plantation, tree removal in small blocks began in 1997 and continued for twenty years resulting in a chronosequence of treatments representing time since tree removal (1, 4, and 19 years). These treatments have received no further management and are being used to evaluate legacies of afforestation on the restoration of northern fescue prairies.

Sampling Design

Aerial photos (National Earth Observation Data Framework Catalogue, 1931), taken prior to the establishment of the Lake Audy plantation, were used to confirm the historic boundaries of the original fescue prairie. Satellite images (Google Earth v.8.0.4.2346, 2016) were used to map the extent of the white spruce plantation and the extent of disturbance resulting from tree removal. Treatment polygons were selected to represent a chronosequence of restoration following tree removal and were grouped according to the year trees were removed (19 years, 4 years, and 1 year post tree removal). Since all trees were planted in 1929, dates of tree removal correspond to the duration of afforestation (68, 83, and 86 years, respectively) (Fig. 2.1). Polygons of uncut plantation forest and control fescue prairie were also included for comparison.

Inside each treatment polygon, windrows of cut trees, burn piles, and sites of excessive soil disturbance created through tree removal were excluded to reduce variability in site conditions. These features were identified using drone images (3 cm/pixel; Phantom 3 Professional, DJI, Shenzhen, China), captured in May and June of

2016. Using GIS (QGIS v. 2.14.2; QGIS Development Team 2016), 5 m buffers were excluded around all features, including roads and polygon boundaries, windrows, burn piles and sites of excessive disturbance. In addition, five metre buffers were excluded around polygon boundaries to account for edge effects between neighbouring treatments and roads (McDonald & Urban, 2006). The boundaries of the resulting polygons were further adjusted to select areas of dense, uniform tree cover using historic aerial photos (1:20,000; National Earth Observation Data Framework Catalogue, 1959). Polygons were created to capture the chronosequence of restoration and include: control fescue prairie (32 392 m^2), remaining plantation (5253 m^2), 1 year restored (27 320 m^2), 4 years restored (3628 m^2) and 19 years restored (6135 m^2)(Fig. 2.1).

Areas remaining inside each collection of treatment polygons were divided into 5 x 5 m grid cells. I then selected a random sample of 20-35 grid cells within each treatment polygon to focus the measurements of plant and seedbank communities (n = 133). The number for each treatment represents approximately 15% of the total number of quadrats possible inside each polygon. Thirty-five quadrats was set as the maximum sampling intensity which I estimated from species-area accumulation curves constructed using plant diversity data from a nearby northern fescue prairie in July 2015 (Pinchbeck, 2015). I chose to use a neighboring northern fescue prairie to develop the minimum quadrat sampling number because this represents the highest diversity that could be encountered. The coordinates for each grid cell were then uploaded into a handheld GPS (Garmin eTrex 20, Garmin International Inc., Olathe, KS, U.S.A., minimum error ± 3 metres)(Fig. 2.1).

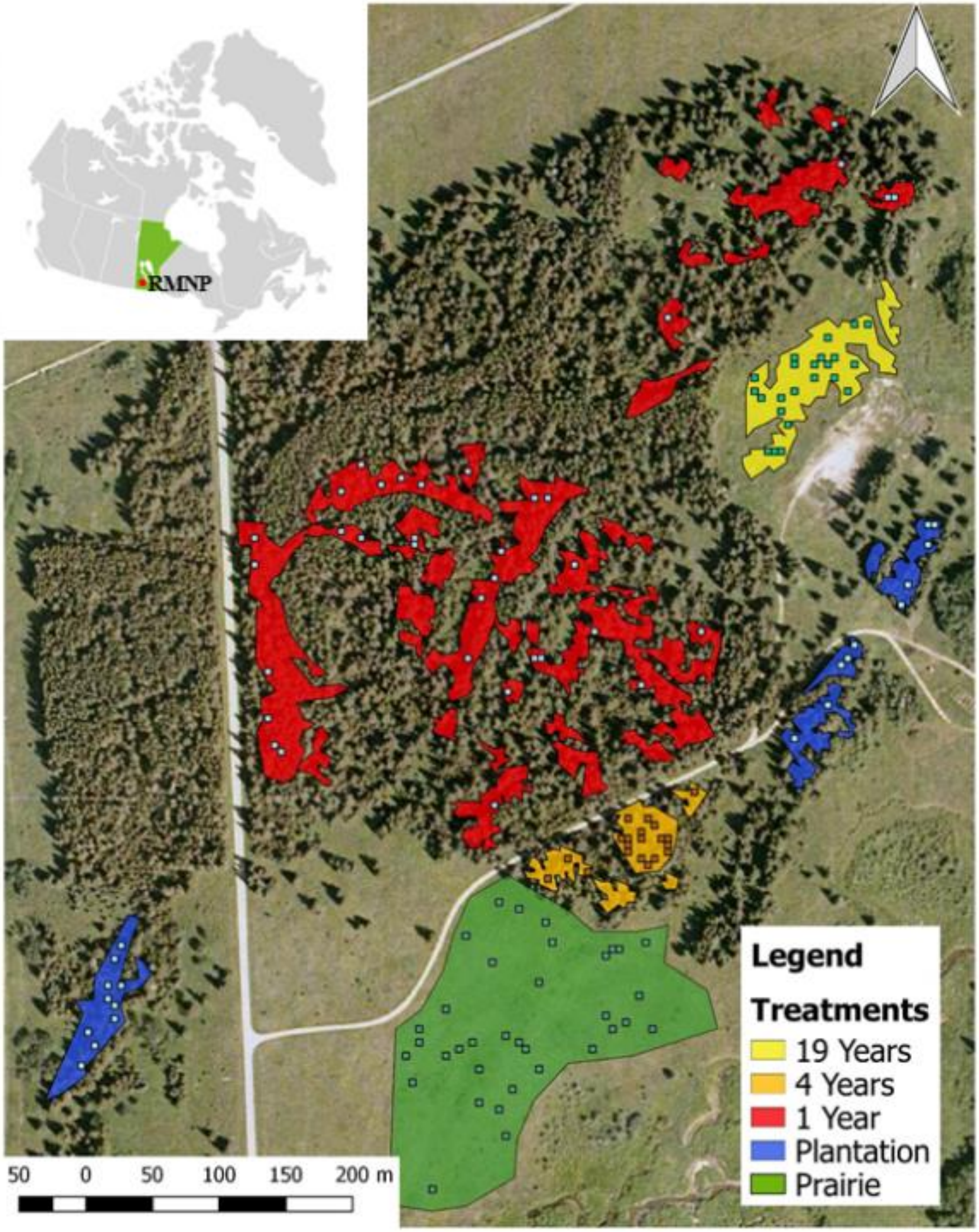


Figure 2.1:
Distribution of sampling quadrats used to evaluate the effects of forest legacies on the restoration of fescue prairies in Riding Mountain National Park (RMNP), Manitoba, Canada. The age of each restoration treatment is denoted by the time since tree removal and the corresponding control fescue prairie and remaining plantation are shown. Quadrats (1 x 1 m) used to sample plant and seed bank communities were selected randomly and are illustrated inside each treatment.

Vegetation Sampling

Plant communities were sampled over six weeks beginning in mid-June 2016 to determine if afforestation legacies correlate with significant differences in the structure, composition, and diversity of plantation, restored, and control fescue prairie communities. The abundance of each herbaceous species was estimated using a pin drop method (Goodall, 1952), inside 1 x1 m quadrats placed in the south-east corner of each 5 x 5 m grid cell. A metal pin (1cm diameter, 70cm length) was dropped 25 times at 20 cm intervals inside each quadrat. At each pin drop, the species of herbaceous plants touching the pin were recorded. Therefore, each species could score up to a maximum of 25 hits per quadrat. This technique yields an estimate of cover for each species based on the percentage of hits per quadrat (Goodall, 1952). Recorded frequency data were then converted to percent cover by taking the proportion of hits out of 25 and multiplying it by 100. For shrub species, percent cover was recorded within the 1m x 1m quadrat using a visual estimate (1-100%).

Seed Bank

I collected soil cores to examine the effect of afforestation on the composition and diversity of prairie seedbank communities and to evaluate the potential of the seed bank to contribute to the restoration of afforested prairies. Soil cores were collected in the fall (October 2016) from the southeast corner of each quadrat (1 x 1 m, n=133) to compare the seedbank with the plant community. Cores (10 cm deep, 7 cm diameter) were placed in sealed plastic bags and stored at 4℃ for 6 weeks to break dormancy (Baskin & Baskin 1989). Following cold stratification, samples were homogenized manually and a subsample was spread in a 1 cm thick layer over soilless potting mix (Sunshine Mix #4, SunGro Horticulture, Hadashville, MB, Canada) inside plastic pots (12.3 X 12.3 cm). Pots were arranged randomly by treatment in a greenhouse and rotated every 2 weeks. Buffer pots with no sample soil were placed around the perimeter of the bench and between treatments to check for cross-contamination between pots. Control pots containing soilless potting mix were placed on a separate bench to check for seed contamination. The samples were misted with water once every 24 hours for 5 minutes and were maintained in 16-hour day (21°C) and 8-hour night (15°C) conditions (Thompson & Grime 1979) under a combination of sodium-iodide lamps and natural lighting. The first germination period was terminated after 10 weeks. Any seedlings that could not be identified were transplanted and allowed to grow until they were identifiable. Once complete, the pots were returned to cold storage (4°C) for a second cold stratification period of 6 weeks and then placed back in greenhouse conditions for an additional 5 weeks. The experiment was terminated after no new seedlings had emerged for two weeks.

Analysis

I evaluated the hypothesis that legacies of afforestation can persist in prairie communities using plant structure, composition, and diversity which I compared between restored treatments and the control fescue prairie. I calculated the mean species richness (s), Shannon-Weaver diversity ($H' = -\sum_{i=1}^{s} p_i log p_i$), Pielou's evenness ($J = H'/\log(s)$) and Sørensen's beta diversity (S = 2a/(2a+b+c), where a = shared species of quadrat 1 and 2, b = species unique to quadrat 1, and c = species unique to quadrat 2)(Legendre &

Legendre, 2012) for all pairwise comparisons within each treatment (control fescue prairie, remaining plantation and 1, 4 and 19 year old restored treatments) and used a one-way ANOVA and Tukey's post hoc tests to determine if significant differences exist between restored treatments. I log-transformed data prior to analysis and assessed the normality of the residuals using the Shapiro-Wilks test (W=0.29-0.98, $0.001<p<0.09$) and quantile-quantile plots of each community measure. I selected the Sørensen index to compare beta diversity among treatments because of the greater weight it assigns to the presence of shared species (Legendre & Legendre, 2012). Abundance-based distance measures of beta diversity, including Chord and Bray-Curtis distances (Magurran & McGill, 2011) yielded similar results and are not presented here. Plant and seed bank community structure was illustrated using species assignments to growth form based on McGregor *et al.* (1986).

I performed redundancy analysis (RDA) on the existing plant and seed bank community data to determine how afforestation legacies are expressed in restored community composition in relation to the control fescue prairie. RDA is a method of constrained ordination that uses eigenvector analysis to determine what proportion of variation observed in the response variable (species composition) can be explained by variation observed in the explanatory variable (treatment) (Legendre & Legendre, 2012). The significance of the RDA model and the variation explained by the treatment was tested using Monte-Carlo permutations to determine if the observed variation explained by each ordination axis was higher than the variation explained for models generated by random permutation of the plant community data along each axis (Oksanen *et al.*, 2017).

To test if plant and seedbank communities are correlated with each other in control and restored prairies I performed a Mantel test to compare the two communities (Legendre & Legendre, 2012). I used the Sørensen dissimilarity index between quadrats to determine if species composition was significantly different between the plant and seed bank communities within each treatment.

All statistical analyses were performed in R v.3.4.0 using the packages ‘stats’ v. 3.4.0 (R Core Team, 2017) and ‘vegan’ v. 2.4-3 (Oksanen *et al.*, 2017)

Results

Plant Community

I found 131 plant species (40 families) in the existing plant community and determined that structure, diversity, and composition of restored northern prairies was related to a legacy of afforestation, however, none of the restored treatments resembled the control fescue prairie even after 19 years following the removal of trees. Structurally, shrubs and exotic forbs were most abundant in recently restored prairies ($F_{4,128}$=27.05, p<0.0001, and $F_{4,128}$=13.06, p<0.0001, respectively) (Table 2.1A, Table 2.3A), whereas the 19 year old restored treatment and the control fescue prairie had the highest abundance of graminoids ($F_{4,128}$=52.22, p<0.0001) (Table 3A). However, most graminoids in the 19-year-old restored treatment were exotic, including two invasive grasses *Poa pratensis* and *Bromus inermis* (Table 2.1A), and lacked *Festuca hallii,* a target species for the restoration of northern fescue prairies, which was also absent from all other restored treatments

Table 2.1: Mean percent cover (±SD) of top ten species in A) aboveground plant community and the mean number of seedlings in the B) seed bank across a restoration chronosequence within a northern fescue prairie affected by afforestation in Riding Mountain National Park, Manitoba, Canada. The time since tree removal is shown in addition to the top ten species from the remaining plantation and control fescue prairie. Taxonomy standardized using VASCAN (Brouillet et al. 2010). Exotic species are in bold. Unidentified *Carex* species may include: *C. capillaris*, *C. deweyana*, *C. hookeriana*, *C. inops* and *C. praticola*.

Plantation		**1 Year**		**4 Years**		**19 Years**		**Prairie**	
A) PLANT COMMUNITY									
Species	% ± S.D.	Species	% ± S.D.	Species	% ± S.D.	Species	% ± S.D.	Species	% ± S.D.
Rubus idaeus	45 ± 37	*Rubus idaeus*	52 ± 32	*Fragaria virginiana*	36 ± 25	***Poa pratensis***	67 ± 39	***Poa pratensis***	93 ± 11
Carex sp.	21 ± 32	*Fragaria virginiana*	22 ± 31	***Poa pratensis***	35 ± 34	***Bromus inermis***	58 ± 40	*Festuca hallii*	54 ± 30
Urtica dioica	14 ± 19	***Poa pratensis***	21 ± 35	***Sonchus arvensis***	35 ± 20	*Carex* sp.	19 ± 23	*Galium boreale*	21 ± 12
Sciurohypnum plumosum	14 ± 25	***Sonchus arvensis***	20 ± 19	*Carex* sp.	31 ± 31	***Sonchus arvensis***	14 ± 20	*Fragaria virginiana*	12 ± 17
Bromus inermis	12 ± 30	***Lappula squarrosa***	19 ± 20	*Rubus idaeus*	25 ± 29	***Cirsium arvense***	13 ± 14	*Achillea millefolium*	9 ± 8
Fragaria virginiana	10 ± 16	***Galeopsis tetrahit***	19 ± 19	***Bromus inermis***	24 ± 32	*Fragaria virginiana*	13 ± 18	*Symphyotrichum laeve*	9 ± 10
Bryum sp.	10 ± 13	***Cirsium arvense***	15 ± 14	***Cirsium arvense***	21 ± 15	*Galium boreale*	8 ± 12	*Carex* sp.	9 ± 10
Prunus virginiana	9 ± 21	*Urtica dioica*	13 ± 13	***Poa compressa***	11 ± 19	*Hesperostipa spartea*	5 ± 13	*Monarda fistulosa*	8 ± 11
Cirsium arvense	8 ± 11	*Bromus ciliatus*	12 ± 21	*Urtica dioica*	7 ± 9	*Achillea millefolium*	4 ± 8	*Thalictrum venulosum*	6 ± 6
Symphoricarpos occidentalis	7 ± 18	*Carex* sp.	9 ± 15	*Symphoricarpos occidentalis*	6 ± 11	*Agastache foeniculum*	4 ± 6	*Artemisia ludoviciana*	6 ± 7
B) SEED BANK									
Species	Num. ± S.D.	Species	Num. ± S.D.	Species	Num. ± S.D.	Species	Num. ± S.D.	Species	Num. ± S.D.
Urtica dioica	3.5 ± 4.7	*Urtica dioica*	6.8 ± 10.9	*Urtica dioica*	9.9 ± 14.1	***Poa pratensis***	3.5 ± 3.9	***Poa pratensis***	1.8 ± 1.8
Lappula squarrosa	1.2 ± 4.0	***Lappula squarrosa***	1.7 ± 3.7	***Sonchus arvensis***	2.2 ± 2.4	***Artemisia absinthium***	1.0 ± 2.3	*Agastache foeniculum*	0.6 ± 1.0
Rubus idaeus	0.7 ± 0.9	***Poa compressa***	0.8 ± 2.2	***Lappula squarrosa***	1.6 ± 2.4	*Poa palustris*	1.0 ± 1.6	*Symphyotrichum laeve*	0.4 ± 0.9
Carex sp.	0.6 ± 1.6	***Cirsium arvense***	0.7 ± 1.0	***Poa pratensis***	1.2 ± 2.3	*Urtica dioica*	1.0 ± 3.3	*Campanula rotundifolia*	0.3 ± 0.7
Campanula rotundifolia	0.6 ± 1.2	***Poa pratensis***	0.7 ± 2.2	***Cirsium arvense***	1.1 ± 1.3	*Agastache foeniculum*	0.7 ± 1.1	*Achillea millefolium*	0.2 ± 0.5
Androsace septentrionalis	0.5 ± 0.8	***Sonchus arvensis***	0.6 ± 1.1	*Rubus idaeus*	0.9 ± 1.9	***Galeopsis tetrahit***	0.5 ± 1.0	*Fragaria virginiana*	0.2 ± 0.4
Heuchera richardsonii	0.4 ± 1.2	*Fragaria virginiana*	0.6 ± 1.9	***Galeopsis tetrahit***	0.8 ± 1.0	***Poa compressa***	0.5 ± 1.1	*Monarda fistulosa*	0.2 ± 0.5
Agastache foeniculum	0.3 ± 0.5	***Galeopsis tetrahit***	0.6 ± 1.2	*Androsace septentrionalis*	0.4 ± 0.9	***Cirsium arvense***	0.4 ± 0.7	*Androsace septentrionalis*	0.1 ± 0.4
Cirsium arvense	0.3 ± 0.7	***Poa annua***	0.5 ± 2.9	*Agastache foeniculum*	0.3 ± 0.6	*Stellaria longipes*	0.3 ± 1.0	*Artemisia dracunculus*	0.1 ± 0.6
Fragaria virginiana	0.3 ± 0.4	*Poa palustris*	0.5 ± 1.7	*Rudbeckia hirta*	0.3 ± 0.9	*Symphyotrichum laeve*	0.3 ± 0.8	*Rudbeckia hirta*	0.1 ± 0.5

Table 2.2: Mean (+/- S.D.) species richness (s), alpha diversity (*H'*) and evenness (J) of the plant community (A) and seed bank (B) across the chronosequence of fescue prairie restoration in Riding Mountain National Park, MB, Canada. The time since tree removal is shown in addition to values from the remaining plantation and control fescue prairie (DOF = 4, 128). Post-hoc test results on treatments means are shown in superscript letters with each letter representing a group that does not have significantly different means at the p<0.05 confidence level.

	Plantation	**1-Year**	**4-Years**	**19-Years**	**Prairie**	F-value	p-value
A) Plant Community							
Richness (s)	12.1 ± 4.0^{b}	12.7 ± 3.2^{b}	12.6 ± 3.7^{b}	10.5 ± 3.7^{b}	17.5 ± 4.9^{a}	13.80	<0.0001
Diversity (*H*')	1.85 ± 0.61^{bc}	2.01 ± 0.34^{ab}	2.07 ± 0.26^{ab}	1.69 ± 0.49^{c}	2.21 ± 0.25^{a}	7.05	<0.0001
Evenness (J)	0.74 ± 0.19^{ab}	0.81 ± 0.06^{a}	0.83 ± 0.4^{a}	0.72 ± 0.14^{b}	0.78 ± 0.04^{ab}	4.17	<0.005
B) Seed Bank							
Richness (s)	4.10 ± 1.71^{bc}	5.60 ± 2.48^{ab}	6.05 ± 2.11^{a}	5.48 ± 2.19^{ab}	3.17 ± 1.48^{c}	10.83	<0.0001
Diversity (*H*')	1.10 ± 0.47^{ab}	1.27 ± 0.47^{a}	1.38 ± 0.46^{a}	1.42 ± 0.48^{a}	0.96 ± 0.47^{b}	5.51	<0.001
Evenness (J)	0.66 ± 0.25^{ab}	0.68 ± 0.19^{a}	0.71 ± 0.17^{a}	0.77 ± 0.14^{a}	0.65 ± 0.26^{b}	4.12	<0.005

Table 2.3: A) Mean percent cover (± SD) and B) mean proportion of seedlings (± SD) according to growth form across all treatments along the fescue prairie restoration chronosequence in Riding Mountain National Park, MB, Canada for both the A) plant community and B) seed bank (DOF = 4, 128). The time since tree removal is shown in years in addition to values from the remaining plantation and control fescue prairie. Post-hoc test results on treatments means are shown in superscript letters with each letter representing a group that does not have significantly different means at the $p<0.05$ confidence level.

	Plantation	**1-Year**	**4-Years**	**19-Years**	**Prairie**		
A) Plant Community						F-value	p-value
Graminoids	22.4 ± 18.9^{c}	24.3 ± 15.5^{c}	45.6 ± 17.5^{b}	68.6 ± 12.2^{a}	60.3 ± 11.3^{a}	52.22	<0.0001
Forbs	31.8 ± 17.0^{bc}	52.2 ± 17.1^{a}	42.1 ± 9.2^{ab}	28.7 ± 13.1^{c}	36.6 ± 10.2^{bc}	13.06	<0.0001
Shrubs	35.4 ± 25.0^{a}	23.4 ± 15.4^{b}	12.3 ± 11.0^{c}	2.6 ± 3.7^{c}	3.0 ± 3.7^{c}	27.05	<0.0001
B) Seed Bank							
Graminoid	0.21 ± 0.28^{b}	0.22 ± 0.29^{b}	0.13 ± 0.16^{b}	0.43 ± 0.28^{a}	0.43 ± 0.30^{a}	6.42	<0.0001
Forbs	0.69 ± 0.29^{ab}	0.75 ± 0.29^{ab}	0.84 ± 0.16^{a}	0.57 ± 0.28^{b}	0.57 ± 0.30^{b}	4.55	<0.005
Shrubs	0.08 ± 0.15^{a}	0.03 ± 0.07^{ab}	0.03 ± 0.07^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	5.01	<0.001
Trees	0.02 ± 0.04^{a}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}	4.62	<0.005

Increasing time since the removal of trees was not associated with the reassembly of the species rich, diverse and heterogenous plant communities characteristic of the control fescue prairie. Species richness ($F_{4,128}$ =13.80, $p < 0.0001$) and Shannon-Weaver diversity ($F_{4,128} = 7.05$, $p < 0.0001$) remained highest in the native prairie and, with the exception of the most recently restored treatments, was lowest in the oldest restored treatment (Table 2.2). Restored prairies were the most homogeneous 20 years following tree removal ($F_{4,128}$ =4.17, $p < 0.005$) (Table 2.2) and did not reach the level of beta diversity observed in the control fescue prairie (Fig. 2.3A).

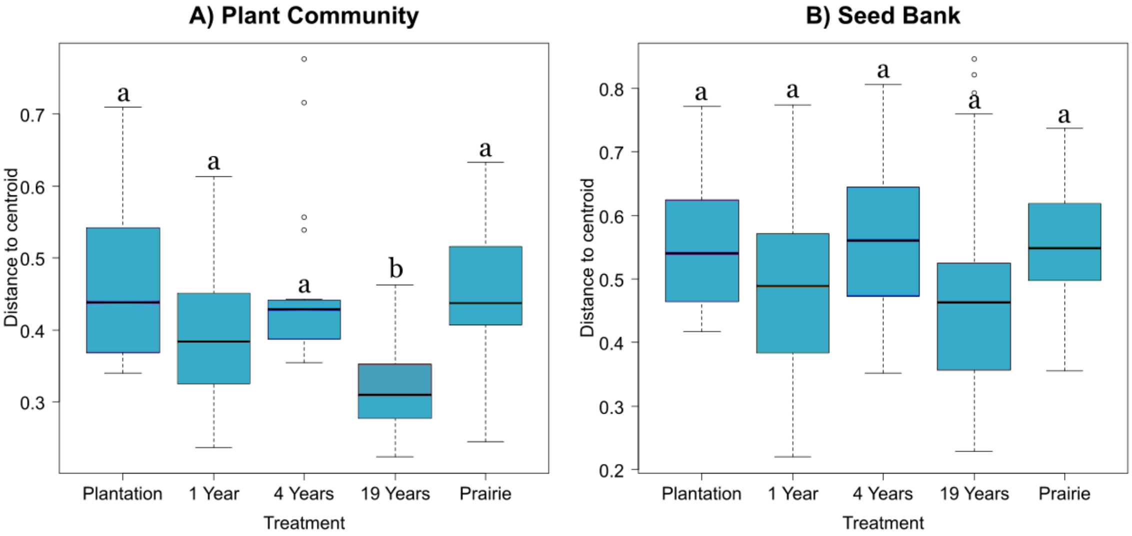
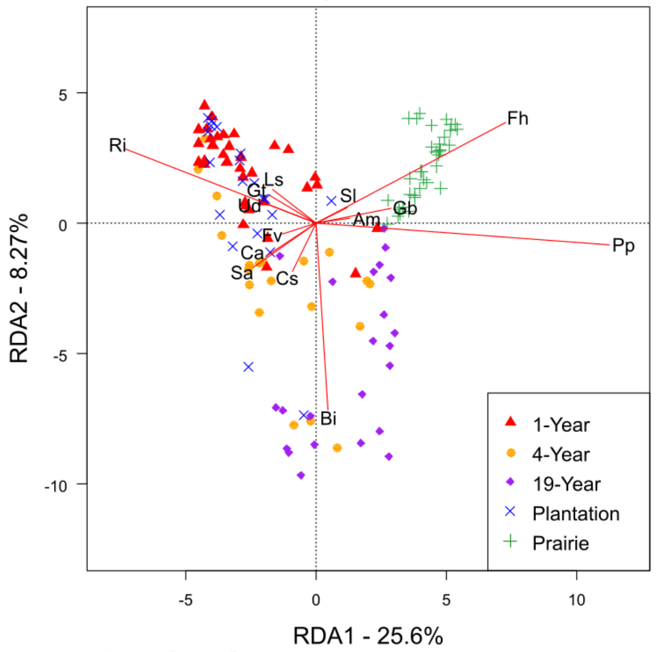
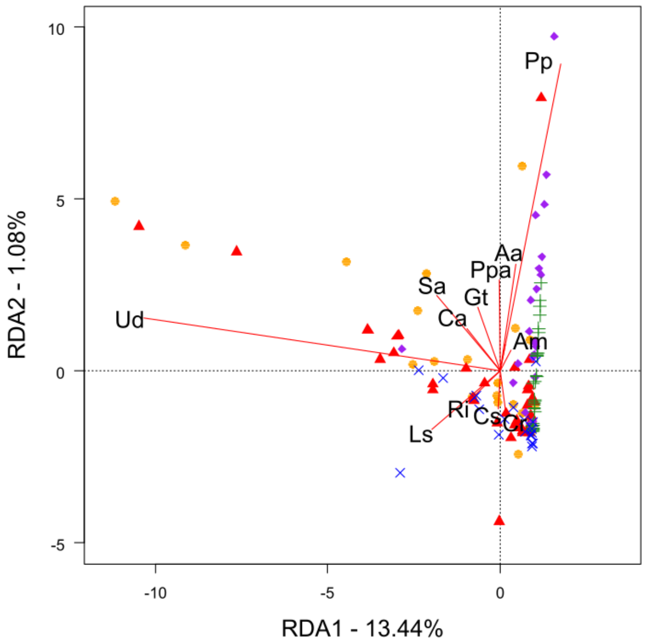


Figure 2.3: Beta diversity of A) the plant community ($F_{4,128}$= 9.11, p<0.0001) and B) the seed bank ($F_{4,128}$= 2.63, p=0.038) using Sørensen's index for the restoration chronosequence at an afforested fescue prairie restoration site in Riding Mountain National Park, MB, Canada. The time since tree removal is shown in addition to values from the remaining plantation and control fescue prairie.

The compositional changes of northern prairie communities following afforestation and tree removal were most evident in the proportion of weedy and invasive species in the plant community. The first axis of the constrained ordination (25.6% of variation explained, $p<0.001$) separated quadrats based on the most abundant grasses in the control fescue prairie, *Poa pratensis*, and *Festuca hallii*, from those in areas where trees had been removed recently which were instead characterized by disturbance-associated species *Urtica dioica*, *Rubus idaeus*, *Sonchus arvensis*, *Cirsium arvense* and *Galeopsis tetrahit* (Fig. 2.2A). The second ordination axis (8.3% of variation explained, $p<0.001$) separated quadrats in older restored areas, invaded by *Bromus inermis*, from all others characterized by the presence of native species *Festuca hallii* or those in prairies recently cleared of trees and characterized by the presence of the shrub, *Rubus idaeus.*



A) Plant Community



B) Seed Bank

Figure 2.2: Redundancy analysis of sampling sites, species and treatments for A) plant community and B) the seed bank along the chronosequence of tree removal at an afforested fescue prairie restoration site in Riding Mountain National Park, MB, Canada. The time since tree removal is shown in addition to values from the remaining plantation and control fescue prairie. Species that have eigenvector elements near zero have been excluded. Am=*Achillea millefolium*, Aa=*Artemisia absinthium*, Bi=*Bromus inermis*, Cr=*Campanula rotundifolia*, Cs=*Carex* sp., Ca= *Cirsium arvense*, Fh=*Festuca hallii*, Gt= *Galeopsis tetrahit*, Gb=*Galium boreale*, Ls= *Lappula squarrosa*, Ppa=*Poa palustris*, Pp=*Poa pratensis*, Ri=*Rubus idaeus*, Sa= *Sonchus arvensis*, Sl= *Symphyotrichum laeve*.

Seed Bank

The structure and diversity of seed banks in all restoration treatments and the remaining forest did not resemble the control fescue prairie, even after 19 years following the removal of trees (Table 2.1B). A total of 1711 seedlings from 62 species emerged over the course of the germination period. The density of germinable seeds per sample ranged from 7 to 427 seeds per 1000 cm^3 of soil. The mean density of seeds per sample for the remaining plantation, 1 year restored, 4 year restored, 19 year restored, and control fescue prairie were 65.3, 120, 140, 83.1, and 34.1 seeds per 1000 cm^3 of soil respectively ($F_{4,128}$=11.74, p<0.0001). No seedlings emerged from both the buffer and control pots over the entire germination period indicating that contamination was not a factor.

Like the aboveground plant community, the remaining plantation and early restored prairies were characterized by a high proportion of forbs ($F_{4,128}$=4.55, p<0.005) both native and exotic (Table 2.1B) and were the only treatments to contain shrubs ($F_{4,128}$=5.01, p<0.001) in the seed bank (Table 2.3B). Seed banks in the oldest tree removal treatment was the most abundant in graminoids ($F_{4,128}$=6.42, p<0.0001) and had no woody species (Table 2.3B). The potential of seed banks to restore afforested prairies was compromised by the absence of native species. Instead, all restored treatments contained a high proportion of disturbance-associated plants including *Urtica dioica, Lappula squarrosa*, and *Galeopsis tetrahit* which were present in the seed bank up to 19 years following tree removal (Table 2.1B). In contrast, *Festuca hallii*, a target species for the restoration of northern fescue prairies, was absent from all restored treatments and most importantly, the control prairie. Although weedy species contributed to the higher species richness ($F_{4,128}$ =10.83, $p < 0.001$) and Shannon-Weaver diversity ($F_{4,128} = 5.51$, $p < 0.001$) of restored treatments (Table 2.2B), invasion of restored prairies by exotic *Bromus* inermis and *Poa pratensis* (Table 2.1) increased the evenness compared with the undisturbed control fescue prairie ($F_{4,128}$ =4.12, $p < 0.005$, Table 2.2B).

Similar to the above-ground plant community, the soil seed bank was also affected by afforestation legacies with the time since tree removal and the presence of invasive species explaining the greatest proportion of variation in the seed bank (Fig. 2.2B). The first two axes of the RDA explained 14.5% of the variation in the seed bank when constrained by time since restoration (treatment). On the first ordination axis seed bank samples were split based on the presence or absence of disturbance-related species characteristic of recently restored areas that included *Urtica dioica*, *Sonchus arvensis* and *Lappula squarrosa* (13.4% of variation explained, p<0.001).

Mantel Test

With the exception of 1 year restored prairies ($r_M = 0.170$, p = 0.026), plant and seed bank communities were not significantly correlated with one another within remaining restored and control fescue prairies (r_M: -0.056 to 0.209, p= 0.026-0.718) (Table 2.4).

Table 2.4: Mantel test scores comparing the similarity between the existing plant community and the seed bank along a restoration chronosequence at an afforested fescue prairie in Riding Mountain National Park, MB, Canada. Similarity matrices based on Sørensen’s presence-absence index (permutations=999).

	Treatment				
	Plantation	**1-Year**	**4-Years**	**19-Years**	**Prairie**
r_M	0.181	0.170	0.209	-0.056	-0.026
p-value	0.080	0.026	0.061	0.718	0.600

Discussion

The results of this study suggest that legacies of afforestation continue to alter the structure, diversity and composition of northern rough fescue prairie plant communities, even 19 years following the removal of trees, and that passive restoration does not occur. In this study, legacies of white spruce afforestation have decreased the abundance of native species and increased the abundance of exotic and invasive species in both the plant community and seed bank and have significantly reduced diversity, evenness and heterogeneity of restored prairies. Furthermore, the absence of plains rough fescue (*Festuca hallii*) in all restored prairies highlights the challenges of restoring northern fescue prairies following afforestation and the limited availability of native propagules in the seedbank.

The increase in diversity in the aboveground plant community after tree removal observed in my study, followed by a sharp decline as the age of the restored prairie increased, could be attributed to the release of competitive pressures and the flush of nutrients that often occurs following tree removal (Palviainen *et al.*, 2004; Alford *et al.*, 2012). Consequently, the observed increase in diversity, characterized by a high abundance of exotic weeds, is consistent with other tree removal experiments, where very few native species were reported in establishing plant communities (Brudvig, 2010; Rossi *et al.*, 2011). However, in my work, many of these weedy species, including *Urtica dioica*, *Lappula squarrosa* and *Galeopsis tetrahit*, were short-lived in the aboveground plant community and were replaced by several exotic invasive graminoids that became dominant in the oldest restored treatment. The resulting loss of species richness, as well as a gradual decrease in the diversity and the heterogeneity of restored communities is common in passively restored prairies (Poschlod *et al.*, 1998; Tognetti *et al.*, 2010;

Zaloumis & Bond, 2011) and demonstrates a major barrier to restoring afforested grasslands without active management.

The success of passive restoration may depend on the composition of species in the afforested prairies. For example, Halpern *et al*. (2012) described an increase in the cover and richness of meadow species following the removal of *Abies grandis* and *Pinus contorta* in Oregon, USA, but that the presence of *Carex inops* subsp. *inops*, a native, invasive graminoid can impair community reassembly (Halpern *et al*. 2016). In the northern Great Plains, northern fescue prairies are threatened by clonal, exotic invaders, including smooth brome (*Bromus inermis*) and Kentucky bluegrass (*Poa pratensis*) that have escaped from cultivation and invade native prairie communities in the absence of disturbance (Sinkins & Otfinowski, 2012). The expected recovery of the richness and diversity of native species in this study did not occur in the 20-year period following tree removal. Consequently, long-term success of restored northern fescue prairies depends on managing invasive species and the maintenance of historic grazing and fire disturbances to reduce the abundance of exotic species and allow native species to establish (Gerling & Bailey, 1994; White *et al.*, 2012, Otfinowski *et al.*, 2017).

Consistent with seed banks from a range of afforested grassland ecosystems (Maccherini & De Dominicis, 2003; Bisteau & Mahy, 2005; Lang & Halpern, 2007; Koyanagi *et al.*, 2011), I found that a legacy of tree growth had depleted the seed bank of prairie species and that disturbance-associated species were instead very abundant in all restored communities (Zylka *et al.*, 2016) preventing passive restoration (Bossuyt & Honnay, 2008; Brudvig, 2010). In this case, the persistence of *Urtica dioica, Lappula squarrosa,* and *Galeopsis tetrahit* across all restoration treatments indicates that these species may characterize future communities upon the introduction of disturbance, regardless of the time since tree removal. The persistence of exotic weeds as a result of plantation legacy suggests that the current seed bank will strongly influence the overlying community initially following tree removal, but this community could be replaced by a small number of invasive exotic species.

Detrimental to the success of restoration in this system was the absence of the majority of fescue prairie species including the characteristic climax species *Festuca hallii*. Other studies have demonstrated a similar low abundance of climax species in restored areas after prolonged periods of tree cover (Bisteau & Mahy, 2005; Ratajczak *et al.*, 2012; Taft & Kron, 2014). In this study, the absence of *Festuca hallii* in restored treatments is likely explained by the sharp decline in viability that this species experiences over time (Romo, 1996) which may exacerbate the effect of afforestation legacies on restoration potential. However, the absence of *F. hallii* combined with the low diversity of species in the control fescue prairie seed bank indicates that there may be additional factors preventing restoration. For example, *F. hallii* produces seed infrequently (Toynbee, 1987) which could affect its ability to create sustainable seed banks. In the northern fescue prairies, native species recruitment in the seed bank has been positively related to regular burning (Ren & Bai, 2017). In this system, fire was suppressed in fescue prairies surrounding the historic plantation and the results point to the importance of integrating surrounding landscapes in the long-term restoration of afforested prairies (Koper *et al.*, 2010; Sengl *et al.*, 2015). As a result, the negative effects of the plantation on prairie restoration may have indirectly extended to the neighbouring native prairie and ultimately reduced its ability to contribute positively to restoration. The connection between surrounding landscapes, prairie species dispersal limitations, and the restoration success of afforested prairies, especially in the highly fragmented northern fescue prairies, has not been explicitly tested (but see: Qiu et al. 2010) and requires further research.

The impact of afforestation on the composition, diversity, and structure of northern fescue prairies, was expressed differently in the plant community and seed bank at this site. These results indicate that only the most recently disturbed treatment's seed bank (1-year restored) was correlated with the aboveground vegetative community and that seed bank composition can be independent of the aboveground vegetation which presents a problem for the restoration of northern fescue prairie communities. The pattern of exotic weed accumulation in the seed bank has been observed in other grasslands, where it is also poorly correlated with the composition of the overlying vegetation (Bisteau & Mahy, 2005; Lang & Halpern, 2007; Vila & Gimeno, 2007, Zylka *et al.*, 2016) indicating that restoration of former communities cannot rely passively on the seed bank. In addition, the prolific nature of many of the ephemeral weeds observed may explain why more recently restored treatments resemble their seed banks closely compared to the native prairie species, which rely more on perennial vegetative growth (Benson & Hartnett, 2006; Bossuyt & Honnay, 2008).

Conclusions

The results of this study suggest that tree removal is insufficient for the restoration of northern fescue prairies and that the persistence of low-diversity plant communities, consisting of invasive grasses, constitutes an important legacy of afforestation and an important barrier to passive restoration. Future work examining how afforestation can modify environmental conditions that leave grassland ecosystems susceptible to invasion by exotic species will be required to determine which types of management, including grazing and fire, will increase restoration success. In addition, exploring prairie species dispersal limitations and considering surrounding landscapes will be important for restoring prairies that have had extended periods of afforestation because this study indicates that the seed bank is not a reliable source of prairie propagules and instead consists mainly of exotic species. Consequently, management of restored prairies should not only focus on reducing the presence of persistent invasive graminoids, rather than the ephemeral exotic forbs that appear immediately following tree removal, but also consider maximizing seed production of native species in areas adjacent to afforested grasslands. These results extend the current knowledge of tree legacy effects on grassland ecosystems by demonstrating how their persistence can impair the success of restoration and can facilitate the assembly of exotic and invasive communities.

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CHAPTER 3: LEGACIES OF AFFORESTATION ON SOIL NEMATODE COMMUNITY COMPOSITION, STRUCTURE, AND DIVERSITY IN A NORTHERN CANADIAN PRAIRIE

Abstract

Aims

I examined how legacies of afforestation affect soil food webs using the composition, structure, and diversity of soil nematodes communities along a prairie restoration chronosequence.

Methods

Vegetation and soil nematode surveys were conducted across a restoration chronosequence of tree removal (2, 5, and 20 years) in a former plantation in Riding Mountain National Park, Canada, established on a northern rough fescue prairie. Nematodes were extracted using sugar-gradient centrifugation, counted and assigned to feeding groups.

Results

Remaining plantation and recently restored prairies were characterized by bacterial feeding nematodes whereas the oldest restored prairie and control prairie were dominated by plant-root feeders. Nematode diversity did not reach the level observed in the prairie even 20 years following the removal of trees. Nematode community diversity and structure was strongly correlated with the composition of restored plant communities. Invasion of restored prairies by exotic grasses corresponded with a strong decline in the diversity of plant-root feeders.

Conclusions

Legacies of afforestation can impact prairie soils years after trees have been removed and can lead to shifts in feeding structure and a loss of diversity in the soil food web. As grasslands continue to decline globally, considering soil faunal communities may help evaluate the outcomes of ecosystem restoration and maintain their key functions.

Introduction

Global changes in climate and intensification of anthropogenic land uses threaten grassland ecosystems around the world (Veldman et al. 2015). Resulting changes compromise vital ecosystem services, including climate and water regulation (Gordon et al. 2010; Lal et al. 2013), biodiversity conservation (Liebman *et al.*, 2013), and biomass production (Tilman et al. 2001), and emphasize the importance of grassland restoration. Recent studies indicate that the resilience and assembly of grassland communities is tied to the functional composition and structure of the soil microbiome (De Deyn et al. 2003; Wubs et al. 2016; Koziol & Bever 2017). Consequently, understanding interactions between the changing structure and composition of plant and soil communities is important for conserving and restoring the function of grassland ecosystems around the world (Kardol & Wardle 2010; Gellie et al. 2017).

Globally, land-conversion, fire suppression, and changes in historic grazing regimes, have resulted in the afforestation of grasslands (Gregory et al. 2015). Afforestation, the establishment of trees in grasslands, has been particularly detrimental to grassland soils because of the significant physical, chemical and biological changes that forest vegetation can have on soil structure and function. For example, changes in the type of carbon storage (Thuille & Schulze, 2006; Pinno & Wilson, 2011), increases in nutrient mineralization (Chen et al. 2008), reductions in pH (Rigueiro-Rodríguez et al. 2012), shifts in fungal and bacterial composition (Liu et al. 2017), and decreased moisture holding capacity (Nosetto et al. 2005), can continue to impact the function of grassland soils and prevent the reassembly of grassland plant communities, long after trees have been removed (Briggs et al. 2005; Crotty et al. 2016; De la Peña et al. 2016).

Lasting effects of forest vegetation on the reassembly of grassland plant communities require that we understand mechanisms underlying the legacies of afforestation on grassland soils including the effects on the soil microbiome (Harris, 2003). Among soil biota, nematodes are one of the most abundant and diverse groups that provide an important model for changes in the structure and function of soil communities (Bongers & Ferris, 1999). Soil nematodes influence root biomass, facilitate nutrient turnover and mediate the functional composition of the soil microbiome (Neher 2010). The immense functional diversity of nematodes combined with their abundance, low motility and sensitivity to environmental conditions has also made this group an important bioindicator of soil health (Bongers and Ferris, 1999; Ekschmitt et al., 2001; Griffiths et al., 2001). The use of nematode feeding groups to analyze trophic structure of the soil microbiome could therefore provide valuable insights into plant-soil interactions in restored ecosystems, including the structural and compositional consequences of afforestation on soil food webs (Ritz & Trudgill, 1999; Kardol & De Long 2018).

I examined the impact of afforestation and subsequent tree removal on soil community assembly in a fragment of the threatened Great Plains rough fescue prairie in north-central Manitoba, Canada. Rough fescue prairies are characterized by the climax species *Festuca hallii* and occupy less than five percent of their original extent in North America, following land conversion, invasion by exotic species, and afforestation (Widenmaier & Strong, 2010; Thorpe et al., 2015). My main research questions were: 1) Do legacies of afforestation influence the structure, diversity, and function of prairie soil food webs; and 2) Do changes in the soil community reflect changes in vegetation along a prairie restoration chronosequence? To test these questions, I examined the structure, functional composition, and diversity of nematode communities along a chronosequence of tree removal in an afforested northern fescue prairie. To my knowledge, this is the first inventory of soil nematode communities in a North American rough fescue prairie.

Methods

Study Region

Research was conducted in Riding Mountain National Park, Manitoba (50°65’ N, 99°97’W) which covers an area of 2978 km^2 and is located in the Boreal Forest Region of north-central Canada (Cody, 1988). This region has daily mean temperatures ranging from -17.5℃ in January to +17℃ in July, with an average annual precipitation of 488 mm (Environment Canada, 2013). The main soil types in the park belong to the Chernozemic soil order and the Grey-Wooded or Grey-Black Transitional Zone groups with the majority developing over surface deposits of glacial till (Ehrlich *et al.* 1958).

Riding Mountain National Park contains a large proportion of the remaining northern rough fescue prairie in Manitoba and western Canada (Trottier, 1986). These prairies are threatened throughout western Canada as a result of land development, altered grazing regimes, exotic plant invasions, and afforestation (Widenmaier & Strong, 2010; Thorpe *et al.*, 2015). Between 1929 and 1950, prior to the establishment of Riding Mountain National Park or soon following its creation, several rough fescue prairies were converted to white spruce plantations in order to supplement the local timber supplies (Higgs, 1993). Over the past 30 years, several former plantations have been removed to initiate the restoration of historic prairies. At these sites, trees were cut in the winter and removed without further management. I selected two sites in Riding Mountain National

Park that have histories of white spruce afforestation and clear chronosequences of tree removal to evaluate the effects of afforestation legacies on prairie plant and soil nematode communities. I observed similar trends in the plant community at both sites (Townsite plantation [22 ha; 50°66’N, 99°95’W]: 1948-2016, Lake Audy plantation [70 ha; 50°45’N, 100°13’W]: 1929-2016)(Chapter 2) and selected the longer restoration chronosequence at the Lake Audy plantation for the detailed comparisons between the plant and soil nematode communities. I acknowledge that the inability to replicate the nematode community analysis at another site due to a lack of comparable restoration chronosequences is a limitation of this study and should be considered when interpreting the results presented. At the Lake Audy plantation, tree removal began in 1997 and continued for 20 years resulting in a chronosequence of restoration with treatments that have been without trees for 2, 5 and 20 years. These treatments have received no further management and are being used to evaluate legacies of afforestation on the restoration of northern fescue prairie communities.

Sampling Design

I used aerial photos (National Earth Observation Data Framework Catalogue, 1931), taken prior to the establishment of the Lake Audy plantation, to confirm the historic boundaries of the original fescue prairie and used satellite images (Google Earth v.8.0.4.2346, 2016) to map the extent of the white spruce plantation and the extent of disturbance resulting from tree removal. Treatment polygons were selected to represent a chronosequence of restoration following tree removal (20 years, 5 years, and 2 year post tree removal) and included areas of uncut plantation and control fescue prairie for comparison. All trees were planted in 1929 so the dates of tree removal correspond to the duration of afforestation (68, 83, and 86 years, respectively). Within each treatment polygon, I selected a random sample of 20-35 quadrats (~ 15% of the total area) to measure plant community structure and composition ((n = 133) and a smaller subset of the same quadrats (n = 60) to measure the structure and composition of soil nematodes.

Vegetation Sampling

I measured the structure and composition of plant communities between June – July 2016 along the chronosequence of tree removal and estimated the abundance of each herbaceous species inside 1 x 1 m quadrats using a pin drop method (Goodall, 1952) . At each pin drop (25 drops/per quadrat), I recorded the species of herbaceous plants touching the pin. This technique allows for presence-absence estimations of cover by sampling a single point in the foliar cover to determine if a species occupies that space. Recorded frequency data were then converted to percent cover by taking the proportion of hits out of 25 and multiplying it by 100. For shrub species, percent cover was recorded within the 1 x 1 m quadrat using a visual estimate (1-100%).

Nematodes

Nematodes were collected to determine if the community structure, diversity and function of soil food webs change following afforestation and if these communities of soil nematodes are related to changes in vegetation along a prairie restoration chronosequence. Sampling was conducted twice (July and September) in the summer of

2017 in order to capture the full diversity of nematodes over the growing season (Barker et al. 1985). Thirty samples (6 samples x 5 treatments) were collected from a random subset of the vegetation sampling quadrats during each sampling period (n = 30 x 2). Soil monoliths (7cm x 7cm x 10cm) for the extraction of nematodes were collected using a tree planting shovel (Bushpro, Vernon, Canada) from the southeast corner of each quadrat. Soil monoliths were placed in sealed plastic bags and stored at 4°C until extraction for up to 3 weeks. Extraction followed the improved centrifugal flotation protocol outlined by Barker et al. (1985) and the resulting nematode suspensions were stored at 5°C for a maximum of 24 hours until nematodes could be counted and identified.

Nematode counts were conducted on each suspension using a dissecting microscope (50x magnification) and a gridded Petri dish. Identification of the first 100 nematodes to genus level followed Jairajpuri and Ahmad (1992) for the Dorylaimida and Bongers (1989) for all other orders of soil nematodes. Nematodes were assigned into functional groups based on their feeding habits (Yeates et al., 1993). Nematodes not found in Yeates et al. (1993) were classified according to the morphological similarities of their feeding apparatus to described nematodes.

Gravimetric moisture content (GMC) was calculated for every sample to allow for the standardization of nematode abundances by dry weight of soil. Ten grams from each sample was homogenized, weighed and dried in a preheated oven at 105°C for 24 hours before being re-weighed to calculate the GMC (Gardner, 1986).

Characterizing Soils

I measured concentrations of nitrate, ammonium, and plant available phosphorus across the remaining plantation, restoration chronosequence treatments, and control fescue prairie to describe soil conditions for both plant and nematode communities. Within each of the five treatments, ten quadrats were randomly selected and sampled three times (May, July and September 2017, n = 50 x 3) to capture seasonal fluctuations in soil nutrients. A Dutch auger (7.5 cm diameter) was used to collect cores from the top 15 cm of soil at the southeast corner of each quadrat. Extracted cores were stored in sealed plastic bags, transferred to a cooler with ice packs, and dried (105°C, 24 h) within 24 hours of collection. Dried samples were analyzed for nitrate and ammonium for all three sampling periods (n = 150) using the methods described by Maynard & Kalra (1993). Plant available phosphorus was determined for samples collected in July only, using the methods of Olsen *et al.* (1954). Mineral nitrogen did not change significantly across the season and ranged from 44.6 to 56.2 mg per kg of dried soil in the plantation and restored treatments while control prairie soils contained on average 79.4 mg per kg of mineral nitrogen ($F_{4,45}$= 12.81, p<0.001). Plant available phosphorus ranged from 0.74 to 0.89 mg per kg of dried soil in the plantation and restored treatments while control prairie soils contained on average 1.15 mg per kg of P ($F_{4,45}$=4.60, <0.005).

Analysis

I evaluated the hypothesis that legacies of afforestation can persist in prairie soil food webs using the structure, composition, and diversity of soil nematode communities. I calculated the mean richness of soil nematode genera (s), Shannon-Weaver diversity (*H*’

$= -\sum_{i=1}^{s} p_i log p_i$), Pielou’s evenness ($J = H$’/log(s)) and beta diversity (Chord distances) for each treatment (control fescue prairie, remaining plantation and 2, 5 and 20 year old restored treatments) and used a one-way ANOVA to test for differences among treatment means (Legendre & Legendre, 2012). I selected Chord distances to compare beta diversity within treatments because of the standardization it applies to quadrats sharing the same species with different absolute abundances (Orlóci, 1967).

I used redundancy analysis (RDA) to test whether legacies of afforestation have altered prairie nematode community abundance and composition. RDA is a method of constrained ordination that uses eigenvector analysis to determine what proportion of variation observed in the response variable (genera composition) can be explained by variation observed in the explanatory variable (treatment) (Legendre & Legendre, 2012). The significance of the RDA model was tested using Monte-Carlo permutations to determine if the observed variation explained by each ordination axis was higher than the variation explained for models generated by random permutation of the nematode community data along each axis (Oksanen *et al.*, 2017).

I tested whether the structure, diversity, and function of soil nematode communities reflect changes in vegetation along a prairie restoration chronosequence using canonical correlation analysis (CANCOR). CANCOR is a form of constrained ordination used to find linear combinations between two multivariate sets of data that maximize their correlation (Legendre & Legendre, 2012). CANCOR is appropriate for analyzing correlations between two ecological communities because it is symmetric and does not assume a factor and response relationship between the two data sets (Legendre & Legendre, 2012). I constructed the correlation matrices using the most abundant plant species and nematode genera representing on average the top 75% of each community across all five treatments ($p = 23$, $q = 21$).

All statistical analyses were performed in R v.3.4.0 using the packages 'stats' v. 3.4.0 (R Core Team, 2017) and 'vegan' v. 2.4-3 (Oksanen *et al.*, 2017).

Results

Time since tree removal and the presence of exotic, invasive species were related to changes in the structure, diversity and composition of the soil nematode community across the prairie restoration chronosequence. After two sampling periods a total of 61 nematode genera were described from 28 different families.

Moving from recently restored to the oldest restored treatment, the structure of soil nematode communities shifted away from bacterial feeding genera ($F_{4,55}=18.06$, $p<0.0001$) and became dominated by plant parasitic genera ($F_{4,55}=21.84$, $p<0.0001$) (Table 3.1 and 3.3). In contrast, the proportion of fungivorous, predacious, and omnivorous nematodes did not change significantly between restoration treatments ($F_{4,55}$= 0.62-1.93, p= 0.12-0.65)(Table 3.3).

Table 3.1: Mean abundance (count per kilogram of soil ± SD) of the top ten genera of soil nematodes across a prairie restoration chronosequence in Riding Mountain National Park, MB, Canada. Top ten genera from each period of tree removal are shown in addition to those found in the remaining white spruce plantation and control fescue prairie. Feeding groups are shown according to Yeates et al. (1993) as superscripts (B = bacterial, F = fungal, PP = plant parasitic, PR = predator).

PLANTATION		**2 YEARS**		**5 YEARS**		**20 YEARS**		**PRAIRIE**	
Genus	Count ± S.D.	Genus	Count ± S.D.	Genus	Count ± S.D.	Genus	Count ± S.D.	Genus	Count ± S.D.
Chronogaster^{B}	1236±760	*Acrobeles*^{B}	1386±1682	*Acrobeles*^{B}	2178±1105	*Tylenchus*^{PP}	7086±4884	*Tylenchus*^{PP}	3975±1590
Acrobeles^{B}	1031±695	*Helicotylenchus*^{PP}	996±2511	*Tylenchus*^{PP}	1621±1918	*Acrobeles*^{B}	840±905	*Acrobeles*^{B}	1861±1186
Tylenchus^{PP}	1014±959	*Tylenchus*^{PP}	979±715	*Chronogaster*^{B}	754±668	*Chronogaster*^{B}	548±526	*Eucephalobus*^{B}	1273±2231
Protorhabditis^{B}	656±270	*Chronogaster*^{B}	867±846	*Eudorylaimus*^{PR}	474±357	*Helicotylenchus*^{PP}	441±814	*Hemicycliophora*^{PP}	1197±1817
Eucephalobus^{B}	422±277	*Eucephalobus*^{B}	732±598	*Eucephalobus*^{B}	445±379	*Eumonhystera*^{B}	429±446	*Helicotylenchus*^{PP}	894±898
Prismatolaimus^{B}	366±442	*Protorhabditis*^{B}	590±752	*Teratocephalus*^{B}	442±446	*Eucephalobus*^{B}	379±375	*Labronema*^{PR}	769±706
Eudorylaimus^{PR}	358±339	*Aphelenchus*^{F}	572±481	*Protorhabditis*^{B}	429±333	*Protorhabditis*^{B}	367±478	*Ditylenchus*^{F}	743±925
Cervidellus^{B}	354±254	*Eudorylaimus*^{PR}	500±531	*Alaimus*^{B}	364±546	*Eudorylaimus*^{PR}	365±421	*Hemicriconemoides*^{PP}	726±1085
Eumonhystera^{B}	338±277	*Cephalobus*^{B}	344±690	*Eumonhystera*^{B}	319±429	*Pratylenchus*^{PP}	340±399	*Criconemella*^{PP}	659±662
Teratocephalus^{B}	251±243	*Eumonhystera*^{B}	330±290	*Cervidellus*^{B}	312±262	*Prismatolaimus*^{B}	328±386	*Tylenchorhynchus*^{PP}	635±639
TOTAL	8956±2718		10610±6023		11337±5732		13846±5256		23006±10960

The richness ($F_{4,55}$= 10.99, $p<0.0001$), diversity ($F_{4,55}$= 5.36, $p<0.001$) and evenness of soil nematode genera ($F_{4,55}$= 4.09, $p<0.005$) declined across the restoration chronosequence and did not resemble the levels of diversity observed in the control prairie, even after 20 years following the removal of trees (Table 3.2). Nematode communities were also more homogenous in the oldest restored treatment and never reached levels of beta diversity similar to those observed in the control prairie ($F_{4,55}$= 13.07, $p<0.0001$)(Fig. 3.1).

Table 3.2: Mean (± S.D.) richness (s), alpha diversity (*H'*), evenness (J) of genera of soil nematodes across the chronosequence of prairie restoration in Riding Mountain National Park, MB, Canada. The time since tree removal in afforested prairies is shown in addition to values from the remaining white spruce plantation and control fescue prairie (DOF = 4, 55). Tukey's post-hoc test results on treatments means are shown in superscript letters with each letter representing groups that do not have significantly different means at the p<0.05 confidence level.

	Plantation	**2-Year**	**5-Year**	**20-Year**	**Prairie**		
						F-value	p-value
Richness (s)	21.1 ± 2.0^{b}	19.5 ± 2.6^{bc}	20.0 ± 2.8^{b}	16.5 ± 3.5^{c}	24.6 ± 2.8^{a}	10.99	<0.0001
Diversity (*H'*)	2.63 ± 0.15^{a}	2.48 ± 0.29^{a}	2.48 ± 0.25^{a}	1.91 ± 0.51^{b}	2.78 ± 0.15^{a}	5.36	<0.001
Evenness (J)	0.85 ± 0.03^{a}	0.82 ± 0.07^{a}	0.81 ± 0.05^{a}	0.66 ± 0.14^{b}	0.86 ± 0.03^{a}	4.09	<0.005

Table 3.3: Mean proportion (± SD) of soil nematodes according to feeding group and mean nematode abundance (per kg of soil) across the chronosequence of prairie restoration in Riding Mountain National Park, MB, Canada (DOF = 4,55). Time since tree removal in afforested prairies is shown and contrasted with communities of soil nematodes from the remaining white spruce plantation and control fescue prairie. Feeding groups were assigned according to Yeates *et al.* (1993). Tukey's post-hoc test results on treatments means are shown in superscript letters with each letter representing groups that do not have significantly different means at the p<0.05 confidence level.

	Plantation	**1-Year**	**4-Years**	**19-Years**	**Prairie**		
						F-value	p-value
Bacteriovores	0.72 ± 0.10^{a}	0.65 ± 0.19^{a}	0.71 ± 0.16^{a}	0.35 ± 0.15^{b}	0.40 ± 0.10^{b}	18.46	<0.0001
Fungivores	0.03 ± 0.02^{a}	0.07 ± 0.03^{a}	0.05 ± 0.05^{a}	0.03 ± 0.03^{a}	0.06 ± 0.03^{a}	1.932	0.118
Plant Parasitic	0.20 ± 0.10^{a}	0.20 ± 0.14^{a}	0.18 ± 0.14^{a}	0.56 ± 0.16^{b}	0.46 ± 0.12^{b}	21.84	<0.0001
Predator	0.04 ± 0.04^{a}	0.05 ± 0.04^{a}	0.06 ± 0.07^{a}	0.04 ± 0.02^{a}	0.04 ± 0.04^{a}	0.621	0.650
Omnivore	0.01 ± 0.01^{a}	0.04 ± 0.09^{a}	0.01 ± 0.01^{a}	0.02 ± 0.02^{a}	0.01 ± 0.01^{a}	1.252	0.300

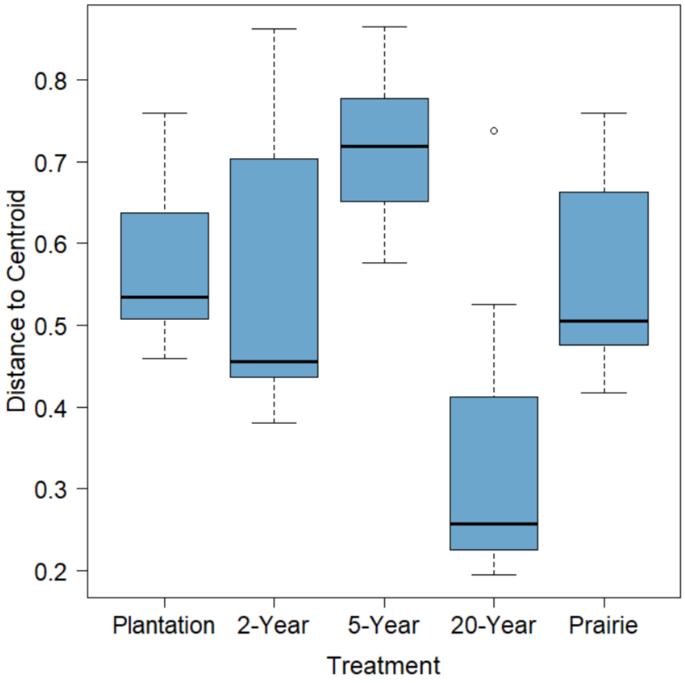

Figure 3.1: Beta diversity within communities of soil nematodes along a prairie restoration chronosequence in Riding Mountain National Park, MB, Canada. Absolute abundances of nematodes per sample were chord-transformed (Orlóci, 1967). Time since tree removal in afforested prairies is shown in addition to values from the remaining white spruce plantation and control fescue prairie.

The impact of afforestation on soil nematode communities was most evident in the abundance and diversity of bacterial and plant parasitic nematodes. The first axis of the constrained ordination (26.3% of variation explained, $p<0.001$) separated treatments based on the age of the restoration treatment (Fig. 3.2). Communities in the oldest restoration treatment were characterized by a high proportion of plant parasitic nematodes (e.g. *Tylenchus*) compared with those most recently restored which were characterized by bacterial feeders (e.g. *Chronogaster*, *Acrobeles*, and *Domorganus*)(Fig. 3.2). The second ordination axis (4.8% of variation explained, $p<0.004$) separated quadrats in the control prairie from those in the restored treatments and the remaining plantation based on the presence of a diverse assemblage of plant parasitic nematodes, including *Hemicycliophora, Criconemella, Hemicriconemoides* (Table 3.1, Fig. 3.2).

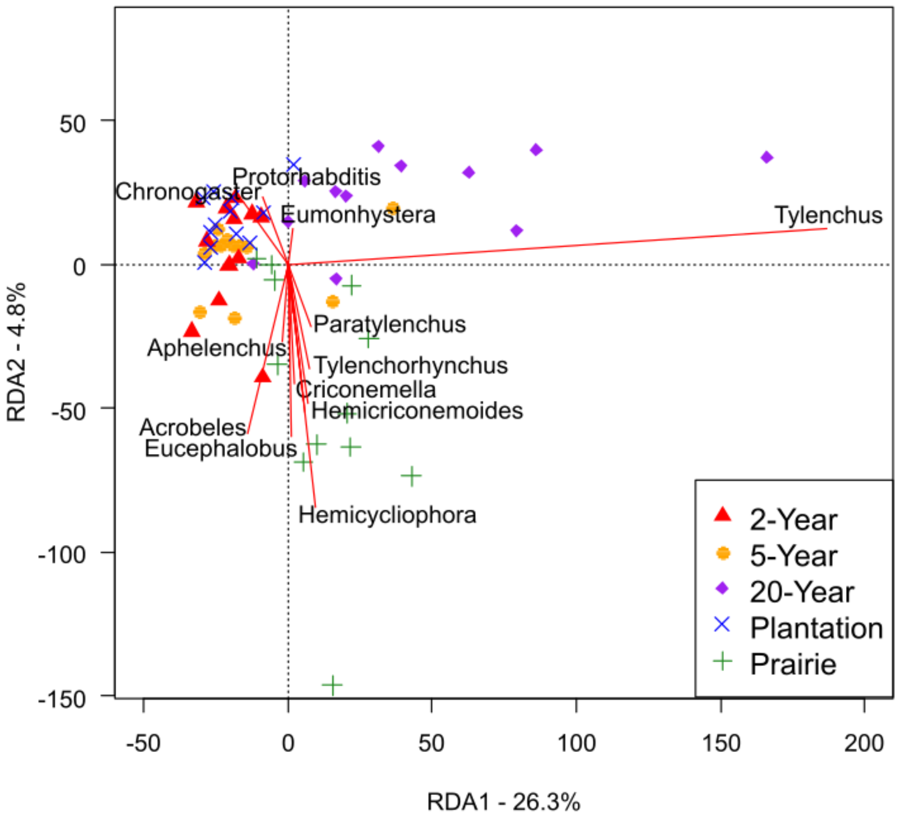

Figure 3.2: Redundancy analysis of individual samples (points), nematode genera (vectors) and restoration treatments (see legend) for communities of soil nematodes along a prairie restoration chronosequence in Riding Mountain National Park, MB, Canada. Time since tree removal in afforested prairies is shown in addition to values from the remaining white spruce plantation and control fescue prairie. For clarity, genera with eigenvector elements near zero have been excluded from the plot.

The structure of soil nematode communities was correlated with the overlying plant community along the chronosequence of prairie restoration (Fig. 3.3). Native prairie plant species including *Festuca hallii*, *Galium boreale*, and *Artemisia ludoviciana* were positively correlated with the majority of plant parasitic nematode genera including *Criconemella*, *Tylenchorhynchus*, *Hemicycliophora* and *Hemicriconemoides* and were negatively correlated with bacterial feeders (Table 3.1, Fig. 3.3). In contrast, native (e.g. *Rubus idaeus*), ruderal (e.g. *Urtica dioica*), and exotic weeds (e.g. *Sonchus arvensis*, *Galeopsis tetrahit*,*Cirsium arvense*) were positively correlated with bacterial feeding genera of nematodes that included *Cervidellus*, *Acrobeles*, and *Protorhabditis* and were negatively correlated with plant parasitic genera. The invasion of the oldest restored prairies by *Bromus inermis*, an exotic invasive graminoid, was most strongly correlated with the nematode genera *Eumonhystera*, *Ditylenchus*, *Pratylenchus*, and *Tylenchus* (Fig. 3.3).

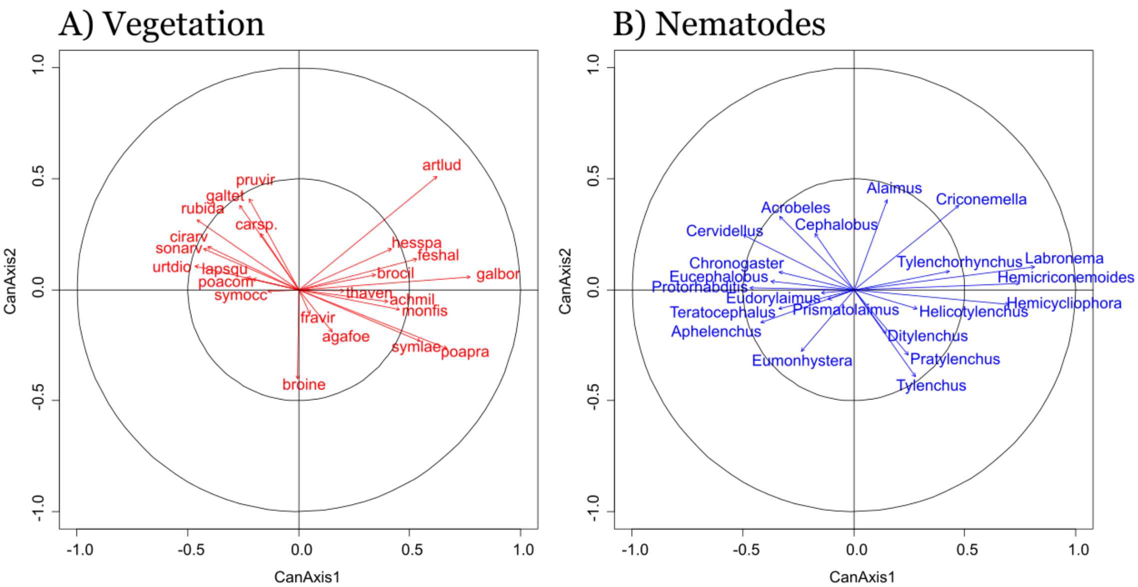

Figure 3.4: Canonical correlation biplot of the vegetation (A) and corresponding soil nematode community (B) across the 20 year tree-removal chronosequence in Riding Mountain National Park, MB, Canada. . Vectors trending in the same direction are positively correlated variables whereas opposing arrows are negatively correlated. Arrows perpendicular to one another represent no correlation. Shortened names used for species of vascular plants: achmil=*Achillea millefolium*, agafoe= *Agastache foeniculum*, ardlud= *Artemisia ludoviciana*, brocil= *Bromus ciliatus*, broine=*Bromus inermis*, carsp.=*Carex* sp., cirarv= *Cirsium arvense*, feshal=*Festuca hallii*, fravir= *Fragaria virginiana*, galtet= *Galeopsis tetrahit*, galbor=*Galium boreale*, hesspa= *Hesperostipa spartea*, monfis= *Monarda fistulosa*, poapra=*Poa pratensis*, rubida=*Rubus idaeus*, sonarv= *Sonchus arvensis*, symlae= *Symphyotrichum leave*, symocc= *Symphoricarpos occidentalis*, thaven= *Thalictrum venulosum*, urtdio= *Urtica dioica*. Plant taxonomy according to VASCAN (Brouillet et al. 2010, http://data.canadensys.net/vascan/search?lang=en).

Discussion

Linkages between above- and below-ground components of terrestrial ecosystems are thought to strongly influence the structure and function of ecosystems and are emerging as an important element in predicting and evaluating the outcomes of restoration (Kardol & Wardle 2010). Below-ground, root morphology, anatomy, and the chemical composition of exudates shape the rhizosphere environment, regulate soil processes, and influence the composition of soil microbial communities (Wang et al. 2015, McCormack 2017). In exchange, feedbacks between soil microorganisms and plants shape plant communities and drive their structure, composition, and diversity (Bever et al. 2012). This work demonstrates that native fescue prairie soil communities are characterized by a diverse assemblage of nematodes that respond structurally and compositionally to changes in the plant community following afforestation. Furthermore, these results illustrate that soil nematode communities begin to structurally resemble those in the native prairie, but that a return to nematode diversity is not reached even after 20 years following the removal of trees from the afforested prairie. In addition, the poor success of plant community restoration (Chapter 2) seemed to correlate with the simple structure and low diversity of soil nematode communities following tree removal.

The difference in soil nematode assemblages of the plantation and control prairie demonstrated the pronounced effects of plant community composition on prairie soils as well as the lasting legacies of afforestation. Structurally, forest plantation communities were dominated by bacterial feeding nematodes and did not contain the diverse assemblage of plant-parasitic nematodes observed in the prairie. Consistent with trends in naturally afforested grasslands (Biederman & Boutton, 2009; Dickie et al., 2011), this study reflects that tree establishment can structurally shift soil food webs towards decomposition-based and dominated by bacterial feeders. In addition, because nematode genera are sensitive to changes in plant species identity (De Deyn et al., 2004), the cause of these structural shifts is likely linked to changes in root quantity and quality (DuPont et al., 2014) and the composition and abundance of litter (Sauvadet et al., 2016). For example, the reduced abundance of plant-parasitic nematodes in the plantation may reflect a change in resource availability (e.g. roots) as the plant community shifted away from prairie forbs and grasses towards a mix of shrubs (e.g. *Rubus idaeus*), exotic species (e.g. *Cirsium arvense* and *Sonchus arvensis*), and weeds (e.g. *Urtica dioica*) (Biederman & Boutton, 2009). The negative correlation of non-prairie plants with plant-feeding nematodes may suggest that there are changes in host plant-soil interactions that are not sufficient for sustaining prairie assemblages of nematodes.

The similarity of soil nematode communities in recently restored prairies reflects potential afforestation legacies as well as the ‘lag’ response of the soil microbiome to abrupt changes in vegetation. As a result, despite the absence of trees, 2 and 5 year restored soil nematode communities resembled the composition and structure of plantation communities. Soil communities under manipulated plant communities have shown comparable compositional lags over a two to five-year period (Li et al., 2007; Holtkamp et al., 2008; Elgersma et al., 2011) with bacterial feeders often the first to respond to changes in nutrient input and disturbance (Ferris et al., 2001). As a result, the abundance of bacterial feeders (e.g. *Acrobeles, Chronogaster,* and *Eucephalobus*) in the recently restored treatments indicates that decomposition is still the main form of resource acquisition in the soil food web and that functionally a return to prairie soil structure and function has not occurred.

Although the composition of nematode feeding groups in the oldest restored treatment began to structurally resemble prairie nematode communities, these nematode communities were the least heterogeneous and diverse across the entire chronosequence and never reached the levels of alpha and beta diversity observed in the control prairie. Structurally, the increased abundance of the plant-parasitic nematode group was correlated with the dominance of a single genus, *Tylenchus* sp.. Compared with other dominant prairie plant-parasitic nematodes, *Tylenchus* species are ubiquitous, relatively small, have weak stylets (Bongers 1988) and are considered common basal fauna in most food webs (Ferris et al. 2001). Dominance of *Tylenchus* in restored communities was mirrored by the low diversity of the prairie plant community, 20 years after tree removal, and its dominance by two exotic invasive grasses *Bromus inermis* and *Poa pratensis* (Coffey & Otfinowski in review). Unlike native prairie species, both *B. inermis* and *P. pratensis* can form, dense, shallow root mats in soils allowing them to exclude prairie grasses and forbs (Gist & Smith 1948; Dong et al. 2014). The observed decline in diversity in both the plant and nematode community in restored prairies invaded by exotic grasses reinforces the strong ties in the response of nematodes to plant community composition (Eisenhauer et al., 2011; Cortois et al., 2017) and highlights the important role of soil invertebrates as potential drivers of grassland succession and diversity (De Deyn et al. 2003). However, this study also demonstrates that a return to prairie nematode diversity does not occur independent of the plant community and that legacies of forest vegetation can continue to influence the structure and composition of soil nematode communities and the reassembly of prairie communities long after the removal of trees. These findings may have important implications for explaining the widespread proliferation of invasive plants in prairie communities and demonstrates the significance of integrating both above- and below-ground biodiversity in predicting the assembly of restored native prairie communities (De Deyn et al., 2004).

Determining the extent to which reduced diversity in the soil food web of afforested prairies is a result of direct interactions between plant and soil communities is a challenge that requires both experimental and field observations. In this study, the emergence of low-diversity communities of nematodes following tree removal, coupled with invasion by exotic grasses, expands on the escape-from-enemy concept of biological invasion to include below-ground enemies (Diez et al. 2010). In afforested prairies, the shift in root morphologies towards a higher proportion of coarse roots, reduced specific root length, and higher root tissue density (Wang et al. 2018) could result in a decreased number of plant-parasitic nematodes (Biederman & Boutton 2009), unable to forage on the roots of trees. Following 65 years of afforestation, the absence of diverse assemblages of root-herbivores may have facilitated the establishment of invasive exotic plants following tree removal. As a result, changes in the soil fauna resulting from afforestation may have altered the selective suppression of dominant plant species and resulted in the dominance of exotic invasive grasses (*sensu* De Deyn et al. 2003). As a result, this has many implications for grasslands threatened by invasive species and suggests that resilience to invasion may be a function of the structure and diversity of the soil food web (Chesson 2002).

In conclusion, these findings indicate that legacies of afforestation and exotic grass invasion present challenges to restoring prairies and I provide evidence for long-term belowground consequences to the diversity and structure of soil food webs and their relationship to aboveground restoration success. In addition, these results reinforce the important role of soil nematode communities as sensitive bioindicators to changes in plant structure, composition, and diversity across a restoration chronosequence. However, future work looking at the morphology, structure, and function of plant roots may be necessary for understanding the interface and drivers of these relationships. This work extends current efforts to incorporate soil faunal communities into the evaluation of restoration success in order to better understand their function in the assembly of restored communities (Perring et al. 2015). As grasslands continue to decline globally, incorporating soil faunal communities offers an important conceptual basis to predict and evaluate the outcomes of ecosystem restoration and maintain key functions of restored ecosystems (Kardol & Wardle 2010).

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CHAPTER 4: GENERAL CONCLUSION

Evaluating restoration of prairie ecosystems using elements of both above and belowground communities can provide a more complete picture of the consequences of historic disturbance and biological invasion (Riggins et al., 2009; Morriën et al., 2017). By incorporating elements of the plant community, seed bank, and soil community into the evaluation of rough fescue prairie restoration I have been able to show that afforestation has created persistent legacies above and belowground and consequently multiple barriers to restoration. Within Riding Mountain National Park afforestation has been identified as a major threat to remaining fescue prairies and measures to restore and maintain the ecological integrity of these ecosystems is a conservation and management priority (Parks Canada 2007; Cornelsen, 2013). The results of my thesis suggest that tree removal alone is insufficient for prairie restoration in afforested areas and that the persistence of low-diversity plant and soil communities constitutes an important legacy of afforestation and an important barrier to restoration. In addition, my work indicates that the seed bank is not a reliable source of prairie species propagules after extended periods of afforestation and that alternative native seed sources will be required to restore afforested prairies. As part of the Parks Canada mandate, my work will contribute to future management decisions, including herbicide use and prescribed burning, to help improve the ecological integrity of remaining fescue prairies in the park by providing feedback on the abundance of exotic species in restored prairies and demonstrating the negative effects of tree establishment on prairie diversity.

The strong correlation observed between plant community diversity and a key taxon within the associated soil food web indicates that future studies will be required to help understand the feedback mechanisms occurring in the rhizosphere that prevent the restoration of afforested prairies. For example, understanding how plant root identity, exudates, architecture, and morphology can shape the rhizosphere will be important for predicting the recovery of afforested prairies following invasion (DuPont et al., 2014; Freschet et al., 2017). In addition, understanding how the soil community influences plant community assembly will also require further work to understand how soils affect the resilience of prairies to invasion (De Deyn et al., 2003; Koziol & Bever, 2017). These future questions are highlighted in Chapter 3 where I demonstrate that the putative response of prairie soil nematode diversity to afforestation and subsequent tree removal parallels the decline in diversity of the plant community. In this case, identifying the drivers and facilitators of prairie community assembly will be an important next step for determining when and where management will be most effective.

In both Chapters 2 and 3, the observation that diversity declines both above and belowground following afforestation and exotic invasion reinforces the importance of understanding biodiversity conservation at multiple trophic levels and subsequently the complexity of defining restoration targets. In many cases, the persistent effects of invasive species on community assembly are difficult to track (Weidenhamer & Callaway, 2010; Xiao et al., 2014) and consequently are not easily anticipated when designing management strategies. In my work, I have been able to show that the consequences of afforestation and exotic species invasion on the composition and diversity of prairie seed banks and soil communities can persist long after trees have been removed and interfere with future restoration.

Overall, my research demonstrates the negative consequences of afforestation on rough fescue prairie communities both above and belowground and illustrates barriers in the seed bank and soil community that may prevent future restoration. The strong correlations between vegetation, seed bank and soil nematodes indicates that afforestation can have widespread legacy effects on the overall composition, structure and diversity of prairie communities. Further research focused on plant-soil interactions will be required to overcome these legacies and increase the success of restoring our prairies.

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